European Polymer Journal 159 (2021) 110709

Contents lists available at ScienceDirect

European Polymer Journal

journal homepage: www.elsevier.com/locate/europolj

Chemical extraction and modification of chitin and chitosan from shrimp shells

Majid Pakizeh [a,b], Ali Moradi [c,d], Toktam Ghassemi [a,*]

[a] Department of Chemical Engineering, Faculty of Engineering, Ferdowsi University of Mashhad (FUM), Mashhad, Iran
[b] Department of Chemical Engineering, Hamedan University of Technology, Hamedan, Iran
[c] Clinical Research Development Unit, Ghaem Hospital, Mashhad University of Medical Sciences (MUMS), Mashhad, Iran
[d] Orthopedic Research Center, Mashhad University of Medical Sciences (MUM), Mashhad, Iran

ARTICLE INFO

Keywords:
Chitin
Chitosan
Chemical extraction
Shrimp shell
Biomaterials

ABSTRACT

Chitin-based materials are one of the most promising abundant polysaccharide biopolymers for developing constructs with advanced functions due to their unique properties; especially in biomedical science and technology. They have versatile applications in pharmaceuticals and biomedicine. Seafood industries produce a large volume of crustacean shells that contains chitin up to about 30% of its volume; a huge waste source with high costs of pollution. These worthy wastes can be converted into additional high grade, low-volume by-products. On commercial scale, chitin/chitosan is mostly extracted chemically from shrimp shells. The present review summarizes the current chemical methods for chitin recovery and production of its most common derivative, the deacetylated form named chitosan, from shrimp shell wastes. The main extraction steps are discussed individually and "should or must to do points" are highlighted. A brief perspective for future researches in this topic are presented at the end.

1. Introduction

1.1. Chitin and Chitosan; Properties and Applications

Chitin, the second most abundant biopolymer on the Earth after cellulose, is a polysaccharide made from N-acetyl-D-glucosamine units which is found mainly in crustacean shells, insects, and microorganisms such as fungi, algae, and yeasts [1]. Extracted chitin is classified as α, β, and γ-chitin according to anti-parallel, parallel, and alternated alignments of the polymer chains (as a combination of α and β structures), respectively (Fig. 1). α-chitin is usually obtained from the crustaceans exoskeletons, specifically from shrimps and crabs; β-chitin can be isolated from squid pens; and γ-chitin is obtained from fungi and yeast [2]. Crustacean shells and fungal mycelia are the industrial resources of chitin [1].

The degree of N-acetylation has a considerable effect on chitin insolubility and limits its swelling properties in water due to the compact structure of the solid state [2,4]. Strong intra- and intermolecular hydrogen bonding network of chitin makes it insoluble in common organic and inorganic solvents. This extremely limits the practical application of chitin. The high hydrophobicity of chitin makes it only soluble in dimethylformamide and lithium chloride, hexafluoroisopropanol and 1, 2-Chloroethanol, and high concentrated inorganic acids like hydrochloric acid (HCl), sulfuric acid (H_2SO_4), and phosphoric acid (H_3PO_4) [5]. Various chemical modifications that can disrupt the hydrogen bonds, without splitting the glucosidic linkages, can improve chitin solubility in water or other solvents. Therefore, chitin derivatives has been developed by applying their polyelectrolite

Abbreviations: DPPH, 2,2-diphenyl-1-picrylhydrazyl; CH_3COOH, Acetic acid; $NaHCO_3$, Bicarbonate of soda; $CaHSO_3$, Calcium bisulfite; $CaCO_3$, Calcium carbonate; $CaCl_2$, Calcium chloride; CO_2, Carbon dioxide; $Ca(OH)_2$, Calcium hydroxide; $Ca_3(PO_4)_2$, Calcium phosphate; DD, Degree of deacetylation; DTT, Dithiothreitol; EDTA, Ethylenediaminetetraa-cetic acid; CH_2O_2, Formic acid; FTIR, Fourier-transform infrared spectroscopy; FPT cycle, Freeze-pump out-thaw cycle; HCl, Hydrochloric acid; H_2O_2, Hydrogen peroxide; ClO^-, Hypochlorite; Mw, Molecular weight; HNO_3, Nitric acid; N_2, Nitrogen; NMR, Nuclear magnetic resonance; H_3PO_4, Phosphoric acid; K_2CO_3, Potassium carbonate; KOH, Potassium hydroxide; $KMnO_4$, Potassium permanganate; RSM, Response surface method; rpm, Revolutions/rotations per minute; RT, Room temperature; $NaHSO_3$, Sodium bisulfite; Na_2CO_3, Sodium carbonate; SDS, Sodium dodecyl sulfate; NaOH, Sodium hydroxide; NaClO, Sodium hypochlorite; Na_3PO_4, Sodium phosphate; Na_2S, Sodium sulfide; Na_2SO_3, Sodium sulfite; H_2SO_4, Sulfuric acid.

* Corresponding author.
E-mail addresses: pakizeh@um.ac.ir, pakizeh@hut.ac.ir (M. Pakizeh), moradia@mums.ac.ir (A. Moradi), toktam.ghasemi@gmail.com (T. Ghassemi).

https://doi.org/10.1016/j.eurpolymj.2021.110709
Received 8 May 2021; Received in revised form 14 August 2021; Accepted 16 August 2021
Available online 19 August 2021

properties, and the presence of reactive functional groups [1]. The free —NH_2 groups within chitin chains allow specific modifications under relatively mild conditions. Chemical modifications (such as deacetylation, phosphorylation, quaternization and carboxymethylation), molecular weight (Mw), the solution pH, ionic strength, and temperature are the main factors that affect chitin solubility [3]. Chitin is mostly converted into deacetylated form, chitosan, a more industrially applicable derivative, by hydrolysis of the acetamide groups with the *trans*-arrangement of the C-2/C-3 substituents in the sugar ring (Fig. 2) [6,7]. The intrinsic properties of chitosan make it a singular bio-based polymer with no actual petrochemical equivalent; a linear, semi-crystalline polysaccharide which should contain at least 60% of D-glucosamine residues (degree of deacetylation (DD) %; The *N*-deacetylation content as the mole fraction of 1 units in the chain, expressed as a percentage).

Chitosan is more soluble in comparison to chitin due to the presence of amino groups at C-2 positions which is utilized to improve the processing methods [9]. Although, chitosan is insoluble in water and most organic solvents; it is soluble in the most aqueous acid solutions such as acetic, citric, formic, and lactic acids [3,5]. The amino groups in chitosan structure are the only positively charged natural polysaccharide that differ it from others such as chitin. Chitosan, as a polyelectrolyte, can be applied for multilayer filming by deposition technique. It is based on the electrostatic interactions via layer-by-layer (LBL) self-assembly of anionic polymer and cationic chitosan. Polycationic properties of chitosan can form electrostatic complexes with oppositely charged macromolecules [10,11].

The amino groups of the D-glucosamine residues enable solubility in diluted acidic aqueous solutions (pH < 6) by NH_2 protonation and exhibit polyelectrolyte properties [1,12]. Protonated amino groups in chitosan make a polycation that can form ionic complexes with various natural or synthetic anionic species; such as lipids, proteins, DNA and some negatively charged synthetic or natural polymers [11,13].

Unlike most natural polysaccharides such as cellulose and agar that are acidic in nature, chitin and chitosan are highly basic. This property causes abilities like solubility in various media, polyelectrolyte behavior, film-forming ability, metal chelation, and structural characteristics in comparison with other acidic polysaccharides [14]. Some of the main chitin/chitosan applications are shown in Fig. 3.

Chitin and chitosan based biomaterials could be formed in various shapes for a wide range of applications such as beads in drug delivery systems [17], microspheres in enzyme immobilization or gene delivery vehicles [18], nano-particles in encapsulation of sensitive drugs [19], shaped objects in orthopedics [20] or contact lenses [21,22], films or coatings [23], fibers, powders, sponges, solutions, gels, tablets, and capsules [13,24].

Biocompatibility, enzymatic biodegradability to normal body constituents, deacetylated amino groups reactivity, chelation ability, adsorption capacity, antimicrobial activity [25], immune-stimulation [26], chemotactic action, binding to mammalian cells, enhancing the reconstruction of connective tissues and growth stimulation [27,28], osteoinduction [29,30], anti-osteoarthritis, fungistatic [31], anti-tumoric, hemostatic, spermicidal, mucoadhesive, anti-cholesteremic, central nervous system depressant, and immune assistance/immune-adjuvant and action as a source of glucosamine and acetyl glucosamine are physiological properties of chitosan [1,8,9,32].

Previous studies have reported two main mechanisms for chitosan antibacterial and antifungal activities: 1) preventing cell permeability to essential materials due to electrostatic interaction of positively charged chitosan with negatively charged groups at cell surfaces, 2) chitosan binding to cell DNA via protonated amino groups that causes inhibition of the microbial RNA synthesis. The mechanism can also be a combination of both named theories too [11,33,34]. The analgesic effect of chitosan might result from protonation of the amino groups of the D-glucosamine residues in the presence of proton ions that are released in the inflammatory area [11]. Besides higher antimicrobial activity and lower toxicity for mammalian cells, chitosan acts as antioxidants by scavenging oxygen radicals such as hydroxyl, superoxide, alkyl and stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals (by acting as hydrogen donors to prevent the oxidative sequence). It possesses anti-tumor activities through inhibiting the tumor cell growth by exerting immune-enhancing effects (by increasing the production of lymphokines and inhibition of tumor cell proliferation by apoptosis induction) [13].

1.2. Chitin Sources and Extraction Methods

α-chitin is the most abundant and stable polymorph which is more common than the β-type, it has an 80% crystallinity index [3,8,16]. Arrangement of microfibrils in α-chitin is strongly fixed by hydrogen bonds that limits its water swelling and permeability compared with β-chitin with a lower content of inter-sheet hydrogen bonds. Also, the proportion of chitin in the source material (such as the raw shells) influences the hardness, permeability, and flexibility [1]. Large available quantity of shrimp and crab shells makes them the most popular source of the white, hard, inelastic, nitrogenous polysaccharide, chitin [35]. Shrimp shells has the lowest inorganic matter (calcium carbonate ($CaCO_3$)) among other marine sources; like crabs, prawns, lobsters, and cuttlefish. Also, its chitin contains a small but significant fraction of de-N-acetylated unit [7]. Although, β-chitin shows much higher reactivity and easier destruction of crystalline structure compared to α-chitin in deacetylation procedure due to the loose arrangement of chitin molecules [36], however, the final chitosan is completely amorphous unlike highly crystalline chitosan from α-chitin. Also, the deacetylation process tends to be accompanied by heavy discoloration even in nitrogen (N_2) for β-chitin. Some treatments such as freeze-pump out-thaw (FPT) cycles can eliminate this drawback of α-phase [37]. Although, β-chitin and chitosan possess high hydrophilicity; they are more suitable for developing advanced functional materials due to their much higher reactivity [38]. However, protein removal in squilla is harder than shrimp, due to stronger protein and chitin linkage [39].

The main sources of raw material for chitin production on commercial scale are crustacean exoskeletons, principally crabs and shrimps [15]. This production is also a solution for the increasingly and costly disposal of marine wastes challenge for the shellfish production and processing industries [5]. In crustaceans, chitin is combined into a complex network with proteins onto which minerals (principally

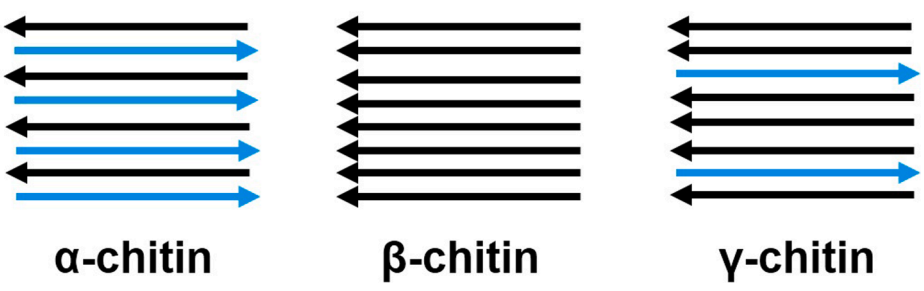

Fig. 1. Polymorphic forms of chitin [3].

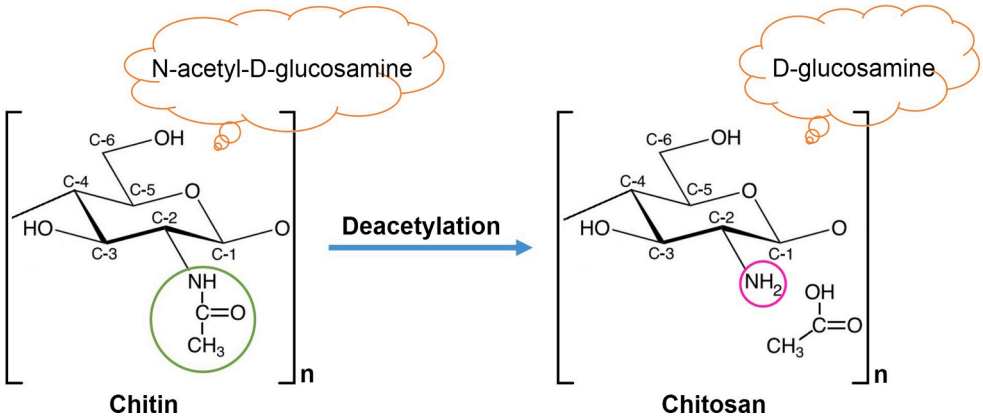

Fig. 2. Chemical structure of chitin and chitosan [8].

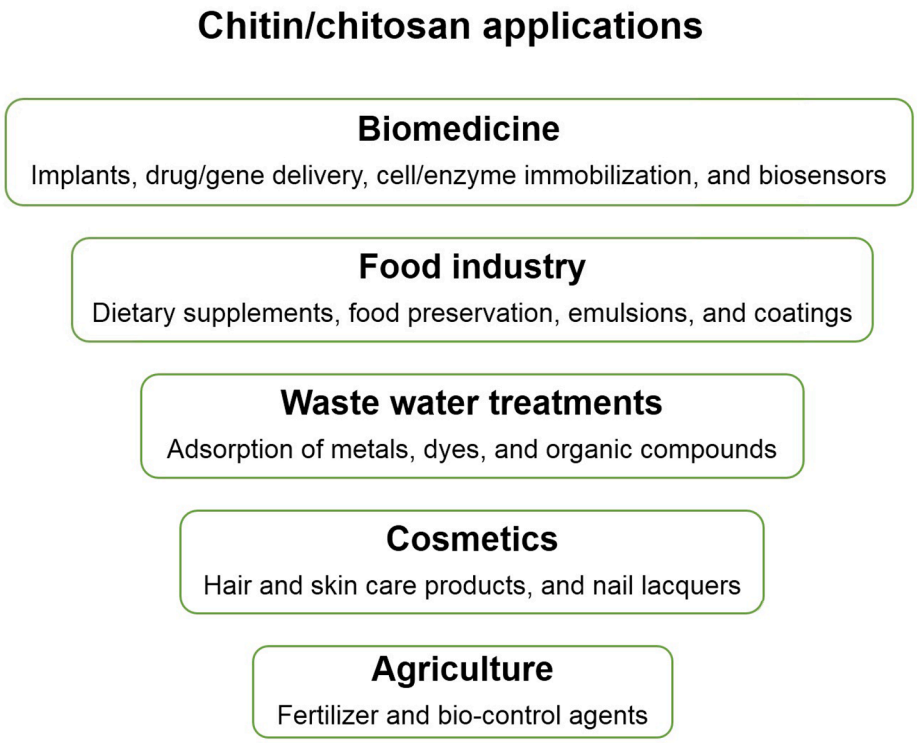

Fig. 3. Chitin/chitosan-based biomaterials applications [4,15,16].

$CaCO_3$) deposits to form the rigid shell. Chitin isolation from shrimps shells is easier due to the thinner wall of the shells [13]. Shrimp shells are composed of chitin, protein, lipids, pigments and flavor compounds [40,41]. Although, the chitin contents of the shells widely vary depending on peeling conditions, the species, the organism part, their nutrition state, climate, stage of reproductive cycle and difficulties in process control [42,43]; however, among fish, shrimp, and crab wastes, shrimp shells was shown to be the best choice with high average Mw, DD, and solubility [44]. Also, shrimp shells are one of the highest chitin content sources among other common chitin origins, such as other marine sources, insects, or fungi's [8]. The value of the global chitosan market size was 1.7 billion dollars in 2019, and has estimated to reach 4.7 billion dollars in 2027, by Allied Market Research website; and shrimp source is and will be the main source. Lower ash content of shrimp shells is the reason of better solubility of the synthesized chitosan compared to fish and crab products. Also, high ash content reduces viscosity and average Mw [44].

Chemical extraction processes, enzymatic hydrolysis, or biological/microbial techniques can be applied for extracting chitin form the shell wastes. Sometimes, a combination of the methods may be performed as the extracting procedure [45]. Chemical procedure is the most common method in chitin extraction techniques in commercial scale [46]. The process involves two main steps: elimination of inorganic matters such as $CaCO_3$ and calcium phosphate ($Ca_3(PO_4)_2$) in dilute acidic medium, usually using HCl solutions (as demineralization), continued with protein extraction in alkaline medium, traditionally by treating sodium hydroxide (NaOH) or potassium hydroxide (KOH) solutions (as deproteinization) [7]. Although, short processing time, high DD of the final chitin, industrial scale, and complete removal of organic salts are chief advantages of the chemical procedure; however, these methods are environmentally unfriendly and the obtained minerals and proteins cannot be used as human and animal nutrients [3]. Safe disposal of chemical wastes increases the production cost of chitin industries. [15]. Protein elimination can be done enzymatically by digestive proteolytic enzymes, such as papain, pepsin, trypsin and pronase [47]. Also, as a more effective method for chitin recovery comparing to chemical

techniques, microbial method can be applied by organic acids (like production of lactic acid by probiotic bacteria) for demineralization step. Higher chitin yield and quality (due to reduction of depolymerization and acetylation degrees), using the protein component as animal feed and elimination of chemical pollution sources are the main advantages of enzymatic or microbial methods [48–52]. However, these new methods have drawbacks such as inconsistency in the research results, higher total cost, and no industry production yet; and hence, more researches should be done.

1.3. Chitin/chitosan chemical extraction scenarios from shrimp shells

The shells contain three major components; 15–40% α-chitin, 20–40% proteins, and 20–50% $CaCO_3$. Pigments and other metal salts are also the minor components [8]. The chemical isolation process is composed of four steps: removal of inorganic $CaCO_3$ (demineralization), elimination of proteins (deproteinization), removing small amounts of pigments (decoloration/bleaching), and converting chitin to chitosan (deacetylation) (Fig. 4). Some studies have changed the arrangement of these steps [13]. Also, some pre-treatments such as grinding for enhancing of solution exposure and few post-treatments like purification techniques may be added for increasing the quality of the final product.

2. Pretreatments

Shrimp shells are freshly collected from the harbor or transferred into plastic containers at −10 to −20 °C until using. All organic matters are separated and after extensive washing, the cleaned shells maybe heated about 20 min at 90-100 C to inactivate endogenous enzymes [53–58]. The cleaned shells are dried, minced to a fine powder, and stored in a closed packs at room temperature (RT) [57], or wetly homogenized in a blender and then kept at − 20 C without any drying step till further use [58,59]. No research has been done to elucidate any difference in the maintaining methods. The shells would be dried under the sun light or in an oven until constant weight [51,60]. Few chemical pretreatments have been suggested for minimizing the use of chemicals and shortening the time periods of the next steps in the extraction procedure (Table 1).

2.1. Guidelines

Although, no general rule has been suggested for the specific chitin source or the unique final application/s, some evoked points from previous studies may be beneficial in future researches as pretreatment steps:

- **Fine grinding**: Due to the higher exposure of chemical agents, samples at small particle sizes will result in the highest degrees of demineralization, deproteinization, decoloration, and deacetylation compared to larger particles at similar conditions. However, particle size is less effective in ash reduction compared to the residual protein [54].
- **Chemical pretreatment**: Acidic/basic pretreatments or chemical washings (like sodium hypochlorite (NaClO)) significantly speed up the following extraction steps and decrease the need for high concentrations of acid and alkali in demineralization and deproteinization steps (up to 60% reduction in the total need for chemicals). In addition to depigmentation and disintegration, chemical pretreatment results in higher Mw chitin/chitosan and leads to substantial energy saving for quick chitin/chitosan extraction from marine resources. Less ash or protein content with no influence on the chitin yield has also been reported. Agitation can effectively accelerate the chemical pretreatments [70,74,75].

Sometimes, commercially important biomolecules like astaxanthin-

Table 1
Pretreatments for chemical chitin/chitosan extraction from shrimp shells.

Procedure	References
Physical Methods:	
- Separating loose tissues	[51,52,617,36,41,45,46,50,54,55,60,62–69]
- Washing/boiling with water/ distillated water /salt water, @ 25–100 °C	
- Cutting/grinding/cryo-grinding/ homogenizing dried/freeze-dried/ freezed shells, from 5 mm to 60 μm	
Chemical Methods:	
After physical treatments of the dried grinded shells:	[7071]; based on [7273]
- Stirring in 7.84 M HCl @ 50 rpm for 3 or 6 h	
- Soaking in 1 M NaOH for 24 h	
- Soaking in 0.28 M HCl at ratio of 1:2.5 w/v for 24 h	

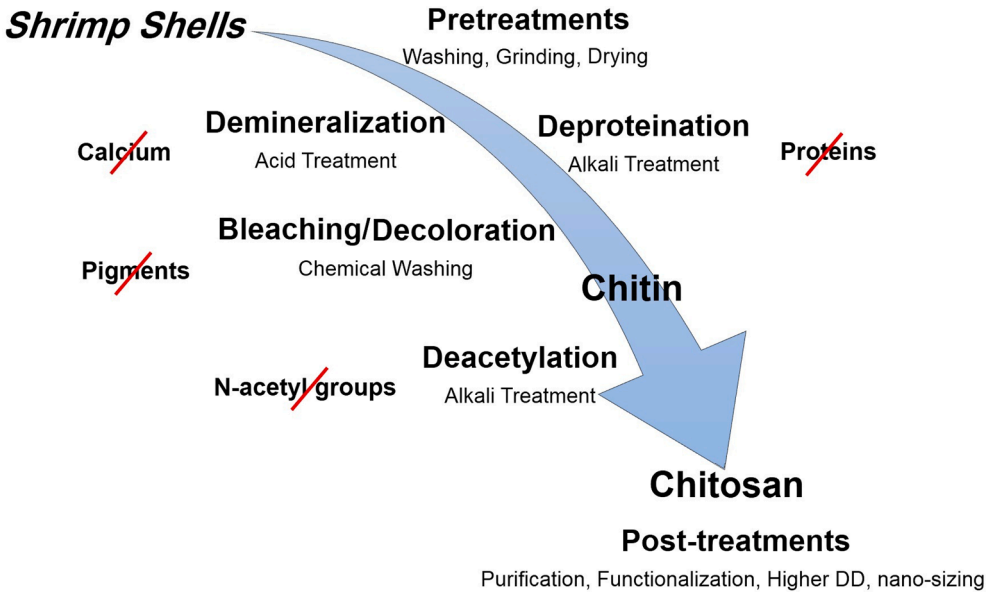

Fig. 4. Extraction process of chitin and chitosan [15].

rich oil are extracted from the shells before starting the main extraction procedure. This step can be performed by supercritical carbon dioxide extraction [76]. Protein can be also extracted by water boiling and mechanically squeezing the residue.

3. Demineralization

Traditional chitin isolation starts with the removal of minerals (primarily calcium and magnesium phosphates and carbonates) by diluted acids like HCl, H_2SO_4, HNO_3, CH_3COOH, and CH_2O_2. Hydrochloric acid is the superior one [1,77,78]. Although, demineralization at RT inside stirred bioreactors is faster and prevents the risk of chitin depolymerization and improves the quality of the final product [46]; however, it has negative effects on the Mw and the DD of the purified chitin [74].

Demineralization reaction causes decomposition of $CaCO_3$ into water-soluble calcium salts (calcium chloride ($CaCl_2$)) followed by carbon dioxide (CO_2) discharge (equation 1):

$$HCl + CaCO_3 \rightarrow CaCl_2 + H_2O + CO_2\uparrow (1)$$

The value of CO_2 gas emission depends on the shrimp mineral content and acid penetration quantity into the shells [7,62]. Based on the stoichiometric formula, two HCl molecules are needed to convert one

Table 2
Demineralization methods for chemical chitin/chitosan extraction from shrimp shells.

Acid Concentration (Molar)	Solid to liquid ratio (g/ml)	Temperature (°C)	Duration	Remained Minerals in Chitin/Chitosan (Ash %)	Consumed acid/ shell dry weight (mole/g)	Explanation	References
1.25 M HCl	1:10	–	1 h	2.4%	0.012	–	[52]
0.55 M HCl	1:11.25	RT	2 h	–	0.019	3 bath	[61]
1 M HCl	1:10	RT	24 h	0.01% Ca*	0.01	constant stirring	[51]
1.2 M HCl	–	20	15 min	Shrimp type I: 0.1% Shrimp type II: 0.5%	–	–	[79]
0.55 M HCl	1:10	RT	1–3 h	~0.02%	0.016	3 bath	[62]
0.8 M HCl	1:3.51:10	RT	12 h	0.5%	0.003 0.008	–	[70]
0.25 M HCl	1:40	40	4 h	I & II_a: ~2.5% II_b: 1.1%	0.01	I: occasionally stirring II: probe sonication (a. sonicate for 1 h @ 41 W/cm2 with leaving in the acid for 3 h with occasional stirring; b. sonicate for 4 h)	[63]
1.7 M acetic acid	1:40	60	6 h	–	0.068	–	[50]
0.25 M HCl	1:40	RT	15–180 min	–	–	several bath	[7]
1 M HCl	1:20	RT	3 h	0.4%	0.02	–	[45] Based on [36]
1.2 M HCl	–	24	15–20 min	0.9%	–	constant stirring	[46]
1.7 M HCl	1:9	RT	6 h	1.4%	0.015	–	[64] ;based on [80]
1 M HCl	1:15	RT	30 min	–	0.015	constant stirring	[65] Based on [81]
1.5 M HCl	1:15	RT	60 min	~0.2%	0.022	constant stirring @ 150 rpm	[66]; based on [82]
0.12 M HCl	1:4	–	24 h	0.2%	0.0005	After the demineralization process, treat the shells with 2% NaOH for 1 h to decompose the albumen into water soluble amino-acids.	[60]; based on [83]
1 M HCl	–	–	–	–	–	–	[71]; based on [72]
1.5 M HCl	1:15	RT	30 min	0.2%	0.022	constant stirring @ ~150 rpm	[67]
0.24 M HCl	1:10	30	12 h	0.3–0.7%	0.0024	–	[54]; based on [84]
1 M HCl	–	75	15 min	–	–	–	[75]
3 M HCl	1:10	1st: 752nd: -	1st:2h2nd:8 min	–	0.03	1st: constant stirring @ 150 rpm2nd: assimilated by microwave method @ 500 W power	[68]
1.7 M acetic acid	1:40	50	4 h	–	0.068	–	[69]
1.2 M HCl	–	32	till no gas evolution	–	–	several bath	[55]
0.4 M HCl	–	RT	30 min	0.03%	–	–	[44]
1 M HCl	1:30	RT	75 min	–	0.03	constant stirring @ 150 rpm	[85]
1.25 M HCl	–	–	–	–	–	constant stirring	[76]; based on [86]
0.8 M HCl	1:3.5	RT	12 h	0.1%	0.003	–	[73]
0.8 M HCl	1:10	25	1 h	–	0.008	constant stirring	[87]; based on [88]
0.3–1.4 M HCl	1:5	RT	16 h	–	0.002–0.007	–	[89]; base on [90]
0.6 M HCl	1:11	30	3 h		0.007	constant stirring @ 300 rpm	[91]

In studies with different experimental conditions, optimum suggested situations had been reported in the table.
In highlighted rows, deproteinization was done before demineralization.
The underlined rows were chemically pretreated.
* This number indicates only residual calcium, not all the mineral residues.

molecule of $CaCO_3$ into $CaCl_2$. Therefore, due to the heterogeneity of the solid material, acid intake should be equal or even greater than the formula for a complete reaction [13]. The cut of CO_2 gas emission is an important indicator for the reaction completion [3]. The pH evolution indicates the reaction state, either towards neutrality for executing reaction (calcium release increases the pH of the supernatant) or persistence of medium acidity in the end of the reaction [61]. The pH variation estimates the optimal reaction time and the exact amount of required acid for a complete reaction for minimizing the hydrolysis of the glycosidic bonds [51].

Most of other minerals react to acid similarly and give soluble salts; afterwards, filtration of the chitin solid phase easily separates the salts by washing. The mineral content of the shells, temperature, extraction time, particle size, acid concentration, and solute/solvent ratio are major factors in demineralization treatments. Higher temperature and finer particle sizes accelerate solvent penetration into the chitin matrix [13]. A summary of previous demineralization procedures have been shown in Table 2.

3.1. Demineralization guidelines

- For a final product with high Mw, only dilute HCl is used to prevent hydrolysis or degradation [81,82]. The required HCl for each gram of dry powder has been reported as 0.01–0.03 mol. The value is not dependent to the acid concentration in the reported range.
- The number and duration of bathes depend on the source. The stability of medium acidity indicates the end of the process or repeated demineralization baths [12,36].
- The percentage of ash content in the final solid indicates the demineralization rate [66]. Increasing the reaction time decreases the ash content of the obtained chitin/chitosan as well as product viscosity and Mw [86]. Commercial chitosan includes around 2% ash content [92]; however, chitosan with<1% ash content has been reported as high quality grade [82].
- In food supplementary industries, HCl can be replaced by acetic acid in demineralization process that supports the food grade quality of the protein; but, the efficacy of protein and ash elimination with acetic acid is less compared to HCl [70].

After each extraction stage like demineralization, deproteinization, bleaching/decoloration, or deacetylation, the treated shells will be filtered in atmospheric/vacuum condition or separated by centrifuge (like at 4000 rpm for 15 min). Then, they would be rinsed with tap, distilled, or deionized water for pH neutrality (pH 7.0). The samples may be lyophilized or dries at RT or oven until reaching a constant weight. Sometimes, the treated shells are grinded again and sieved before the next step [44,50,55,60,61,63,65,67].

4. Deproteinization

Principally, the protein content in biomaterials is the main reason for allergic reactions; therefore, radical deproteinization is crucial for biomedical applications [3]. However, some of active materials in shrimp waste including lipoprotein concentrates, protein hydrolysates, and a carotenoid extract, considerably increase the antioxidant properties of chitosan-based solutions [93]. Also, addition of shrimp protein concentrate significantly reinforces the chitosan polymeric matrix due to changes in film structure and water content [94].

The deproteinization step interrupts proteins and chitin chemical bonds by depolymerizing the biopolymer. Commonly, the demineralized shells are subjected to alkaline solutions including: NaOH, sodium carbonate (Na_2CO_3), soda ($NaHCO_3$), KOH, potassium carbonate (K_2CO_3), calcium hydroxide ($Ca(OH)_2$), sodium sulfite (Na_2SO_3), sodium bisulfite ($NaHSO_3$), calcium bisulfite ($CaHSO_3$), sodium phosphate (Na_3PO_4) and sodium sulfide (Na_2S). NaOH and KOH are the illustrious reactants [3,95]. However, NaOH treatment leads to biopolymer hydrolysis, decreasing Mw, and partial chitin deacetylation [13]. Deproteinization depends on alkali concentration, temperature, and shells mass to the alkali ratio (w/v). The solution temperature and solid-solvent ratio are the most critical factors [7,41]. Decoloration of the medium at the end of deproteinization indicates completion of the process [36,61,62].

Deproteinization process is slow due to limited access to the reactives and the various kinds of possible interactions with proteins of the R chitin structure [51]. Covalent bonds between chitin and proteins form stable complexes, like glycoproteins. Irradiation of proteins degrades their structures and shortens the deproteinization time [96]. Table 3 compares the deproteinization procedures for chemical chitin/chitosan extraction from shrimp wastes

4.1. Deproteinization guidelines

Some basic issues to be considered for deproteinization stage include:

- High deproteinization temperatures (>65 °C) decrease the Mw possibly due to long chain polymer breakage. Modifying the incubation time and temperature can yield chitin/chitosan with high viscosity and Mw [46,51].
- The protein and moisture content of the shrimp shells determine the optimum ratio of the demineralized shells to alkali solution [46]. The required mole of NaOH to each gram of the dry powder has been reported as 0.002–0.09. The reported value may depend on the alkali concentration.
- The solution clarity determines the duration of time for deproteinization process and the numbers of baths; no change in the medium color indicates the washout of protein or end of the treatment [36].
- The rate of deproteinization is evaluated by protein concentration in the supernatant [67].
- Use of ultrasound will increase the crystallinity, reduce the particle sizes, and erode the surface of the final product. Although, high sonication time brings about high DD, however, it causes a product with medium and low Mw. Also, it results in chitosan with lower whiteness index due to oxidation of the pigments in the skeleton [91].

5. Bleaching/decoloration

If a colorless product is required, a bleaching/decoloration step is added as an additional step. An organic solvent like acetone eliminates pigments such as melanin and carotenoids [16,46]. Residual pigments can cause side effects in biomedicine. Therefore, chitin and chitosan need to be highly purified [13]. The pink color of chitosan is treated with bleaching or sun-drying. However, this step will decrease the viscosity and Mw [39]. Table 4 represents the suggested techniques for bleaching/decoloration of chitin.

No study has compared the bleaching/decoloration methods and hence, no general cues can be suggested. However, color measurement has been introduced as a standard evaluating technique for determining the efficiency of the bleaching/decoloration process. High lightness value or less yellowness index are preferable.

6. Deacetylation

The simplest modification of chitin is *N*-deacetylation, which transforms it into chitosan by removal of acetyl group [1]. Alkaline treatment mostly improves chitin solubility due to the rupture of the main chain and destruction of the crystalline structure [102]. Deacetylated chitin is usually achieved by severe alkaline hydrolysis treatment at high temperatures. Concentrated NaOH or KOH (40–50%) are commonly used [3,6,103]. The dielectric constant, as a measure of the amount of electric potential energy, is high for aqueous NaOH solution compared to

Table 3
Comparision of deproteinization procedures for chemical chitin/chitosan extraction from shrimp shells.

Alkali Concentration (Molar)	Solid to liquid ratio (g/ml)	Temperature (°C)	Duration	Remained protein in chitin (%)	Consumed NaOH/ shell weight (mole/ g)	Explanation	References
0.75 M NaOH	1st bath: 1:1.52nd bath: 1:1	~100	1st: 15 min 2nd: -	5.1*	0.002	2 bath	[52]
0.3 M NaOH	–	80–85	1 h	–	–	3 bath	[61]
1 M NaOH	1:15	<70	24 h	<1%	0.015	vigorous stirring	[51]
2.25 M NaOH	–	65	90 min	–	–	–	[79]
0.3 M NaOH	–	80–85	1 h	–	–	3 bath	[62]
0.75 M NaOH	1:2.5	RT	24 h	0.8%	0.002	–	[70]
0.25 & 1 M NaOH	1st stage: 1:40 2nd stage: -	40	2–6 h	~3%	–	1st: sonicate for 0, 1, and 4 h in 0.25 M NaOH 2nd: soak for 2 h in 1 M NaOH	[63]
1 M NaOH	–	105–110	–	–	–	several bath	[36]
0.5 M NaOH	1:30	90	2 h	–	0.015	–	[50]
1.0 M NaOH	1:20	70	24 h	–	–	several bath	[7]
1 M NaOH	1:20	70	–	–	0.02	–	[45]; based on [36]
2 M NaOH	1:4	45	4 h	~0.8%	0.008	constant stirring @ 100 rpm	[46]
2.5 M NaOH	–	65	6 h	–	–	–	[64];based on [80]
0.9 M NaOH	1: 10	65	2 h	–	0.009	constant stirring	[65] Based on [81]
2 M NaOH	1:20	46	125 min	3.1%	0.04	–	[66]
1 M NaOH	–	1st stage: Boiling 2nd stage: cooling @ RT	1st: 1 h 2nd: 30 min	–	–	–	[60]; based on [97] & [98]
1 M NaOH	–	–	–	*4.8%	–	–	[71]; based on [72]
2 M NaOH	1:20	45	2 h	~4%	0.04	–	[41]
1 M NaOH	1:10	90	12 h	~3%	0.01	–	[54]; based on [84]
1 M NaOH	–	100	20 min	–	–	–	[75]
2.5 M NaOH	1:10	I: 80	I:2h II: 5 + 3 = 8 min	–	0.025	I: conventional method II: assimilated by microwave method, 5 min @ power 160 W, then 3 min @ power 350 W	[68]
0.89 M KOH	–	1st stage: boiling 2nd stage: -	1st: 6 h 2nd: 72 h	–	–	2nd: This stage was done after bleaching to remove the residual proteins	[99]
0.5 M NaOH	1:30	80	2 h	–	0.015	constant stirring	[69]
1.2 M NaOH	–	1st bath:100 2nd bath:32	1st bath: 1 h 2nd bath: 18 h	–	–	2 bath	[55]
0.75 M NaOH	–	80	30 min	8% (chitosan protein)	–	–	[44]
3 M NaOH	1:30	RT	75 min	–	0.09	Constant stirring @ 150 rpm	[85]
0.75 M NaOH	1st bath: 1:1.5	Boiling	1st bath: 15 min 2nd bath: 30 min	–	–	–	[76]; based on [86]
0.75 M NaOH	1:2.5	RT	24 h	>1% (chitosan protein)	0.002	–	[73]
19 M NaOH	1:10	110	3 h	–	0.19	stirring	[87]; based on [88]
1 M NaOH	1:5	RT	20 h	–	0.005	–	[89]; based on [90]
Deionized water	–	–	10–40 min	2–6%	–	high-frequency ultrasonic bath	[91]

In studies with different experimental conditions, optimum suggested situations had been reported in the table.
In highlighted rows, deproteinization was done before demineralization.
The underlined rows were chemically pretreated.
* This number only reports N_2 %.

anhydrous KOH. This property favors the deacetylation reaction with the NaOH solution [62].

Chitin consists of crystalline and amorphous domains. Unlike amorphous domains that are deacetylated fast and highly hydrated, crystalline domains are slowly deacetylated and insoluble. After complete amorphization, crystalline domains break and the proportions of deacetylation and soluble fraction increase. This mechanism is proposed for α-chitin deacetylation [104]. The amount of N-acetyl-D-glucosamine residues decreases during the deacetylation reaction in spite of increasing the amounts of chitin with lower Mw, chitosan, their oligomers, and D-glucosamine residues due to the degradation reactions [2]. In addition to the source and the isolation process of original chitin; the

Table 4
Previous suggested techniques for bleaching/decoloration of chitin/chitosan.

Agent Concentration	Solid to liquid ratio (w/v) (g/mL)	Temperature (°C)	Duration	Explanation	References
10% Hypochlorite (ClO^-)	–	–	5 min	–	[52]; based on [100]
Hydrogen peroxide (H_2O_2)/33% HCl (9/1 (v/v))	–	–	–	Mild oxidizing treatment for removing pigments	[61,62]
1st step: Potassium permanganate ($KMnO_4$) + oxalic acid + H_2SO_4 2nd step: Ethanol	–	–	1st: - 2nd: 6 h	1st: Mild oxidizing treatment for removing pigments 2nd: Eliminating traces of protein and coloring materials	[36]
1st step: Ethanol 2nd step: Acetone	1st: 1:10 2nd: 1:10	1st: Hot 2nd: Boiling	1st: 3 h 2nd: 3 h	Removing any impurities, pigments and other unpleasant materials	[7,45,69]
H_2O_2	1:9	–	-	-	[64] ;based on [8087]; based on [101]
	1:10	-	3 h	30% H_2O_2	
1st step: Acetone 2nd step: 0.315% sodium hypochlorite (NaClO)	1st: 1:10 2nd: 1:10	RT	1st: 10 min 2nd: 5 min	1st: Decolorizing; filter, and dry for 2 h @ RT 2nd: Bleaching	[65,6667]; based on [81,82]
$KMnO_4$ + Oxalic acid	–	–	–	Discoloring	[71]; based on [72]
3% NaClO	–	100	10 + 10 = 20 min; The step was done twice	Stirring	[75]
1st step: NaClO solution (17 g NaClO in 1 L of 0.3 M sodium acetate buffer @ pH 4.0)	–	80	2 h	Bleaching	[99]
1 M H_2O_2	–	32	24 h	–	[55]
1st step: Acetone 2nd step: 0.315% NaOCl	1st: 1:10 2nd: 1:10	RT	1st: 10 min2nd: 15 min	Stirring @150 rpm	[85]

Similar methods have been reported in one raw, and all details was included.

reaction time, temperature, concentration, and nature of alkaline reagent are the main parameters in deacetylation reaction [105].

Enzymatic or chemical methods can be applied for transforming chitin into chitosan. Although, traditional chemical chitosan production is usually used by industries due to its suitability for mass production; it has some drawbacks like inconsistency in Mw and DD, and environmental hazards. These inconsistencies are due to uncontrollable hydrolysis and chemical modifications that produce undesired by-products and huge amounts of aqueous waste [4,12].

Chitin deacetylation through enzymatic processes has shown good efficiency. However, high cost and low productivity limit their products to low Mw and amorphous chitin at laboratory scale [106,107].

The most studied and used techniques in chitin chemical deacetylation are homogeneous or heterogeneous alkaline hydrolyses at RT or high temperature, respectively. Heterogeneous process is preferred for industrial purposes due to the pretreatment of the starting chitin as well as the long reaction time (several days) for the homogeneous process [37,103]. Heterogeneous conditions lead to non-uniformly accessibility of the block copolymers in the deacetylation reaction and different physicochemical properties against randomly deacetylated chitosan under homogeneous conditions [108]. High solubility of the products and the independency of the reaction to the initial crystalline structure are advantages of homogeneous deacetylation, with good efficiency and low degradation [37]. Heterogeneous deacetylation preferentially happens in the chitin amorphous region, then continues toward the inside of the crystalline region [108]. Therefore, the alkali concentration controls the reaction rate on the solid surface or the reactant diffusion from bulk fluid to the solid surface [109]. Heterogeneous deacetylation is manipulated by two mechanisms: reaction and diffusion. So, the reaction picks up principally with increasing the temperature or NaOH concentration [80]. Table 5 represents the deacetylation methods of α-chitin in suspension techniques.

6.1. Solid-phase deacetylation

In these techniques, unlike the homogenious and heterogenious methods that have at least one liquid phase, the deacetylation reaction totally goes forward in solid state. The deacetylation reaction of chitosan production is performed in a two-screw extruder with controlled heating. The extruder is fed with a mixture of chitin and solid NaOH in a defined ratio. When the mixture mass moves through the extruder, it is exposed to high pressure and shear deformation at the defined temperature. The final product is a chitosan mixture with different degrees of deacetylation, relying on process conditions. The mixture is rinsed with water until neutrality and then is dried at RT. The molar ratio of chitin to NaOH and temperature are the main factors that influence the DD, solubility, and Mw of the obtained chitosan. Although, in the common suspension method DD, Mw, and solubility are higher, the solid-phase technique needs shorter processing time (0.1 h instead of 2 h) and minor NaOH to chitin ratio. The optimum condition for DD (90%) has been achieved by 1:5 M ratio of chitin to NaOH in 200 °C; but, the maximum Mw has been obtained at 180 °C with the same ratio [121].

6.2. Deacetylation guidelines

The extraction method, particle size of raw chitin, temperature, reaction time, concentration and rate of reagents to chitin, and the reaction atmosphere affect the Mw and DD of chitosan in deacetylation step [122,123]. The yields of extracted chitosan increase with decreasing of chitin particle size and increasing the concentration of NaOH solution used in deacetylation step [54]. However, severe conditions of deacetylation cause chitin hydrolysis and make a strong depolymerization [37]. Also, the effect of the solution-to-chitin ratio has been insignificant in the range of 1:5 to 1:45 (g/mL) in heterogeneous deacetylation [80].

6.2.1. Purification

Highly purified chitin results in high viscosity and Mw chitosan in deacetylation process. However, it does not guarantee complete acetic acid release or deacetylation [46].

6.2.2. Homogenizing

Wet grinding has been reported as the most efficient method in chitin activation [112]. The use of disintegrator or homogenizer for 20–30 min or 5 min sonication and 25 min swelling with magnetic stirring for suspending chitin in alkali will result in a stable, non-layered, and homogeneous suspension which efficiently promotes complete deacetylation [112,117]. Although, sonication is an excellent method to improve the sample exposure to chemical reagents, deterioration of the sonicator

Table 5
Deacetylation methods of α-chitin (suspension techniques, in homegenious & heterogenious state).

Alkali Concentration (Molar)	Solid to liquid ratio (w/v) (g/ml)	Temperature (°C)	Duration	Chitosan characteristics	Consumed NaOH/chitin weight (mole/g)	Explanation	References
12 M NaOH	I: 1:17 II: 1:30	I: 4/190 II: 90	I: 16 h/ 270 sec II: 30 h	I DD: 98% II DD: 96%	I: 0.20 II: 0.36	I: Flash method; in the presence of saturated aqueous vapor II: Conventional method; under stirring	[110]
19 M NaOH	–	135	3.5 h	DD: 90% (unknown measuring method)	–	α-chitin was extracted from prawn shells	[111]
25 M NaOH	1:10	90–95	2 h	Viscosity: 110 cP DD%: 86 Ash content: 0.4%	0.25	–	[52]
13 M KOH	0.5:30	up to 90	–	DD: 90% Intrinsic viscosity: 582 ml/g Mw: 125,000 g/mol	–	96° ethanol + mono-ethylene glycol as solvent	[61]; based on [105]
8 M NaOH	1:25	RT or heated	–	DD: 89%	0.2	Dry chitin was mechanically disintegrated and suspended in alkali solution. The alkaline suspension of chitin was frozen in a cryostat and thawed at RT. The formed gel was mechanically disintegrated into 3–5 mm particles.	[112]
19 M NaOH	–	136	1 h	DD > 99% Mw:320,000 g/mol	–	–	[79]
19 M NaOH	1:60	120	3*3h = 9 h	DD: 99% Intrinsic viscosity: 801 ml/g Mw: 190,000 g/mol	1.14	3 bath, under N_2 stream with stirring; Adding NaBH4 or thiophenol to chitin at 1:1 (w/w) to prevent polymer degradation.	[62]; based on [38]
19 M NaOH	1:10	110	4 h	Mw: 423,700; 490,700; 518,000 g/mol for three crab types	0.19	under N_2 atmosphere; taken out samples of the reactor every 15 min during 4 h; α-chitin was extracted from carb shells	[113]
19 M NaOH	1: 22.2	I: 110 II: 100	I: 40 min II: 30 min	I DD%: ~60 II 1st deacetylation: DD %: ~70 II 2nd deacetylation: DD%: ~90	0.42	under argon atmosphere II: 6–7 freeze-pump out-thaw (FPT) cycles	[37]; based on [104]
19 M NaOH	1:20	100	4 h	DD%: ~25%	0.38	stirring	[63]; based on [114]
14 M NaOH	–	–	3 h	DD %: ~ 95% Intrinsic viscosity: ~ 500 ml/g	–	Reflux chitin in the solution in an autoclave under 2 atmospheres pressure; facilitated by steeping 1 day in the solution @ RT before heating	[36]
4 M NaOH	–	–	–	–	–	@ pH = 9	[50]
17 M NaOH	I: 1:15	I: 110	I:2–10 h II: 6–15 min	–	I: 0.26	I: traditional heating; facilitated by steeping 24 h in the solution @ RT before heating II: steeping as I, microwave radiation @ 600 W	[7]
19 M NaOH	1:10	–	10 min	–	0.19	irradiate @ 1400 W	[115]
17 M NaOH	–	–	I: 3 h II: 35 min	I DD%: 93 II DD%: 95 Viscosities for I: <400 cP II: 400 cP	–	I: heating in an autoclave pressure of 2 Pa; facilitated by steeping 1 day in the solution @ RT before heating II: steeping as I, microwave radiation @ 400 W	[45]; based on [116]
19 M NaOH	1:20	105	4 h	Viscosity: ~900 cP	0.38	under 2 bar nitrogen atmosphere	[46]
19 M NaOH	–	120	–	DD%: 92 Mw: 2,200 g/mol Apparent viscosity @ 25 °C : 46 cP	–	–	[64]; based on [80]
19 M NaOH	1:15	100	5–6 h	I DD%: 76 Viscosity: 106 cP Mw: 128,500 g/mol II DD%: 80 Viscosity: 26 cP Mw: 57,900 g/mol	0.28	II: irradiation of solid samples @ 25 kGy, with a dose rate of 1.1013 kGy/h in air (after deacetylation step)	[65]
19 M NaOH	1:50	90–100	3–5 h	DD%: 80 Mw: 12 g/mol Intrinsic viscosity: 100 ml/g	0.95	under constant stirring	[66]; based on [38]
19 M NaOH	–	100/RT	2 h/30 min	Mw: 1,599,558 g/mol DD%: 90	–	Wash the residue continuously with the 50% NaOH and filter in order to retain the chitosan	[60]
19 M NaOH	–	–	–	DD% 1st deacetylation: 75 2nd deacetylation: 89 Mw: 43,020 g/mol	–	–	[71]; based on [72]

(*continued on next page*)

Table 5 (*continued*)

Alkali Concentration (Molar)	Solid to liquid ratio (w/v) (g/ml)	Temperature (°C)	Duration	Chitosan characteristics	Consumed NaOH/chitin weight (mole/g)	Explanation	References
17 M NaOH	1:50	100	2 h	DD%: 82 Mw: 12,000 g/mol	0.85	–	[67]
19 M NaOH	1:10	–	10 min	DD%: 95.2 Mw: 4,467,100 g/mol	0.19	irradiating @ 1400 W	[54]; based on [115]
14 M NaOH	1:23	Up to 105	30 + 60 = 90 min	DD%: >70 Mw: 130,000 g/mol	0.32	Pre-sonication of 5 min followed by 25 min of magnetic stirring was performed before the deacetylation process; assisted by high intensity ultrasound irradiation @ $\nu = 20$ kHz coupled to a 0.5 in (1.3 cm) stepped probe; α-chitin was extracted from prawn shells	[117]
19 M NaOH	1:20	I: 100	I: 2 h II: 8 min	I DD: 82% Mw: 123,000 g/mol II DD: 83% Mw: 1,410,000 g/mol	0.38	I: conventional method II: assimilated by microwave method @ 350 W	[68]
19 M NaOH	–	–	20 sec	DD%: ~85	–	Response surface optimization (RSM); @ 720 W microwave power	[69]
10 M KOH	–	90	6 h	DD%: 78 Mw: 6,273 g/mol	–	–	[44]
19 M NaOH	1:50	90	50 min	–	0.95	constant stirring	[85]; based on [8]
14 M NaOH	1:10	95	2 h	DD%: 76 Viscosity: 1031 cP	0.14	–	[76]; based on [86]
12.5 M NaOH	1:5	65	12 h	DD%: 85 Mw: 235,000 g/mol	0.06	–	[73]
19 M NaOH	1:10	121	15 min	–	0.19	autoclaved	[87]; based on [118]
19 M NaOH	1:50	100	3–5 h	DD%: 83	0.95	constant stirring	[119]; based on [120]
19 M NaOH	1:10	65	20 h	DD%: 65	0.19	–	[89]; based on [90]
19 M NaOH	1:4	1st step: 70 2nd step: 115	1st: 2 h 2nd: 2 h	DD%: 73–94 Mw: 1,000–87,000 g/mol	0.08	constant stirring @ 700 rpm with condensing system for avoiding NaOH loss due to evaporation and enhancement of NaOH/chitin contact	[91]

Most of the deacetylation processes have been done inside a reactor.
In highlighted rows, the initial α-chitin is not extracted from shrimp shells.
Underlined numbers present DD% which was measured by infrared (IR) spectrometry; more caution should be attended due to being less sensitive and probable misjudgments of this method [37].

probe tip in high NaOH solution should be considered. A reasonable solution is to sonicate the powder in a dilute NaOH medium first, then, soak the residue in concentrated media [63]. However, ultrasonic irradiation reduces the average Mw of the obtained polymer due to depolymerization [117].

6.2.3. Steeping

Soaking chitin in strong NaOH solution at RT, before heating, highly alleviates the deacetylation reaction [116].

6.2.4. Freeze-pump out-thaw (FPT) cycles

The cycles improve the reaction rate through opening the crystalline structure of α-chitin and increasing the permeability to the alkali solution. This mechanism results in the higher sensitivity of deacetylation reaction to temperature and includes advantages of both homogeneous and heterogeneous methods. This technique produces fully deacetylated, high Mw chitosan. The total deacetylation time reduces drastically and the alkali degradation/hydrolysis is limited. However, chitosans obtained by standard methods have a more disturbed structure due to the effect of severe treatment conditions. Time elongation of the freeze/thaw procedure, increasing the temperature gradient and improving the chitin-solution interface will improve the process. Although, cryo-activation by moderate acid–base treatment as well as severe acid–base results higher Mw chitosan, but, only severe acid–base conditions increase the DD degree. Cryo-chitosan has an amorphous structure with weak bonds between its molecules and is not electrified during disintegration [112]. Deacetylation reaction is independent of the initial crystallinity of the samples by applying this technique. So, α-chitin with a denser structure can be deacetylated as effective as β-chitin [37].

6.2.5. Alkali concentration

α-chitin deacetylation is commonly performed with 40–50% aqueous alkali, NaOH or KOH in most cases, at 100–160 °C for a few hours [6,124]. Higher Mw is obtained with KOH and higher DD results with NaOH medium [61]. The needed NaOH to dried chitin powder has been used in the range of 0.2–2.2 mol/gr. The wide applied range demonstrates the probable effect of alkali concentration or other reaction conditions such as agitating type, heating method and the treatments in previous steps.

6.2.6. Microwave heating

Less deacetylation time (from hours to few minutes) and superior Mw and crystallinity will be gained by microwave irradiation instead of traditional heating [7,45,125].

6.2.7. Process time

The duration of deacetylation reaction depends upon the chitin source. The Mw of chitosan decreases with increasing the reaction duration for higher DD [62]. Application of several baths instead of single bath will shorten the process time, at the cost of more chemicals usage.

6.2.8. Minimizing hydrolysis/degradation

Alkali concentration, the processing time and temperature, dissolved

oxygen and shear stress result in chitosan degradation and influence its properties [126,127]. Reduction of the amount of alkaline solution can be done by using water-miscible organic solvents such as acetone as diluting agent. Although, the use of diluting agents enables adequate deacetylation reaction with much less alkali, the DD and the viscosity of the obtained chitosan have been less compared to those gained with aqueous NaOH alone, at the same temperature and time period [128]. Due to the insolubility of high chitin concentration in diluted alkali mediums and progression of deacetylation reaction at low polymer concentrations, the chitin concentrations in diluted alkaline solutions should be low (2.5–4%) [112]. Also, the process time can be shorten by replacing the processing conditions with reactive extrusion [121], flash treatment [110], or microwave accelerated process [115]. Flash treatment proceeds faster with a higher activation energy for the deacetylation reaction and induces structure modifications. In particular, higher crystallinity indexes and specific area values are observed together with changes in the local and chain conformation. Also, the flash treated chitosan has shown>80% increase in water retention value in comparison with the conventional deacetylated product [110]. In the reaction medium, oxygen concentration can be reduced by changing the reaction atmosphere. Applying inert atmospheres like N_2 and argon [5], sodium thiophenolate (Tiophenol) as an oxygen scavenger and reaction catalyst [103,129] or sodium borohydride ($NaBH_4$) prevent the oxidation [128] and reduce degradation rate in harsh acid/base treatments. However, adding thiophenol never allows to reach a true 100% DD [129]. Presence of $NaBH_4$ in the deacetylation medium can also increase the DD% of the final product [62]. Although, addition of these agents leads into increasing the Mw of chitosan [74,103].

7. Post-treatments

The deacetylated chitin, chitosan, should be purified or separated from the alkali solution. It can be simply filtered or firstly centrifuged for cut-off the chitosan into low, medium, and high Mw and then filtered. Also, the final product can be fractioned by ultrafiltration through the membrane using a combination of two different solvents, like sodium nitrate and sodium acetate [130]. Sometimes the filtrate is rinsed with chemical solvents such as alcohols or acetone for residue elimination. This step can act as the bleaching/decoloration step too [62,85,110]. If fine chitosan particles are needed, the dried product is pulverized [52]. The final dried chitosan could be stored in air tight containers for future use [131].

7.1. Chitosan purification

For biomedical and pharmaceutical applications, the obtained chitosan must be purified. This process was categorized into three steps [60,132]:

7.1.1. Insolubles removal

This step eliminates the insoluble chitosan which is insufficiently deacetylated. A homogenous solution of 1 mg of chitosan into 1 ml of acetic acid 1% (v/v) is prepared under magnetic stirring. The solution would be passed through filter paper (22 μm) to remove the insolubles.

7.1.2. Re-precipitation

Filtered chitosan might be precipitated by titration with 1 M NaOH till pH = 8.5. Then, the chitosan will be severally washed with distilled water and followed by centrifuging at 8,000 to 10,000xg. Dithiothreitol (DTT) was added to all the steps as reducing agent to provide more consistency and reproducibility between the batches.

7.1.3. Demetallisation

1 ml of 10% w/v sodium dodecyl sulfate (SDS) was added to the retrieved chitosan, with stirring at RT overnight. Next, 3.3 ml of 5% w/v ethylenediaminetetraa-cetic acid (EDTA) was poured for precipitation of heavy metals with EDTA, with stirring at RT for 2 h. The water insoluble precipitate will be collected by centrifugation at 5000xg for 30 min. Then, the residue was rinsed several times with distilled water by re-suspending and re-centrifugation for 30 min. the final chitosan was oven dried [133].

Some papers have reported similar procedure with different agent concentrations or deleting the last step (demetallisation); such as dissolving chitosan in 2% acetic acid and re-precipitating it in 5 M NaOH [4,5,7].

7.2. Chitosan with higher DD%

Few applicable methods are highlighted in published researches for having chitosan with higher DD% including:

7.2.1. Successive baths vs. single bath

Repeating the alkali treatment will result in high deacetylated chitin (90–95%). Changing the bath will shorten the process time for lower DDs too (<90%). This technique will produce chitosan with higher Mw due to shortening time elongation and avoiding chitin hydrolysis or degradation [103,128,129,134].

7.2.2. FPT cycles

FPT cycles plus use of thiophenol has suggested to produce fully deacetylated chitosans [37].

7.2.3. Re-dissolving

DD% is higher than 80% in soluble fraction of chitosan as opposed to the insoluble fraction with<20% in both suspension and solid state methods. Therefore, filtered solution of chitosan in acetic acid might have higher DD% [121].

7.2.4. Re-precipitation

The precipitated chitosan at high pH values, like precipitated by titration with NaOH, shows higher DD% [121].**Gamma radiation:** Irradiation at 25 kGy, with a dose rate of 1.1013 kGy/h in air (after deacetylation step), increased DD% from 76 into 80. However, it reduced viscosity and average Mw of the gained chitosan [65].

7.2.5. Domard et al. method

600 mg of acetate form chitosan (a freeze-dried solution of chitosan in 5% acetic acid aqueous solution) was dissolved into 16 ml water. Next, 80 ml dimethylsulphoxide was poured to form gel. 2 g NaOH dissolved into 2 ml H_2O was dropped into the solution to spread the precipitate under fast stirring. Then, 1.6 ml thiophenol was added. The reactor, with N_2 atmosphere, was placed in a 100 °C oil bath for 1 h with stirring. After, obtained chitosan was washed and filtered 3 times with water and 3 times with methanol. The dried polymer passed the above procedure 3 times. The method result 100% DD without much decrease in Mw [129].

7.2.6. Mima et al. method

A small amount of 1.5–2% chitosan aqueous solution in 2% acetic acid was poured into a large amount of 1 M NaOH solution. Then, the chitosan passed the alkali treatment. The final chitosan was reported up to 99% DD. The α-chitin source was crab shells [134].

7.3. Chitin/chitosan nano-whiskers or fibers

Chitin forms micro-fibrillar alignment in the shrimp exoskeletons. The fibrils have about 2 nm diameter and are infixed in a protein matrix [135]. Chitin acid hydrolysis or defibrilisation can disperse them into rod-like particles [136]. These nano whiskers showed an enhanced adsorption [99] and also they are so favorite in manufacturing of engineered scaffolds. Chitin nano-whiskers (from shrimp shells) were performed by the modified conditions based on the following method. A

summary of the technique is represented in Fig. 5:

7.3.1. Chitin nano-whiskers

- Hydrolyze the chitin in 3 M HCl (1: 100 w/v (g/ml)) for 3 × 1.5 h at 105 °C (under boiling condition)
- Centrifuge and repeatedly wash the residue
- Dialyze the suspensions of chitin nano-whiskers in distilled water till pH = 6–7
- Perform three successive 2 min sonication and filtration on the obtained dispersion
- Add HCl solution to the suspension until the pH = 2.5 and homogenize [99,137–139]

7.3.2. Chitosan nano-whiskers

- Treat an aqueous 6% solution of the chitin nano-whiskers in 60% NaOH (1:5 (v/v)) at 150 °C for 3 h; under N_2 atmosphere
- Dialyze the obtained nano-whiskers chitosan in distilled water till pH = 7; DD = 95% [139].

7.4. Chitosan depolymerization

High Mw chitosan depolymerizes into low Mw in hot concentrated acid solutions such as HCl, H_2SO_4, or H_3PO_4 effectively. And, they are recovered from the solutions by precipitation at pH = 8–10 with lowered temperatures [140].

8. To the Future

In recent decades, many noteworthy studies has been performed about chitin/chitosan extraction methods, purification techniques and modification/functionalization procedures. However, some objects need more attention and few related issues still remain with no serious researches done. Some of the critical research areas in this field are outlined in below:

8.1. Chitin/chitosan characterization

Chitin/chitosan properties are principally depended on the natural origin and the extraction process [15]. Also, the ash and protein contents assess the quality [53]. Nowadays, the current approach for chitin/chitosan-based biomaterials is focused on the characterizing the result products rather than in targeting predefined properties by controlling the production process [11].

There are two main questions unanswered in this area. First, no gold standard method has been introduced for characterizing the extracted chitin/chitosan. For instance DD%, a determinant factor for chitin-based material properties, has been measured by titration, elemental analysis, FTIR or H NMR. The DD value is highly affected by the analytical methods which is employed [141,142]. It is also true for viscosity measuring. Chitin/chitosan yields are defined in different references in the papers too. The inhibitory effects of bacteria growth depend on the tested bacteria, with greater antimicrobial activity against gram positive bacteria than gram negative bacteria [54]. Gram-positive effects correlate positively with the material Mw, but, decrease of the solution viscosity increases these effects [45]. Another dropped out essential factor is the yield of chitin/chitosan which specifies the extraction efficiency. Few researches have reported it just as a number in a range of 0.1–0.3 based on different incomparable references [61,85]. Therefore, no logical comparison is available for the reported properties of the produced materials. Lack of consistency in assessment of main parameters result in contrary physicochemical characteristics with similar functionality [143]. There is a need to a technical paper for comparing various measuring techniques and introducing gold standards in chitin/chitosan characterization. Second, the desirable goals or compulsory constrains for physicochemical properties of chitin/chitosan are application-dependent and often still unknown, e. g. when the DD reaches to the plateau at high values, it does not vanish by use of current suggested treatments like FPT cycles [37] or the formation of stable complex by covalently protein bonded to chitin makes 100% deproteinization difficult [67]. Table 6 presents reported characteristics of two common in use commercial products. No specific properties, not in a similar range was reported.

8.2. Chitin/chitosan modifications

Higher efficiency, better engineered functionality and acceptable consistency are the requested modifications for chitin-derived materials in industries. These modifications are categorized into optimizing the

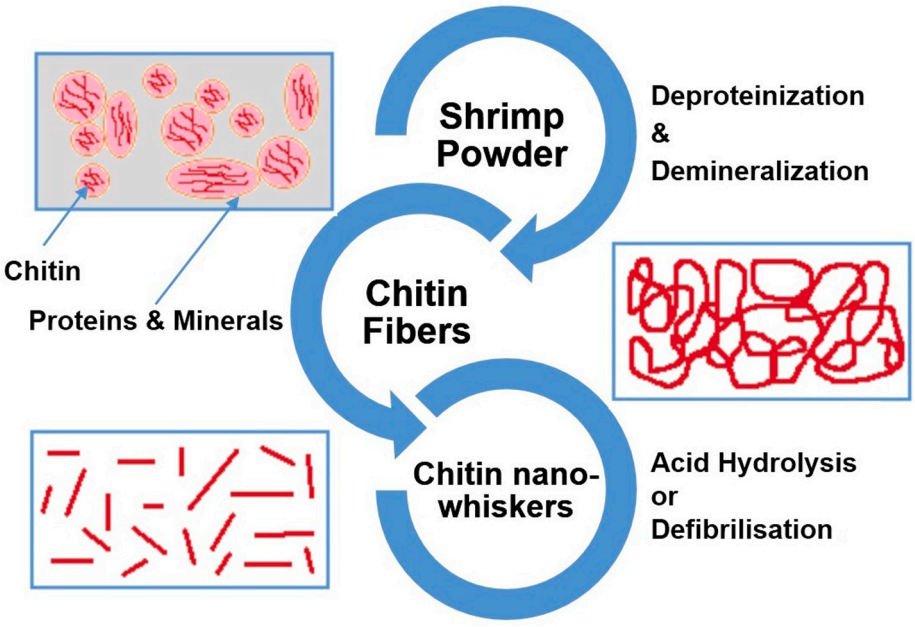

Fig. 5. Schematic representation of the protocol for the manufacturing of chitin nano-whiskers [99].

Table 6
Chitosan characteristics of two reliable commercial brands, from shrimp shells.

Sigma-Aldrich*	DD%≥75%
Product number: C3646	Soluble in acetic acid; insoluble in H_2O and organic solvents
	Bulk density: 0.15-0.3 g/cm^3
Glentham Life Science^	DD%≥90%
Product number: GC1909	Mw: ~200,000 g/mol
	Sulphated Ash ≤ 1.5%
	Heavy metal ≤ 10 ppm

* https://www.sigmaaldrich.com/catalog/product/sigma/c3646?lang=en&region=IR
^ https://www.glentham.com/en/products/product/GC1909/

process conditions of the extracting methods and functionalizing the chitin/chitosan products for the defined goals.

8.2.1. Optimization

Adjustment of the process parameters enables us to produce chitin/chitosan with tailored characteristics [16,117]. The following topics have been suggested as controversial issues in optimizing the current chemical procedures:

8.2.1.1. Optimal process conditions. Effective process parameters such as the nature and concentration of alkali reagents, temperature, reaction time and repetition of the alkaline treatment principally influence the chitin-based Mw, viscosity, distribution of acetyl groups and DD [16,61]. Also, chitin/chitosan main functionalities like solubility, biodegradability, absorbability, mechanical strength, emulsification, antioxidant properties, mucoadhesiveness, analgesic and haemostatic properties are positively or negatively correlated with the DD or/and Mw [2,11,54,144,145]. These major characteristics are sometimes correlated or related in opposite direction. Therefore, they cannot be adjusted without altering other factors. Some of these relations have been published previously as strong correlation of Mw with viscosity and DD [44], or negative correlation between WBC with DD, Mw, viscosity, and moisture content [65], or chitosan with a higher DD tends to have a higher antimicrobial activity [146]. Also, some correlations are only trusted in a specific range, i.e. the solid-solvent ratio was not a significant parameter on demineralization step [67].

No final rule has been confirmed for the optimum *sequence of chemical extraction steps*. However, in some of previous papers, acid wash was suggested as starter due to stronger reagents can break the crystal blocks more rapidly and they may lead to a more efficient extracting.

Residual proteins are one of the most problematic features of chitin production from crustaceans [63]. The ultimate goal is production of protein-free chitin due to the higher solubility of the gained chitosan and elimination of probable allergic reactions [11,66]. Application of more efficient purification processes can improve the physicochemical properties of the synthesized chitin/chitosan [44].

Another crucial problem is *the low reactivity of chitin* in deacetylation reaction. The problem dictates using high concentrations of reagents and prolonged reaction times that leads to degradation of the polysaccharide chains [147].

Crystallinity is maximum for a %DD equal to 0 or 100% (chitin or fully deacetylated chitosan, respectively), and has a lower value for intermediate %DD. Besides, the distribution of acetyl residues along chitosan will also affect its crystallinity. Polymer crystallinity is inversely related to its *biodegradation rate*. As a result, smaller chitosan chains will be more rapidly degraded into oligosaccharides than chitosan with higher Mw.

Biocompatibility of chitosan-derived materials rises with DD. While the number of positive charges increases, the interaction between the cells and chitosan increases which tends to improve the biocompatibility of the construct [11]. Specifying the accurate coefficient value and the sign of this correlation are crucial.

All the steps of the extraction method should preferably be designed in one experiment series; sometimes individual or discrete studies lead to wrong results, due to the relation of steps together. For instance, deproteinization procedure plays a crucial role for deacetylation step. High temperature deproteinization step weakens the functional group bonding, and causes an easier release of acetyl groups during the deacetylation process [46].

8.2.1.2. Mechanical strength. Blending/composite of chitin/chitosan with other biopolymers such as PLA (Polylactic acid), PLGA (polylactic–glycolic acid), collagen, alginate, etc. increases their mechanical properties [16]. Also, tensile strength positively correlates with DD% [134]. Chitin nano-fibrils, as a reinforcing material, can also be employed for synthesizing biodegradable nano-composites as scaffolds for tissue engineering [16].

8.2.1.3. Hydrothermo-chemical method. In this technique, all the procedure was performed in two steps: demineralization in acid medium and deproteinization and deacetylation simultaneously in basic medium [148]. This simple method decreases the production time, and might produce a chitosan with a better yield and a high DD [149].

8.2.1.4. Sonication. Considerable amount of insoluble materials in the final chitosan products can be due either to poor alkali distribution or to inadequate agitation during the reaction [128]. High-intensity ultrasonication makes easier accessibility of acetyl groups to reagents and provoking changes in the crystalline structure of chitin and its morphology are helpful for deacetylation reactions [63]. Although, sonication may enhance deproteinization and deacetylation process, this treatment did not improve the elimination of minerals and may even be detrimental due to depolymerization. Chitin source, the shell composition and structural arrangement, affect the output of ultrasound-assisted extraction [63].

8.2.1.5. Crosslinking. Commonly, chitosan is crosslinked with reagents such as glutaraldehyde, epichlorohydrin, diisocyanate, or 1,4-butanediol diglycidyl ether due to less stability of the chitosan because of more hydrophilicity and especially the pH sensitivity [13,150].

8.2.1.6. Microwave heating method. The application of microwaves in chemistry intensely reduces the reaction time in comparison to conventional process heating (from hours to minutes) [139]. Scientists hope to substitute the conventional method with this technique in chemical extractions. Microwave heating method often extensively heats inside the material in comparison with the outer layers. Therefore, it is hard to measure the reaction temperature properly, particularly in dense and solvent less media [3,125]. Although, simple rapid microwave heating has advantages like ultra-pure products, high yields and energy efficiency in a wide temperature range, and less side-products. However, difficulty in heat force control, water evaporation, and using dangerous closed containers are the main disadvantages of this method in chitin/chitosan extraction [125]. As reported, microwave heating causes the lower required energy and reaction time with a higher DD, yield, and purity of extracted chitosan by reducing unwanted side reactions [3,69]. Structure, morphology, and chemical composition of produced chitosan have been reported similar for both conventional and microwave heating method. But, in comparisons of methods, conflicting results were published for increase or decrease of Mw [68,69].

An effective deacetylation process without discoloration and the lowest alkaline degradation indicates appropriate deacetylation conditions [38,104]. Optimization by statistical tools and mathematical modelling, such as response surface method (RSM), Taguchi method, artificial neural networks modelling, and genetic algorithms might be beneficial in identifying the optimum conditions [46,123,151].

8.2.2. Functionalization

Polysaccharide nature of chitin/chitosan-derived materials and the presence of reactive functional groups (hydroxyl/acetyl amino groups) dispose them to undergo various chemical modifications for construction of complex molecular architectures [8]. These groups participate in reactions like alkylation, quaternization, acylation, hydroxylation, phosphorylation, thiolation, methylation, azylation, nitration, sulphonation, xanthation or N-succinylation, react with aldehydes and ketone o-acetylation, H-bond with polar atoms, cross-link, graft copolymerize, etc. [11,60]. Functionalization can improve chitin/chitosan solubility (in water or organic solvents), and the chemical and mechanical stability. Therefore, the properties of chitin-based materials and their functionalities can be exceptionally adjusted for their effective unlimited applications as new functional biomaterials in many fields [3]. Some of the most in use derivatives are mentioned below:

8.2.2.1. Graft copolymerization. This approach is one of the mostly investigated and promising techniques to lots of molecular designs. It modifies the physicochemical properties of chitin and chitosan such as antibacterial effects, chelating and complexation for various applications. [152]. This method forms functional derivatives by covalent binding of a molecule onto the polymer backbone. The free amino groups on deacetylated units and the hydroxyl groups on the acetylated units can be grafted. Grafting would be started by variable initiators like Fenton's reagent, ceric ammonium nitrate, ammonium and potassium persulfate, potassium diperiodatocuprate and ferrous ammonium sulfate; or enzymes and γ-irradiation [3].

8.2.2.2. Carboxyalkyl chitin/chitosan. This material involves the acidic groups on the polymer chain by addition of carboxyl groups as potential anionic charge with pH dependent solubility [3].

8.2.2.3. Quaternization. This function is gained by introducing the quaternary ammonium functionality into the biopolymer chain. These derivatives improve antimicrobial and antifungal activities of chitin/chitosan and, also enhance their absorption potential [3].

8.2.2.4. Carboxymethylation. It is a water-soluble derivative which is obtained during the reaction of alkali-chitin/chitosan with monohalocarboxylic acid [1].

8.3. Mechanisms

Chitin/chitosan-based materials exhibit unique physicochemical properties; but, there is no comprehensive understanding of these specific functionalities. For instance, the immunological and antimicrobial activities of chitin derivatives and their ability to stimulate connective tissue reconstruction should be studied in detail [1]. For the biomaterial application in nano scale, the potential toxic effects are the major challenges [16]. Some physical properties like solubility strongly depend on random distribution of the acetyl groups along the chain in addition to DD [13,111], the mechanism should be understood. No adverse effect has yet been reported so far on the quality and yield of the final product when demineralization and deproteinization steps are reversed [95]. Also, some studies have claimed that eliminating the decoloration or deproteinization steps has no effects on DD, ash, and protein content [67]. However, a conceptual experiment design is needed for a concise consensus. Proteins are immunogenic; so, purification is compulsory. No permissible value for protein content is identified based on the cycle. New accelerating methods like ultrasonication and microwave irradiation are introduced for manufacturing chitin/chitosan derivatives, no confirmed theory is yet available [63]. For example, uncontrollable temperature growth or specific effect of microwave activation are two supposed hypothesis in microwave heating [3,125]. A part of chitosan degradation is supposed due to shear stress. The probable mechanism should be studied.

9. Conclusion

Polysaccharides, like chitin and chitosan, are promising biopolymers for developing desirable advanced functions due to their unique structures, properties, and differences with synthetic polymers [8]. Pharmaceutical industries need a wide range of refined chitin-based materials to meet the required standards; e.g. low Mw oligomers for wound healing constructs (with more effective antibacterial activity for a greater range of bacteria [66]), high Mw for tissue engineering applications, and in addition high DD for drug delivery systems [60].

The crustacean shells are a serious source of pollution due to their low decomposition rate. Shrimp shells, as a high-value by-product with a superior chitin content in comparison with crabs, has a yield of 15% in chemical extraction processes on industry scale [12,45,75]. However, no standard method has been adopted for preparation of pure chitin yet.

We reviewed all aspects of the chemical procedure for chitin/chitosan extraction from shrimp shells in detail. Also, unknown areas and dropped-out issues were mentioned for future steps; such as the probability of substitution of the efficiently enzymatic methods with the current chemically processes in industries [1], comparing other of chitin sources (β or γ) with α-type in physiochemical properties and their abilities in the desired modifications [1,7,44,62] or importance of random distribution of the acetyl groups along the chain in physical properties [13,111].

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors would like to acknowledge financial support from the Ferdowsi University of Mashhad (FUM), Mashhad, Iran.

References

[1] J. Synowiecki, N.A. Al-Khateeb, Production, properties, and some new applications of chitin and its derivatives, (2003).
[2] G.A. Roberts, Chitin chemistry, Macmillan International, High. Educ. (1992).
[3] H. El Knidri, R. Belaabed, A. Addaou, A. Laajeb, A. Lahsini, Extraction, chemical modification and characterization of chitin and chitosan, Int. J. Biol. Macromol. 120 (2018) 1181–1189.
[4] T. Philibert, B.H. Lee, N. Fabien, Current status and new perspectives on chitin and chitosan as functional biopolymers, Appl. Biochem. Biotechnol. 181 (4) (2017) 1314–1337.
[5] M. Hayes, B. Carney, J. Slater, W. Brück, Mining marine shellfish wastes for bioactive molecules: Chitin and chitosan ndash, Part A: Extraction methods, Biotechnol. J.: Healthcare Nutrit. Technol. 3 (7) (2008) 871–877.
[6] D. Horton, D. Lineback, N-deacetylation, chitosan from chitin, Methods Carbohydrate Chem. 5 (1965) 403–406.
[7] F. Al Sagheer, M. Al-Sughayer, S. Muslim, M. Elsabee, Extraction and characterization of chitin and chitosan from marine sources in Arabian Gulf, Carbohydr. Polym. 77 (2) (2009) 410–419.
[8] K. Kurita, Chitin and chitosan: functional biopolymers from marine crustaceans, Mar. Biotechnol. 8 (3) (2006) 203.
[9] P.K. Dutta, J. Dutta, V. Tripathi, Chitin and chitosan: Chemistry, properties and applications, (2004).
[10] Z.S. Haidar, R.C. Hamdy, M. Tabrizian, Protein release kinetics for core–shell hybrid nanoparticles based on the layer-by-layer assembly of alginate and chitosan on liposomes, Biomaterials 29 (9) (2008) 1207–1215.
[11] F. Croisier, C. Jérôme, Chitosan-based biomaterials for tissue engineering, Eur. Polym. J. 49 (4) (2013) 780–792.
[12] S. Hajji, I. Younes, O. Ghorbel-Bellaaj, R. Hajji, M. Rinaudo, M. Nasri, K. Jellouli, Structural differences between chitin and chitosan extracted from three different marine sources, Int. J. Biol. Macromol. 65 (2014) 298–306.
[13] I. Younes, M. Rinaudo, Chitin and chitosan preparation from marine sources, Structure, properties and applications, Marine drugs 13 (3) (2015) 1133–1174.
[14] E.C. Society, M.G. Peter, EUCHIS'99: Proceedings of the 3rd International Conference of the European Chitin Society, Potsdam, Germany, Aug. 31-Sept. 3, 1999, Univ. Potsdam, Univ.-Bibliothek, Publ.-Stelle2000.

[15] S. Kaur, G.S. Dhillon, The versatile biopolymer chitosan: potential sources, evaluation of extraction methods and applications, Crit. Rev. Microbiol. 40 (2) (2014) 155–175.
[16] M. Yadav, P. Goswami, K. Paritosh, M. Kumar, N. Pareek, V. Vivekanand, Seafood waste: A source for preparation of commercially employable chitin/chitosan materials, Bioresources Bioprocess. 6 (1) (2019) 8.
[17] M. Yadollahi, S. Farhoudian, H. Namazi, One-pot synthesis of antibacterial chitosan/silver bio-nanocomposite hydrogel beads as drug delivery systems, Int. J. Biol. Macromol. 79 (2015) 37–43.
[18] S. Özbas-Turan, C. Aral, L. Kabasakal, M. Keyer-Uysal, J. Akbuga, Co-encapsulation of two plasmids in chitosan microspheres as a non-viral gene delivery vehicle, J. Pharm. Pharmaceut. Sci 6 (2003) 27–32.
[19] M.A. Ghaz-Jahanian, F. Abbaspour-Aghdam, N. Anarjan, A. Berenjian, H. Jafarizadeh-Malmiri, Application of chitosan-based nanocarriers in tumor-targeted drug delivery, Mol. Biotechnol. 57 (3) (2015) 201–218.
[20] Y. Zhang, M. Ni, M. Zhang, B. Ratner, Calcium phosphate—chitosan composite scaffolds for bone tissue engineering, Tissue Eng. 9 (2) (2003) 337–345.
[21] M. Kazemi Ashtiani, M. Zandi, P. Shokrollahi, M. Ehsani, H. Baharvand, Chitosan surface modified hydrogel as a therapeutic contact lens, Polym. Adv. Technol. 31 (4) (2020) 741–748.
[22] S. Xin-Yuan, T. Tian-Wei, New contact lens based on chitosan/gelatin composites, J. Bioactive Compatible Polymers 19 (6) (2004) 467–479.
[23] J.D. Bumgardner, R. Wiser, P.D. Gerard, P. Bergin, B. Chestnutt, M. Marini, V. Ramsey, S.H. Elder, J.A. Gilbert, Chitosan: potential use as a bioactive coating for orthopaedic and craniofacial/dental implants, J. Biomater. Sci. Polym. Ed. 14 (5) (2003) 423–438.
[24] P. Jollès, R.A. Muzzarelli, Chitin and chitinases, EXS(Basel) (1999).
[25] R.C. Goy, D.D. Britto, O.B. Assis, A review of the antimicrobial activity of chitosan, Polímeros 19 (3) (2009) 241–247.
[26] D.A. Zaharoff, C.J. Rogers, K.W. Hance, J. Schlom, J.W. Greiner, Chitosan solution enhances both humoral and cell-mediated immune responses to subcutaneous vaccination, Vaccine 25 (11) (2007) 2085–2094.
[27] R. Muzzarelli, G. Biagini, A. Pugnaloni, O. Filippini, V. Baldassarre, C. Castaldini, C. Rizzoli, Reconstruction of parodontal tissue with chitosan, Biomaterials 10 (9) (1989) 598–603.
[28] T.-L. Yang, Chitin-based materials in tissue engineering: applications in soft tissue and epithelial organ, Int. J. Mol. Sci. 12 (3) (2011) 1936–1963.
[29] R.F.B. de Souza, F.C.B. de Souza, A. Thorpe, D. Mantovani, K.C. Popat, Â. M. Moraes, Phosphorylation of chitosan to improve osteoinduction of chitosan/xanthan-based scaffolds for periosteal tissue engineering, Int. J. Biol. Macromol. 143 (2020) 619–632.
[30] R. Muzzarelli, M. Mattioli-Belmonte, C. Tietz, R. Biagini, G. Ferioli, M. Brunelli, M. Fini, R. Giardino, P. Ilari, G. Biagini, Stimulatory effect on bone formation exerted by a modified chitosan, Biomaterials 15 (13) (1994) 1075–1081.
[31] J. Movaffagh, F. Bazzaz, A.T. Yazdi, A. Sajadi-Tabassi, M. Azizzadeh, E. Najafi, N. Amiri, H.B. Taghanaki, M.H. Ebrahimzadeh, A. Moradi, Wound Healing and Antimicrobial Effects of Chitosan-hydrogel/Honey Compounds in a Rat Full-thickness Wound Model, Wounds: a compendium of clinical research and practice 31(9) (2019) 228-235.
[32] R. Muzzarelli, M. Mattioli-Belmonte, B. Muzzarelli, G. Mattei, M. Fini, M. Biagini, Medical and veterinary applications of chitin and chitosan, Proceedings of 7th International Conference on Chitin, Chitosan, and Euchis' 97 (1997) 580–589.
[33] X. Fei Liu, Y. Lin Guan, D. Zhi Yang, Z. Li, K. De Yao, Antibacterial action of chitosan and carboxymethylated chitosan, J. Appl. Polymer Sci. 79 (7) (2001) 1324–1335.
[34] D. Raafat, K. Von Bargen, A. Haas, H.-G. Sahl, Insights into the mode of action of chitosan as an antibacterial compound, Appl. Environ. Microbiol. 74 (12) (2008) 3764–3773.
[35] J. Ornum, Shrimp waste must it be wasted, Info Fish 6 (92) (1992) 48–52.
[36] E.S. Abdou, K.S. Nagy, M.Z. Elsabee, Extraction and characterization of chitin and chitosan from local sources, Bioresour. Technol. 99 (5) (2008) 1359–1367.
[37] G. Lamarque, M. Cretenet, C. Viton, A. Domard, New route of deacetylation of α-and β-chitins by means of freeze− pump out− thaw cycles, Biomacromolecules 6 (3) (2005) 1380–1388.
[38] 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., Part A: Polym. Chem. 31 (2) (1993) 485–491.
[39] A. Domard, G.A. Roberts, K.M. Varum, Advances in chitin science, Domard, A (1997) 410.
[40] F. Shahidi, J. Synowiecki, Isolation and characterization of nutrients and value-added products from snow crab (Chionoecetes opilio) and shrimp (Pandalus borealis) processing discards, J. Agric. Food. Chem. 39 (8) (1991) 1527–1532.
[41] M. Benhabiles, N. Abdi, N. Drouiche, H. Lounici, A. Pauss, M. Goosen, N. Mameri, Protein recovery by ultrafiltration during isolation of chitin from shrimp shells Parapenaeus longirostris, Food Hydrocolloids 32 (1) (2013) 28–34.
[42] E. Johnson, Q. Peniston, Utilization of shellfish waste for chitin and chitosan production, ABSTRACTS OF PAPERS OF THE AMERICAN CHEMICAL SOCIETY, AMER CHEMICAL SOC 1155 16TH ST, NW, WASHINGTON, DC 20036, 1979, pp. 34-34.
[43] M. Apetroaei, A.-M. Manea, G. Tihan, R. Zgarian, V. Schroder, I. Rău, Improved method of chitosan extraction from different crustacean species of Romanian Black Sea coast, UPB Scientific Bulletin, Series B: Chemistry and Materials Science 79 (1) (2017) 25–36.
[44] S. Kumari, S.H.K. Annamareddy, S. Abanti, P.K. Rath, Physicochemical properties and characterization of chitosan synthesized from fish scales, crab and shrimp shells, Int. J. Biol. Macromol. 104 (2017) 1697–1705.
[45] A. Alishahi, A. Mirvaghefi, M. Tehrani, H. Farahmand, S. Shojaosadati, F. Dorkoosh, M.Z. Elsabee, Enhancement and characterization of chitosan extraction from the wastes of shrimp packaging plants, J. Polym. Environ. 19 (3) (2011) 776–783.
[46] M. Bajaj, J. Winter, C. Gallert, Effect of deproteination and deacetylation conditions on viscosity of chitin and chitosan extracted from Crangon crangon shrimp waste, Biochem. Eng. J. 56 (1–2) (2011) 51–62.
[47] P. Sandford, Chitosan: commercial uses and potentials, Skjak-Brack, G., Anthonsen, T., Sandford, PA Chitin and chitosan. Sources, chemistry, biochemistry, physical properties and applications. London and New York: Elsevier (1989) 51.
[48] G. Allan, J. Fox, N. Kong, A critical evaluation of the potential sources of chitin and chitosan, in: Proceedings of the first international conference on chitin/chitosan, MIT Sea Grant Program, Cambridge, MA, 1978, pp. 64-78.
[49] M. Hansen, A. Illanes, Applications of crustacean wastes in biotechnology, Springer, Fisheries processing, 1994, pp. 174–205.
[50] A. Khanafari, R. Marandi, S. Sanatei, Recovery of chitin and chitosan from shrimp waste by chemical and microbial methods, J. Environ. Health Sci. Eng. 5 (1) (2008) 1–24.
[51] A. Percot, C. Viton, A. Domard, Optimization of chitin extraction from shrimp shells, Biomacromolecules 4 (1) (2003) 12–18.
[52] A. Gopalakannan, G.I. Jasmine, S. Shanmugam, G. Sugumar, Application of proteolytic enzyme, papain for the production of chitin and chitosan from shrimp waste, J. Mar. Biol. Assoc. India 42 (2000) 167–172.
[53] M. Shrinivas Rao, K. Aye Nyein, T. Si Trung, W.F. Stevens, Optimum parameters for production of chitin and chitosan from squilla (S. empusa), J. Appl. Polym. Sci. 103 (6) (2007) 3694–3700.
[54] M.M. Samar, M. El-Kalyoubi, M. Khalaf, M.A. El-Razik, Physicochemical, functional, antioxidant and antibacterial properties of chitosan extracted from shrimp wastes by microwave technique, Ann. Agric. Sci. 58 (1) (2013) 33–41.
[55] O. Gbenebor, S. Adeosun, G. Lawal, S. Jun, S. Olaleye, Acetylation, crystalline and morphological properties of structural polysaccharide from shrimp exoskeleton, Eng. Sci. Technol., Int. J. 20 (3) (2017) 1155–1165.
[56] H. Bonfante-Alvarez, G. De Avila-Montiel, A. Herrera-Barros, M. Torrenegra-Alarcon, A.D. Gonzalez-Delgado, Evaluation of Five Chitosan Production Routes with Astaxanthin Recovery from Shrimp Exoskeletons, Chem. Eng. Trans. 70 (2018) 1969–1974.
[57] K. Jellouli, A. Bayoudh, L. Manni, R. Agrebi, M. Nasri, Purification, biochemical and molecular characterization of a metalloprotease from Pseudomonas aeruginosa MN7 grown on shrimp wastes, Appl. Microbiol. Biotechnol. 79 (6) (2008) 989.
[58] I. Younes, S. Hajji, V. Frachet, M. Rinaudo, K. Jellouli, M. Nasri, Chitin extraction from shrimp shell using enzymatic treatment. Antitumor, antioxidant and antimicrobial activities of chitosan, Int. J. Biolo. Macromolecules 69 (2014) 489–498.
[59] L. Manni, O. Ghorbel-Bellaaj, K. Jellouli, I. Younes, 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. 162 (2) (2010) 345–357.
[60] Y.S. Puvvada, S. Vankayalapati, S. Sukhavasi, Extraction of chitin from chitosan from exoskeleton of shrimp for application in the pharmaceutical industry, Int. Curr. Pharm. J. 1 (9) (2012) 258–263.
[61] M. Rhazi, J. Desbrieres, A. Tolaimate, A. Alagui, P. Vottero, Investigation of different natural sources of chitin: influence of the source and deacetylation process on the physicochemical characteristics of chitosan, Polym. Int. 49 (4) (2000) 337–344.
[62] A. Tolaimate, J. Desbrieres, M. Rhazi, A. Alagui, Contribution to the preparation of chitins and chitosans with controlled physico-chemical properties, Polymer 44 (26) (2003) 7939–7952.
[63] G.T. Kjartansson, S. Zivanovic, K. Kristbergsson, J. Weiss, Sonication-assisted extraction of chitin from North Atlantic shrimps (Pandalus borealis), J. Agric. Food. Chem. 54 (16) (2006) 5894–5902.
[64] A. Kucukgulmez, M. Celik, Y. Yanar, D. Sen, H. Polat, A.E. Kadak, Physicochemical characterization of chitosan extracted from Metapenaeus stebbingi shells, Food Chem. 126 (3) (2011) 1144–1148.
[65] F. Ocloo, E. Quayson, A. Adu-Gyamfi, E. Quarcoo, D. Asare, Y. Serfor-Armah, B. Woode, Physicochemical and functional characteristics of radiation-processed shrimp chitosan, Radiat. Phys. Chem. 80 (7) (2011) 837–841.
[66] M. Benhabiles, R. Salah, H. Lounici, N. Drouiche, M. Goosen, N. Mameri, Antibacterial activity of chitin, chitosan and its oligomers prepared from shrimp shell waste, Food Hydrocolloids 29 (1) (2012) 48–56.
[67] M. Benhabiles, N. Drouiche, H. Lounici, A. Pauss, N. Mameri, Effect of shrimp chitosan coatings as affected by chitosan extraction processes on postharvest quality of strawberry, J. Food Meas. Charact. 7 (4) (2013) 215–221.
[68] H. El Knidri, R. El Khalfaouy, A. Laajeb, A. Addaou, A. Lahsini, Eco-friendly extraction and characterization of chitin and chitosan from the shrimp shell waste via microwave irradiation, Process Saf. Environ. Prot. 104 (2016) 395–405.
[69] M. Nouri, F. Khodaiyan, S.H. Razavi, M. Mousavi, Improvement of chitosan production from Persian Gulf shrimp waste by response surface methodology, Food Hydrocolloids 59 (2016) 50–58.
[70] K.N. Aye, W.F. Stevens, Improved chitin production by pretreatment of shrimp shells, J. Chem. Technol. Biotechnol. Int. Res. Process, Environ. Clean Technol. 79 (4) (2004) 421–425.
[71] M. Teli, J. Sheikh, Extraction of chitosan from shrimp shells waste and application in antibacterial finishing of bamboo rayon, Int. J. Biol. Macromol. 50 (5) (2012) 1195–1200.

[72] J. Dutta, P. Dutta, P. Dutta, Chitin and Chitosan: Opportunity & Challenges, Dutta, PK, Ed (2005) 1.
[73] T.S. Trung, N. Van Tan, N. Van Hoa, N.C. Minh, P.T. Loc, W.F. Stevens, Improved method for production of chitin and chitosan from shrimp shells, Carbohydr. Res. 489 (2020), 107913.
[74] M.C. Gortari, R.A. Hours, Biotechnological processes for chitin recovery out of crustacean waste: a mini-review, Electronic J. Biotechnol. 16 (3) (2013) 14.
[75] M. Kaya, T. Baran, M. Karaarslan, A new method for fast chitin extraction from shells of crab, crayfish and shrimp, Nat. Prod. Res. 29 (15) (2015) 1477–1480.
[76] P. Aneesh, R. Anandan, L.R. Kumar, K. Ajeeshkumar, K.A. Kumar, S. Mathew, A step to shell biorefinery—Extraction of astaxanthin-rich oil, protein, chitin, and chitosan from shrimp processing waste, Biomass Convers. Biorefin. (2020) 1–10.
[77] H.K. No, E.Y. Hur, Control of foam formation by antifoam during demineralization of crustacean shell in preparation of chitin, J. Agric. Food. Chem. 46 (9) (1998) 3844–3846.
[78] A. Percot, C. Viton, A. Domard, Characterization of shrimp shell deproteinization, Biomacromolecules 4 (5) (2003) 1380–1385.
[79] V. Ramos, N. Rodrıguez, M. Dıaz, M. Rodrıguez, A. Heras, E. Agullo, N-methylene phosphonic chitosan. Effect of preparation methods on its properties, Carbohydrate Polymers 52 (1) (2003) 39–46.
[80] K.L.B. Chang, G. Tsai, J. Lee, W.-R. Fu, Heterogeneous N-deacetylation of chitin in alkaline solution, Carbohydr. Res. 303 (3) (1997) 327–332.
[81] H.K. No, S.P. Meyers, K.S. Lee, Isolation and characterization of chitin from crawfish shell waste, J. Agric. Food. Chem. 37 (3) (1989) 575–579.
[82] H.K. No, S.P. Meyers, Preparation and characterization of chitin and chitosan—a review, J. Aquat. Food Prod. Technol. 4 (2) (1995) 27–52.
[83] T.S. Trung, W.W. Thein-Han, N.T. Qui, C.-H. Ng, W.F. Stevens, Functional characteristics of shrimp chitosan and its membranes as affected by the degree of deacetylation, Bioresour. Technol. 97 (4) (2006) 659–663.
[84] J. Synowiecki, N.A.A.Q. Al-Khateeb, Mycelia of Mucor rouxii as a source of chitin and chitosan, Food Chem. 60 (4) (1997) 605–610.
[85] H. Srinivasan, V. Kanayairam, R. Ravichandran, Chitin and chitosan preparation from shrimp shells Penaeus monodon and its human ovarian cancer cell line, PA-1, Int. J. Biol. Macromol. 107 (2018) 662–667.
[86] P. Madhavan, K. Ramachandran Nair, Utilization of prawn waste: isolation of chitin and its conversion to chitosan, Fishery Technol. 11 (1) (1974) 50–53.
[87] H.A.S. Al Hoqani, A.-S. Noura, M.A. Hossain, M.A. Al Sibani, Isolation and optimization of the method for industrial production of chitin and chitosan from Omani shrimp shell, Carbohydr. Res. 492 (2020), 108001.
[88] S. Chatterjee, M. Adhya, A. Guha, B. Chatterjee, Chitosan from Mucor rouxii: production and physico-chemical characterization, Process Biochem. 40 (1) (2005) 395–400.
[89] F. Hisham, M.M. Akmal, F. Ahmad, K. Ahmad, Facile extraction of chitin and chitosan from shrimp shell, Mater. Today:. Proc. 42 (2021) 2369–2373.
[90] M. Hossain, A. Iqbal, Production and characterization of chitosan from shrimp waste, J. Bangladesh Agric. Univ. 12 (1) (2014) 153–160.
[91] D. Vallejo-Domínguez, E. Rubio-Rosas, E. Aguila-Almanza, H. Hernández-Cocoletzi, M. Ramos-Cassellis, M. Luna-Guevara, K. Rambabu, S. Manickam, H.S. H. Munawaroh, P.L. Show, Ultrasound in the deproteinization process for chitin and chitosan production, Ultrason. Sonochem. 72 (2021), 105417.
[92] V. Mohanasrinivasan, M. Mishra, J.S. Paliwal, S.K. Singh, E. Selvarajan, V. Suganthi, C.S. Devi, Studies on heavy metal removal efficiency and antibacterial activity of chitosan prepared from shrimp shell waste, 3, Biotech 4 (2) (2014) 167–175.
[93] M.Y. Arancibia, A. Alemán, M.M. Calvo, M.E. López-Caballero, P. Montero, M. C. Gómez-Guillén, Antimicrobial and antioxidant chitosan solutions enriched with active shrimp (Litopenaeus vannamei) waste materials, Food Hydrocolloids 35 (2014) 710–717.
[94] M.Y. Arancibia, A. Alemán, M.E. López-Caballero, M.C. Gómez-Guillén, P. Montero, Development of active films of chitosan isolated by mild extraction with added protein concentrate from shrimp waste, Food Hydrocolloids 43 (2015) 91–99.
[95] E. Johnson, J. EL, Utilization of shellfish waste for chitin and chitosan production, (1982).
[96] M. Mahlous, D. Tahtat, S. Benamer, A.N. Khodja, Gamma irradiation-aided chitin/chitosan extraction from prawn shells, Nucl. Instrum. Methods Phys. Res., Sect. B 265 (1) (2007) 414–417.
[97] P. Lertsutthiwong, N.C. How, S. Chandrkrachang, W.F. Stevens, Effect of Chemical Treatment on the Characteristics of Shrimp Chitosan, J. Metals, Mater. Minerals 12 (1) (2002) 11–18.
[98] G. Lamarque, J.-M. Lucas, C. Viton, A. Domard, Physicochemical behavior of homogeneous series of acetylated chitosans in aqueous solution: role of various structural parameters, Biomacromolecules 6 (1) (2005) 131–142.
[99] S. Gopi, A. Pius, S. Thomas, Enhanced adsorption of crystal violet by synthesized and characterized chitin nano whiskers from shrimp shell, J. Water Process Eng. 14 (2016) 1–8.
[100] G. Hall, C. Reid, Scale up of lactic fermentation for the recovery of chitin from prawn waste, Chitin and chitosan, the versatile environmental friendly modern materials, University Kebangsaan, Malaysia, 1995, pp. 47–51.
[101] K. Gadgey, S. Dey, Development of chitin and chitosan from Narmada riverside crab shells, Int. J. Mech. Eng. Technol. 8 (7) (2017) 298–307.
[102] T. Sannan, K. Kurita, Y. Iwakura, Studies on chitin, 1. Solubility change by alkaline treatment and film casting, Die Makromolekulare, Chemie: Macromolecular Chem. Phys. 176 (4) (1975) 1191–1195.
[103] P. Le Dung, M. Milas, M. Rinaudo, J. Desbrières, Water soluble derivatives obtained by controlled chemical modifications of chitosan, Carbohydr. Polym. 24 (3) (1994) 209–214.
[104] G. Lamarque, C. Viton, A. Domard, Comparative study of the first heterogeneous deacetylation of α-and β-chitins in a multistep process, Biomacromolecules 5 (3) (2004) 992–1001.
[105] P. Broussignac, Chitosan: a natural polymer not well known by the industry, Chim. Ind. Genie Chim 99 (9) (1968) 1241–1247.
[106] A. Martinou, D. Kafetzopoulos, V. Bouriotis, Isolation of chitin deacetylase from Mucor rouxii by immunoaffinity chromatography, J. Chromatogr. A 644 (1) (1993) 35–41.
[107] I. Tsigos, V. Bouriotis, Purification and characterization of chitin deacetylase from Colletotrichum lindemuthianum, J. Biol. Chem. 270 (44) (1995) 26286–26291.
[108] K. Kurita, T. Sannan, Y. Iwakura, Studies on chitin, 4. Evidence for formation of block and random copolymers of N-acetyl-D-glucosamine and D-glucosamine by hetero-and homogeneous hydrolyses, Die Makromolekulare Chemie: Macromolecular Chem. Phys. 178 (12) (1977) 3197–3202.
[109] O. Levenspiel, Chemical reaction engineering, Ind. Eng. Chem. Res. 38 (11) (1999) 4140–4143.
[110] B. Focher, P. Beltrame, A. Naggi, G. Torri, Alkaline N-deacetylation of chitin enhanced by flash treatments. Reaction kinetics and structure modifications, Carbohydrate Polymers 12 (4) (1990) 405–418.
[111] K. Kurita, M. Kamiya, S.-I. Nishimura, Solubilization of a rigid polysaccharide: controlled partial N-acetylation of chitosan to develop solubility, Carbohydr. Polym. 16 (1) (1991) 83–92.
[112] S. Nemtsev, A. Gamzazade, S. Rogozhin, V. Bykova, V. Bykov, Deacetylation of chitin under homogeneous conditions, Appl. Biochem. Microbiol. 38 (6) (2002) 521–526.
[113] G. Galed, B. Miralles, I. Paños, A. Santiago, Á. Heras, N-Deacetylation and depolymerization reactions of chitin/chitosan: Influence of the source of chitin, Carbohydr. Polym. 62 (4) (2005) 316–320.
[114] R. Muzzarelli, Human enzymatic activities related to the therapeutic administration of chitin derivatives, Cellular Mol. Life Sci. CMLS 53 (2) (1997) 131–140.
[115] A. Sahu, P. Goswami, U. Bora, Microwave mediated rapid synthesis of chitosan, J. Mater. Sci. - Mater. Med. 20 (1) (2009) 171–175.
[116] K. Kurita, Controlled functionalization of the polysaccharide chitin, Prog. Polym. Sci. 26 (9) (2001) 1921–1971.
[117] W.G. Birolli, J.A. de Moura Delezuk, S.P. Campana-Filho, Ultrasound-assisted conversion of alpha-chitin into chitosan, Appl. Acoust. 103 (2016) 239–242.
[118] D.D. Salman, W.S. Ulaiwi, A. Qais, Preparation of Chitosan from Iraqi Shrimp Shell by Autoclave, Studying Some Physiochemical Properties and Antioxidant Activity, J. Pharm. Sci. Res. 10 (12) (2018) 3120.
[119] S. Abirami, D. Nagarajan, V.S. Antony, A. Mini Varsini, A. Sugasini, D.A. Anand, Extraction, characterization, and utilization of shrimp waste chitin derived chitosan in antimicrobial activity, seed germination, preservative, and microparticle formulation, (2020).
[120] K. Kurita, K. Sugita, N. Kodaira, M. Hirakawa, J. Yang, Preparation and evaluation of trimethylsilylated chitin as a versatile precursor for facile chemical modifications, Biomacromolecules 6 (3) (2005) 1414–1418.
[121] S. Rogovina, T. Akopova, G. Vikhoreva, Investigation of properties of chitosan obtained by solid-phase and suspension methods, J. Appl. Polym. Sci. 70 (5) (1998) 927–933.
[122] K.T. Hwang, S.T. Jung, G.D. Lee, M.S. Chinnan, Y.S. Park, H.J. Park, Controlling molecular weight and degree of deacetylation of chitosan by response surface methodology, J. Agric. Food. Chem. 50 (7) (2002) 1876–1882.
[123] C.-C. Pădureţu, R. Isopescu, I. Rău, M.R. Apetroaei, V. Schröder, Influence of the parameters of chitin deacetylation process on the chitosan obtained from crab shell waste, Korean J. Chem. Eng. 36 (11) (2019) 1890–1899.
[124] H.K. No, Preparation of chitin and chitosan, Chitin handbook (1997) 475–489.
[125] M.A. Surati, S. Jauhari, K. Desai, A brief review: Microwave assisted organic reaction, Arch. Appl. Sci. Res. 4 (1) (2012) 645–661.
[126] P.R. Rege, L.H. Block, Chitosan processing: influence of process parameters during acidic and alkaline hydrolysis and effect of the processing sequence on the resultant chitosan's properties, Carbohydr. Res. 321 (3–4) (1999) 235–245.
[127] P. Methacanon, M. Prasitsilp, T. Pothsree, J. Pattaraarchachai, Heterogeneous N-deacetylation of squid chitin in alkaline solution, Carbohydr. Polym. 52 (2) (2003) 119–123.
[128] I. Batista, G.A. Roberts, A novel, facile technique for deacetylating chitin, Die Makromolekulare Chemie 191 (2) (1990) 429–434.
[129] A. Domard, M. Rinaudo, Preparation and characterization of fully deacetylated chitosan, Int. J. Biol. Macromol. 5 (1) (1983) 49–52.
[130] S.A. Lopatin, M.S. Derbeneva, S. Kulikov, V. Varlamov, O. Shpigun, Fractionation of chitosan by ultrafiltration, J. Anal. Chem. 64 (6) (2009) 648–651.
[131] M. Benhabiles, N. Abdi, N. Drouiche, H. Lounici, A. Pauss, M. Goosen, N. Mameri, Fish protein hydrolysate production from sardine solid waste by crude pepsin enzymatic hydrolysis in a bioreactor coupled to an ultrafiltration unit, Mater. Sci. Eng., C 32 (4) (2012) 922–928.
[132] R.Q. Qian, R.W. Glanville, Methods for purifying chitosan, Google Patents (2005).
[133] R. Qian, R. Glanville, Methods for purifying chitosan. Providence Health System, US Patent Number-6896809 (2005).
[134] S. Mima, M. Miya, R. Iwamoto, S. Yoshikawa, Highly deacetylated chitosan and its properties, J. Appl. Polym. Sci. 28 (6) (1983) 1909–1917.
[135] J. Li, J. Revol, E. Naranjo, R. Marchessault, Effect of electrostatic interaction on phase separation behaviour of chitin crystallite suspensions, Int. J. Biol. Macromol. 18 (3) (1996) 177–187.

[136] R. Marchessault, F. Morehead, N. Walter, Liquid crystal systems from fibrillar polysaccharides, Nature 184 (4686) (1959) 632–633.
[137] A. Morin, A. Dufresne, Nanocomposites of chitin whiskers from Riftia tubes and poly (caprolactone), Macromolecules 35 (6) (2002) 2190–2199.
[138] K. Gopalan Nair, A. Dufresne, Crab shell chitin whisker reinforced natural rubber nanocomposites. 1. Processing and swelling behavior, Biomacromolecules 4 (3) (2003) 657–665.
[139] T. Lertwattanaseri, N. Ichikawa, T. Mizoguchi, Y. Tanaka, S. Chirachanchai, Microwave technique for efficient deacetylation of chitin nanowhiskers to a chitosan nanoscaffold, Carbohydr. Res. 344 (3) (2009) 331–335.
[140] A. Zamani, M. Taherzadeh, Production of low molecular weight chitosan by hot dilute sulfuric acid, BioResources 5 (3) (2010) 1554–1564.
[141] T.A. Khan, K.K. Peh, H.S. Ch'ng, Reporting degree of deacetylation values of chitosan: the influence of analytical methods, J Pharm Pharmaceut Sci 5 (3) (2002) 205–212.
[142] S. Kumari, P. Rath, A.S.H. Kumar, T. Tiwari, Extraction and characterization of chitin and chitosan from fishery waste by chemical method, Environ. Technol. Innovation 3 (2015) 77–85.
[143] D. Knorr, J. Klein, Production and conversion of chitosan with cultures of Mucor rouxii or Phycomyces blakesleeanus, Biotechnol. Lett. 8 (10) (1986) 691–694.
[144] E.S. Abdou, S.S. Elkholy, M.Z. Elsabee, E. Mohamed, Improved antimicrobial activity of polypropylene and cotton nonwoven fabrics by surface treatment and modification with chitosan, J. Appl. Polym. Sci. 108 (4) (2008) 2290–2296.
[145] C.M. de Moura, J.M. de Moura, N.M. Soares, L.A. de Almeida Pinto, Evaluation of molar weight and deacetylation degree of chitosan during chitin deacetylation reaction: used to produce biofilm, Chem. Eng. Process. Process Intensif. 50 (4) (2011) 351–355.
[146] A.B.V. Kumar, M.C. Varadaraj, L.R. Gowda, R.N. Tharanathan, Characterization of chito-oligosaccharides prepared by chitosanolysis with the aid of papain and Pronase, and their bactericidal action against Bacillus cereus and Escherichia coli, Biochem. J 391 (2) (2005) 167–175.
[147] R. Muzzarelli, Enzymatic synthesis of chitin and chitosan, Chitin, Occurrence of chitin, 1977, pp. 5–17.
[148] T. Truong, R. Hausler, F. Monette, P. Niquette, Valorisation des résidus industriels de pêches pour la transformation de chitosane par technique hydrothermo-chimique, Revue des sciences de l'eau/journal of Water Sci. 20 (3) (2007) 253–262.
[149] H. El Fargani, R. Lakhmiri, A. Albourine, O. Cherkaoui, M. Safi, Valorization of shrimp co-products "Pandalus borealis": Chitosan production and its use in adsorption of industrial dyes, vol 7 (2016) 1334-1346.
[150] Y. Wei, S. Hudson, J. Mayer, D. Kaplan, The crosslinking of chitosan fibers, J. Polym. Sci., Part A: Polym. Chem. 30 (10) (1992) 2187–2193.
[151] C.-C. Pădureţu, R.D. Isopescu, C.L. Gîjiu, I. Rău, M.R. Apetroaei, V. Schröder, Optimization of chitin extraction procedure from shrimp waste using Taguchi method and chitosan characterization, Mol. Cryst. Liq. Cryst. 695 (1) (2019) 19–28.
[152] J. Wang, C. Chen, Chitosan-based biosorbents: modification and application for biosorption of heavy metals and radionuclides, Bioresour. Technol. 160 (2014) 129–141.