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Review



Alternative processes to produce chitin, chitosan, and their oligomers

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ABSTRACT

Sustainable recovery of chitin and its derivatives from shellfish waste will be achieved when the industrial production of these polymers is achieved with a high control of their molecular structure, low costs, and acceptable levels of pollution. Therefore, the conventional chemical method for obtaining these biopolymers needs to be replaced or optimized. The goal of the present review is to ascertain what alternative methods are viable for the industrial-scale production of chitin, chitosan, and their oligomers. Therefore, a detailed review of recent literature was undertaken, focusing on the advantages and disadvantages of each method. The analysis of the existing data allows suggesting that combining conventional, biological, and alternative methods is the most efficient strategy to achieve sustainable production, preventing negative impacts and allowing for the recovery of high added-value compounds from shellfish waste. In conclusion, a new process for obtaining chitinous materials is suggested, with the potential of reducing the consumption of reagents, energy, and water by at least 1/10, 1/4, and 1/3 part with respect to the conventional process, respectively.

1. Introduction

As Crini (2019) mentioned in his historic review, studies related to chitin and chitosan fulfilled 220 years of development in 2018 (Crini, 2019). To date, however, researchers have not determined the optimal mass production process of these multipurpose biopolymers from cuticles of various crustaceans (Hirano, 2012). The industrial production of chitin and chitosan began in Japan in 1971 (Crini, 2019). From that time to the present, the most common industrial production process for chitin, chitosan, and their oligomers is the chemical method (or conventional method), which consists of the liberation (fractionation) of the components that accompany the chitin (proteins, minerals, lipids, and pigments) through their denaturalization or dissolution, and the posterior deacetylation and/or depolymerization of the chitin to obtain chitosan or oligomers of chitin and chitosan (Santos et al., 2020; Younes & Rinaudo, 2015). The reason that led to this process's birth is the chitin's semi-crystalline structure, which makes it insoluble in most organic and inorganic solvents (Casadidio et al., 2019). This restriction made other processing methods, such as chitin solvent extraction, unfeasible to implement at an industrial level until the second decade of the 21st century (525 Solutions, 2017; Mari Signum, 2018; Shamshina, 2019; Shamshina & Berton, 2020).

Disadvantages of the chemical method include: (i) harsh conditions (too acid or too alkaline) (Mahmoud et al., 2007); (ii) large amounts of generated waste; (iii) no recovery of other by-products such as proteins, minerals, pigments, and lipids (Chen et al., 2016; Kerton et al., 2013; Yan & Chen, 2015; Yang & Yan, 2018); (iv) use of large amounts of water; (v) production of large amounts of effluents that require pre-

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treatment to be discharged; (vi) high energy demand; (vii) high investment in equipment (Bastiaens et al., 2019; Devi & Dhamodharan, 2018; Gómez-Ríos et al., 2017; Lopes et al., 2018; Orrego & Salgado, 2014); and (viii) a poor structural control of chitin, chitosan, and the oligomers obtained, resulting in materials with a high dispersity in their degree of polymerization (X) and molar fraction of acetylation (f_A), and a random pattern of acetylation (P_A) (Cord-Landwehr et al., 2019, 2020; Wattjes et al., 2020). Conversely, advantages include: (i) high yield, with almost 50 % more chitin production per kilogram of shellfish waste than the biological method; (ii) short processing times; (iii) high relative-purity of the products, e.g., chitin without traces of protein, minerals, pigments, or lipids; (iv) a lower initial investment, and (v) a shorter payback period (Gómez-Ríos et al., 2017; Lopes et al., 2018). At present, the chemical method is preferred over other methodologies for the large-scale production of chitin and chitosan. However, a more conscious market of the structure-function relationships of chitins and chitosans, specifically of how their biological functionalities and physicochemical properties change with the X, f_A, and P_A (Bellich et al., 2016; Cord-Landwehr et al., 2019, 2020; Wattjes et al., 2020), and the global demand for more sustainable practices are driving the transition to more ecological processes, with more control of the chemical structure of products (Barber et al., 2013; Casadidio et al., 2019; Kaur & Dhillon, 2015; Kerton et al., 2013; Nano3Bio, 2017). The crux of this transition is to develop new physical or chemical transformation technologies or methods that allow the production of *high-value products* more efficiently (Barber et al., 2013; Naqvi & Moerschbacher, 2017). These new technologies or methods base their economic viability and comparative advantages on a considerable reduction of reagents (e.g., acids or bases), solvents and energy; better use of feedstock (shellfish waste) by obtaining new commercial by-products; and high specificity of the generated products. The first and second facts make these new technologies more eco-friendly (*lower waste output*) and cost-effective, while the third adds value to the final products.

The objective of this review is to ascertain what methods exist to produce chitin, chitosan, and their oligomers **from shellfish waste**. A detailed review of recent literature was undertaken, focusing on the advantages and disadvantages of each method. The analysis of available data allows suggesting a new method resulting from the combination of technologies to facilitate the transition towards more sustainable processes (Junceda-Mena et al., 2023; Lv et al., 2023; Mohan et al., 2022).

2. Chitin and chitosan

In this review, the description of the natural hierarchical order in which chitin is immersed in shellfish waste sustains the non-emergence of a new process of obtaining chitin and its derivatives more ecologically and cost-efficient compared to the conventional method at an industrial level until the second decade of the 21st century.

2.1. Chemical structure of chitin

Fully acetylated chitin (poly-*N*-acetylglucosamine) is a linear nitrogen-containing natural polysaccharide constituted by 2-acetamide-2-deoxy-β-D-(+)-glucose (GlcNAc) units linked via β-(1,4)-glycosidic bonds. Its IUPAC name, poly (1,4′-*O*-β-2-acetamide-2-deoxy-D-glucose), refers to a structure of one polysaccharide composed of six-membered cyclic monosaccharides derivatives, D-glucose, linked through di-equatorial glycosidic bonds, in which the acetamide group has replaced the hydroxyl radical of carbon 2 (see Fig. 1-B) (Wade, 2007; Zargar et al., 2015). The chemical structure of fully acetylated chitin (see Fig. 1-A) is a straight-chain homopolymer composed of n units of *N*-acetylglucosamine (GlcNAc) (see Fig. 1-A). In general, the chitin is partially deacetylated due to the action of the chitin deacetylase present in living organisms that contain chitin or due to deacetylation caused by extraction processes (Einbu, 2007, Chapter 1; Niaounakis, 2014, Chapter 1; Roberts, 1992, Chapter 1; Tsvetkov et al., 2020).

The molar fraction of acetylation of commercial chitin can vary between 0.9 and 1.0, and thus the presence of 2-amino-2-deoxy-β-D-(+)-glucose (Glucosamine, GlcN) units in their chain structures make it a copolymer (see Fig. 4). On the other hand, the linearity of chitin macromolecules is because the β-(1,4)-glycosidic bonds allow a stretched conformation in the form of a regular zigzag, giving rise to strong intramolecular forces such as hydrogen bonds (Pillai et al., 2009). As observed in Fig. 1-C, these secondary bonds occur between the hydrogen of the hydroxyl of carbon 3 and the oxygen found in the cyclic structure of the previous ring, and between the hydrogen of the hydroxyl of carbon 6 and the oxygen of the acetylamino functional group of the monosaccharide prior to this. The rigidity generated by these hydrogen bonds added to the considerable volume of the substituent acetamide ($-NHCOCH_3$) not only leads to inflexibility of the chains but also limits rotation along the single bonds (glycosidic) that link the monosaccharides, resulting in highly stable chain structural conformations (Khoushab & Yamabhai, 2010).

2.2. Crystal structure of chitin

Chitin occurs in three crystalline polymorphic forms: α, β, and γ. These three variants differ in their degree of hydration, unit cell size

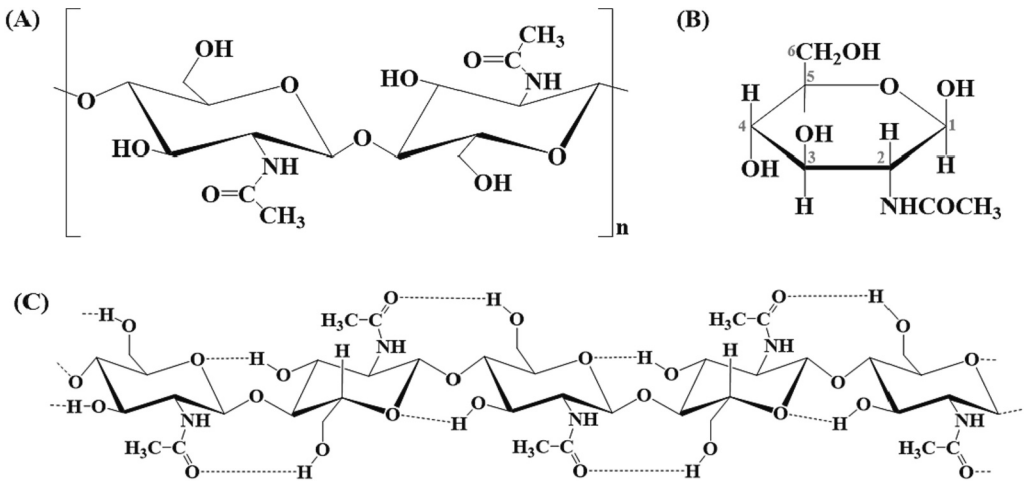


Fig. 1. (A) Structure of fully acetylated chitin; both GlcNAc units are equal. Note that both are inverted to allow a reasonable valence angle in glycosidic oxygen. (B) An *N*-acetyl-D-glucosamine monosaccharide. (C) Chemical structure of fully acetylated chitin with intramolecular hydrogen bonds between neighboring saccharide rings.

(minimum crystalline structure), and the number of chitin chains per unit cell. The α-chitin is the most common form, being present in living organisms such as arthropods, fungi, crustaceans, and the *cysts of Entamoeba* (Jang et al., 2004; Toldra & Kim, 2017). β-chitin is less frequent and typically found in specimens such as squid pens or tubes produced by tubeworms (*vestimentiferan pogonophora*) (Kim, 2014). Meanwhile, γ-chitin is an unusual variant of α-chitin found in the stomach of *Loligo* and *Ptinus* beetle cocoon fibers (Kaya et al., 2017; Rinaudo, 2006).

Crystallographic studies have determined that α-chitin is the most crystalline, stable, and compact form of chitin (Sikorski et al., 2009). The chains of α-chitin are arranged in an anti-parallel manner (↑↓↑), allowing hydrogen bonds to occur between chains in two directions. Conversely, the chains of β-chitin are arranged in parallel (↑↑↑), allowing only the appearance of hydrogen bonds in one direction. In the γ-polymorph, the chains are arranged in a pattern whereby two parallel chains are in one direction (↑↑↓), while the third is in the opposite direction (Al-Hmoud et al., 2020).

The chain arrangement described above is seen more clearly in the proposed crystalline structures for α- and β-chitin shown in Fig. 2-A and -B. Both poly-morphologies organize the chitin chains into tightly linked sheets. In the case of α-chitin, the anti-parallel arrangement of the chains allows the formation of intermolecular hydrogen bonds along the **a-** and **b**-axes. For β-chitin, the parallel arrangement of the chains only allows the formation of an intermolecular hydrogen bond along the **a**-axis. In both poly-morphologies, the intermolecular bond along the **a**-axis corresponds to the strong C-O···NH hydrogen bonds. Only in α-chitin, the intermolecular bond along the **b**-axis corresponds to a hydrogen bond between two hydroxyl groups (Rinaudo, 2006). This means that α-chitin has a lower unit cell dimension along the **b**-axis (i.e., more compact) than the β- and γ-chitin. This is due to the intersheet hydrogen bonds in this direction. These intersheet bonds are responsible for the lack of swelling in the water of α-chitin, whereas β-chitin, in which this intersheet bonding is absent, swells readily in water and forms hydrates. It has been suggested that the greater resistance to swelling of α-chitin is a major reason for its much greater prevalence in nature (Roberts, 1992).

2.3. Chitin in nature

Chitin is one of the most abundant natural polymers in nature (Cauchie, 1997, 2002). This polysaccharide is synthesized by arthropods, mollusks, fungi, yeasts, and algae, being found in ordered crystalline microfibrils in exoskeletons, membranes, and cell walls. These structural components provide mechanical support, protection against predators and pathogens, and mobility and other essential functions to these living organisms. With only one known exception, the microscopic diatom unicellular algae, chitin in nature, is linked to other structural components such as proteins, glycoproteins, proteoglycans, and other

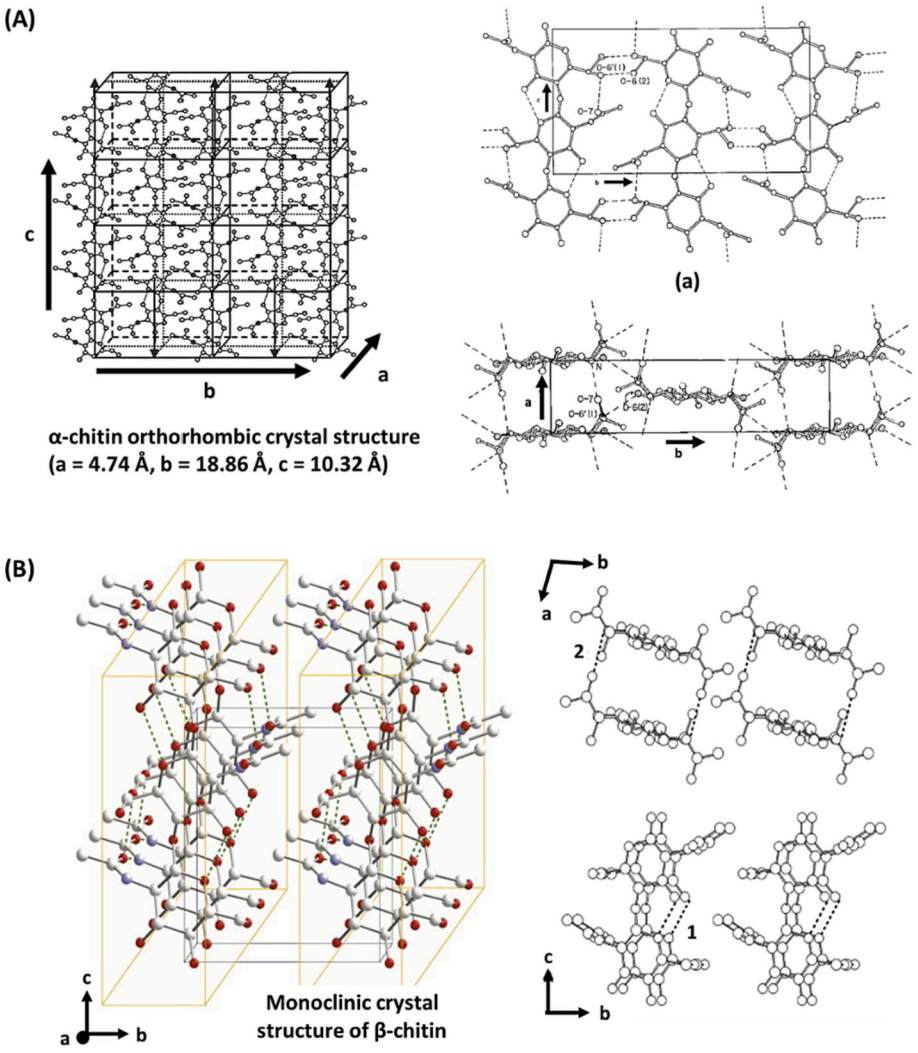


Fig. 2. (A) Crystal structure of α-chitin, and (B) crystal structure of β-chitin. The dotted lines denote (1) intramolecular and (2) intermolecular hydrogen bonds. (Adapted with permission from (Raabe et al., 2007; Yui et al., 2007). Copyright (2007) Elsevier Science Ltd).

sugars. For example, it is covalently linked to glucans directly or through peptide bridges in fungal cell walls. On the other hand, chitin is always associated with specific proteins through covalent and non-covalent bonds in insects and other invertebrates, giving rise to ordered structures that then undergo mineralization with inorganic particles (Brown & Walsh, 2019; Einbu, 2007; Younes & Rinaudo, 2015).

From the industrial point of view, the primary source of chitin is exoskeletons (cuticles) of crustaceans such as crabs, lobsters, and shrimp. The arthropod cuticle is a hierarchical structure, where the unit GlcNAc represents the lowest hierarchical level. From this, chitin chains are formed, arranged in crystalline forms representing the second hierarchical level (see Fig. 3). In α-chitin, the chains are arranged anti-parallel to each other, resulting in the orthorhombic crystal structure shown in Fig. 2-A. In β-chitin, the chains are parallel, generating a monoclinic crystal symmetry as shown in Fig. 2-B. Finally, γ-chitin is a mixture of both α- and β-chitin, with two parallel chains followed by an anti-parallel chain. Although all three of these polymorphic forms can be found in parts of the same organism, crystallin α-chitin predominates in the arthropod cuticle (Muzzarelli et al., 2011; Raabe et al., 2006). Typically, 18 to 25 chitin chains are arranged anti-parallel forming sections of α-chitin crystallites. These molecules are then peripherally enveloped by helically arranged proteins, thus creating nano-fibers with diameters between 2 and 5 nm. Subsequently, these chitin-protein nanofibrils are aggregated together to form thicker fibers of 50 to 250 nm in diameter, representing the fourth hierarchical level. At the next level, these thicker fibers are arranged parallel to each other, creating flat matrices, overlapping sheets, forming stacks where each sheet is rotated at a constant angle, and giving rise to a twisted plywood-type pattern or Bouligand structure. This helical structural arrangement forms the three main layers of the cuticle, with different pile heights (see Fig. 3) (Fabritius et al., 2009; Muzzarelli, 2011; Muzzarelli et al., 2011; Raabe et al., 2006). On the other hand, the organic material between the *exo*- and *endo*-cuticle is combined with inorganic nanoparticles arranged according to the hierarchical organization of chitin-protein fibers. This inorganic phase consists mainly of calcium carbonate, calcium phosphate, and Mg-calcite. Hence, the cuticle is considered a hierarchical composite material that combines high mechanical strength and functional versatility (Muzzarelli et al., 2011).

2.4. Chemical and crystal structure of chitosan as a derivative of chitin

According to the nomenclature proposed by the European Chitin Society (EUCHIS), the difference between chitin and chitosan relies on insolubility and solubility, respectively. For example, in an aqueous solution of acetic acid 0.1 mol/L, the insoluble material should be called chitin, and the soluble, chitosan (Giraldo et al., 2023; Kumirska et al., 2010). However, most manuscripts relate the difference between these polysaccharides to the molar fraction of acetylation. Thus, chitin has a f_A greater than or equal to 0.5, while chitosan has a f_A lower than 0.5 (see Fig. 4) (Aranaz et al., 2009; Dutta et al., 2004; Pillai et al., 2009; Ravi Kumar, 2000; Rinaudo, 2006; Younes & Rinaudo, 2015).

The term chitosan refers to a linear copolymer composed of GlcN and GlcNAc monomeric units with different topologies (random, alternate, and block) (Kumirska et al., 2010, 2009; Weinhold et al., 2009). Although this polysaccharide exists naturally in some microorganisms such as *ascospore* yeast (Briza et al., 1988), its primary source is chitin extracted from fishing industry waste (e.g., arthropod exoskeletons and squid pens) (Kurita, 2006). Hence, chitosan is the main derivative of chitin.

Like chitin, chitosan can also present parallel and anti-parallel arrangements of its chains (α, β, and γ) (Faria et al., 2016). However, the chemical and biochemical reactivity of its polymorphic forms is greater than chitin due to the free amino groups distributed along the chains. On the other hand, the conventional chitosan obtained from the deacetylation of α-chitin presents a hydrated polymorphic crystallinity, in which chitosan macromolecules form a double helix extended in the crystal (Dumitriu, 2004). The hydrated form of chitosan has double-helical symmetry reinforced by a hydrogen bond along the **c**-axis, typical in polysaccharides with β-(1,4)-glycosidic bonds, such as cellulose and chitin (see Fig. 5-A). On the other hand, the adjacent chitosan chains along the **b**-axis are linked through two hydrogen bonds CO···NH, and the sheets formed as a result of these bonds are stacked along the **a**-axis, but unlike chitin, these are linked through hydrogen bonds via water molecules (see Fig. 5-B) (Ogawa et al., 2004; Okuyama et al., 2000).

Different studies have demonstrated that the molar fraction of acetylation and the weight-average molecular mass are the primary factors that influence the crystal structure of chitin and chitosan: (i) a decrease in the molar fraction of acetylation is correlated with lower degrees of crystallinity, and (ii) an increase in the weight-average molecular mass is correlated with lower degrees of crystallinity (Kumirska

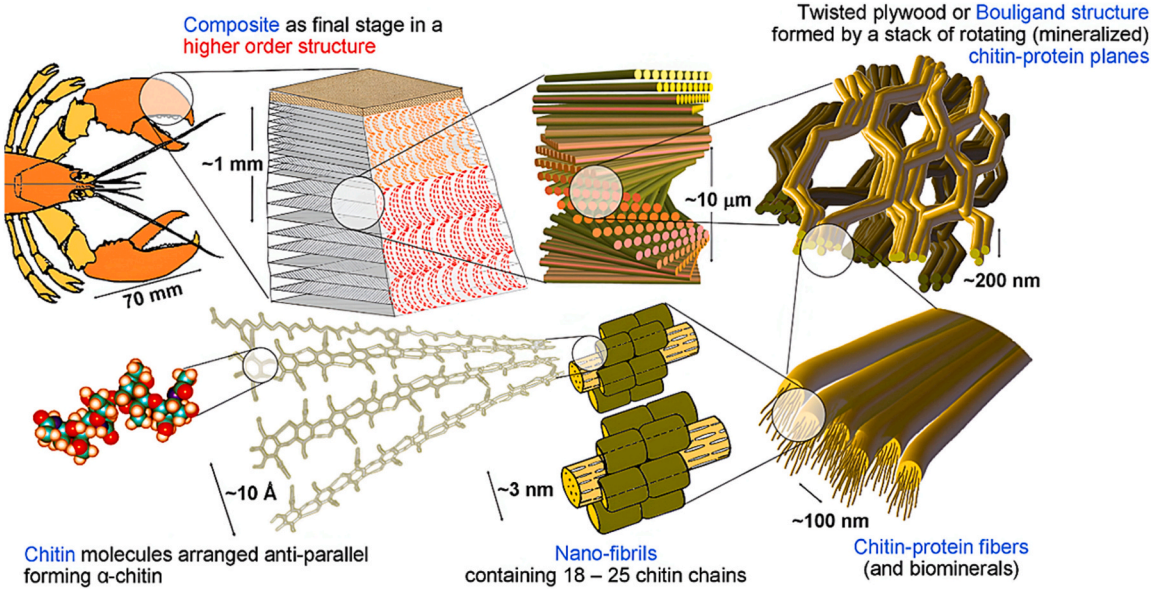


Fig. 3. Hierarchical microstructure of the lobster cuticle *H. americanus*. (Adapted with permission from (Raabe et al., 2007, 2005). Copyright (2007) Elsevier Science Ltd).

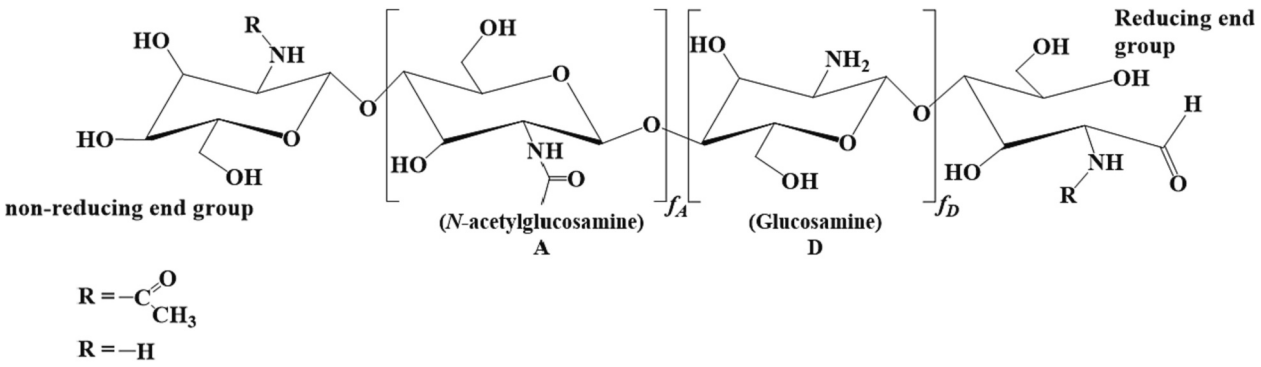


Fig. 4. Chemical structure of chitin or chitosan: when $f_A \geq 0.5$ corresponds to chitin, and when $f_A < 0.5$ correspond to chitosan. (Reprinted with permission from (Giraldo et al., 2023). Copyright (2023) Elsevier Science Ltd).

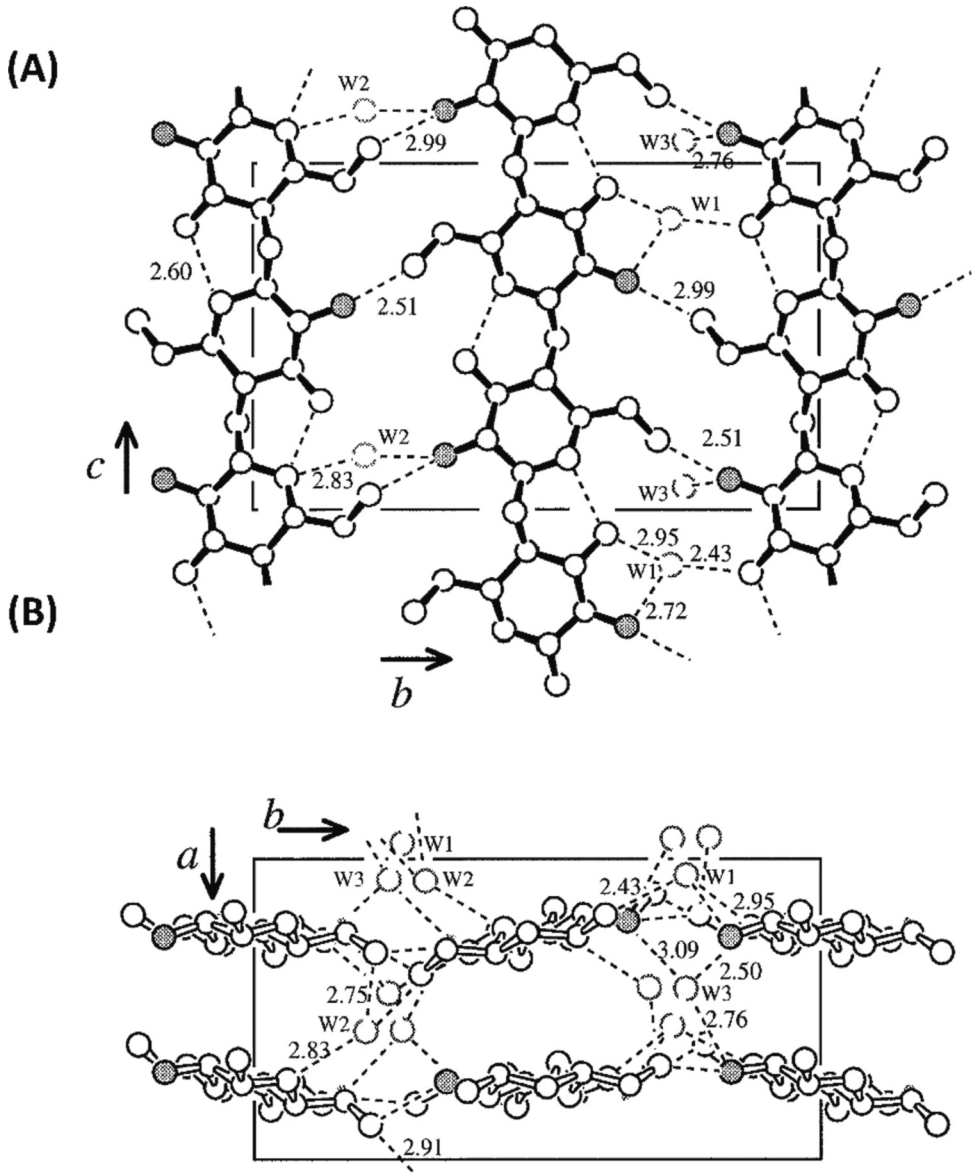


Fig. 5. Crystal structure of hydrated chitosan. Dotted lines denote hydrogen bonds; gray circles denote nitrogen atoms; and lines with the letter w denote water molecules. (Reprinted with permission from (Okuyama et al., 1997). Copyright (1997) American Chemical Society).

et al., 2010; Ogawa & Yui, 1993). Regarding the effects on the crystalline structure by the weight-average molecular mass, the method of deacetylation or acetylation must be considered; chitosan samples prepared under homogeneous conditions do not show significant change when this parameter is varied (Facchinatto et al., 2020). On the other hand, the obtention of polymer samples of chitin and chitosan with

patterns of acetylation different from the random pattern is scarce. As far we know, no studies in the consulted literature correlate this parameter's variation with the crystal structure of these biopolymers.

3. Characterization parameters

Chitin and chitosan are structurally characterized by three parameters: molar fraction of acetylation (f_A), degree of polymerization (X), and pattern of acetylation (P_A) (Sreekumar et al., 2018; Wattjes et al., 2020). Given that studies on the production of chitin or its derivates aim to achieve the total structural control of the materials obtained, and considering that studies focusing on the applications of these materials relate their chemical structures to their functionalities, it is mandatory to report at least the f_A and X of these biopolymers (Cord-Landwehr et al., 2019).

3.1. Molar fraction of acetylation, f_A

It is a characterization parameter that indicates the relationship between the moles of GlcNAc (n_{GlcNAc}) and the total moles ($n_{GlcNAc} + n_{GlcN}$) in a sample, as expressed by Eq. (1) (Cord-Landwehr et al., 2019; Jiang et al., 2003; Lavertu et al., 2003; Rehman et al., 2023). Its inverse is the molar fraction of deacetylation ($f_D = 1 - f_A$), which indicates the relationship between the moles of GlcN (n_{GlcN}) and the total moles in a sample (Giraldo & Rivas, 2020). This relationship is equal to the number-average molar fraction of acetylation ($\bar{f}_{A_n}$) of the macromolecules that make up the sample (Mnatsakanyan et al., 2013).

$$f_A = \frac{n_{GLcNAc}}{n_{GlcNAc} + n_{GlcN}} \tag{1}$$

Dispersity of the molar fraction of acetylation ($Đ_{f_A}$) indicates the inter-molecular homogeneity ($Đ_{f_A} = 1$) or heterogeneity ($Đ_{f_A} > 1$) of the molar fraction of acetylation of the macromolecules that make up the sample, as expressed by Eq. (2) (G.A.F. Roberts, 1997).

$$Đ_{f_A} = \frac{\bar{f}_{A_{n-max}}}{\bar{f}_{A_n}} \tag{2}$$

Where $\bar{f}_{A_{n-max}}$ is the highest number-average molar fraction of acetylation found after implementing a separation method (Bahrke et al., 2002; Berth & Dautzenberg, 2002; Cord-Landwehr et al., 2019; Mnatsakanyan et al., 2013; Nguyen et al., 2009; Ottoy et al., 1996; Roberts, 1997; Thevarajah et al., 2017).

3.2. Pattern of acetylation, P_A

It is a characterization parameter that indicates how GlcNAc (A) and GlcN (D) units are distributed along the macromolecules that make up a chitin or chitosan sample. As linear copolymers, three chain architectures (topologies) can exist: alternating, random, and block-wise patterns or distributions of GlcNAc (A) and GlcN (D) units, as shown in Fig. 6 (Cord-Landwehr et al., 2019; Kumirska et al., 2010, 2009; Ottsy et al., 1996; Varum et al., 1991; Vårum et al., 1991; Wattjes et al., 2019; Weinhold et al., 2009).

This parameter can be measured using ^{1}H or ^{13}C NMR spectroscopy, normalizing the relative experimental intensities of a chitin or chitosan sample according to the Bernoulli statistical model (Kumirska et al., 2010). The distribution of GlcNAc and GlcN units along the macromolecules that make up a chitin or chitosan sample can show a perfect alternating pattern if $P_A = 2.0$; an alternating-dominated pattern if $1.5 < P_A < 2.0$; a random-dominated pattern if $0.5 < P_A < 1.5$; probably a perfect random pattern if $P_A = 1.0$, a block-dominated pattern if $0 < P_A < 0.5$, and a perfect block-wise pattern if $P_A = 0$ (Weinhold et al., 2009). It is important to note that when $P_A = 1.0$, it is not a sufficient condition to declare a perfect random **disorder** distribution of the GlcNAc and GlcN units along the macromolecules that make up a chitin or chitosan sample because within the **statistical randomness** exists the **probability** that appears block-wise or alternating distributions (Cord-Landwehr et al., 2019).

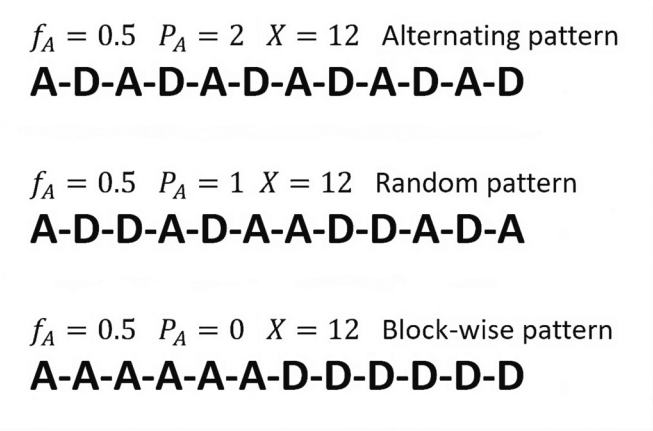


Fig. 6. Theoretical patterns of acetylation found in chitin or chitosan samples. A and D denote GlcNAc and GlcN units, respectively.

3.3. Degree of polymerization, X

In general, samples of chitin and its derivatives are heterogeneous in terms of size distribution of the macromolecules, i.e., their molecular mass refers to an average value. Polymer chemistry distinguishes four central average values for the molecular mass of the polymers: number-average molecular mass ($\overline{M}_n$), weight-average molecular mass ($\overline{M}_w$), viscosity-average molecular mass ($\overline{M}_v$), and z-average molecular mass ($\overline{M}_z$) (Cord-Landwehr et al., 2019). As heterogeneous systems, $\overline{M}_n < \overline{M}_v < \overline{M}_w < \overline{M}_z$, and their dispersities ($Đ_M$) are commonly expressed by Eq. (3). Theoretically, dispersity of the molecular mass indicates the inter-molecular homogeneity ($Đ_M = 1$) or heterogeneity ($Đ_M > 1$) of the molecular mass of the macromolecules in a sample (Standard Test Method for Determining the Molar Mass of Chitosan and Chitosan Salts by Size Exclusion Chromatography with Multi-Angle Light Scattering Detection (SEC-MALS), 2013).

$$Đ_M = \frac{\overline{M}_w}{\overline{M}_n} \tag{3}$$

The degree of polymerization (X) is the number of structural units (GlcNAc + GlcN) per polymer chain in a sample. For chitin and its derivatives, X can be determined by Eq. (4).

$$X = \frac{Mf_A}{M_{GlcNAc}} + \frac{M(1-f_A)}{M_{GlcN}} \tag{4}$$

Where M_{GlcNAc} (203.194 g/mol) and M_{GlcN} (161.157 g/mol) are the molecular mass of GlcNAc and GlcN units, respectively. M can be: $\overline{M}_n$, $\overline{M}_v$, $\overline{M}_w$, or $\overline{M}_z$, and therefore X is an average value, e.g., number-average degree of polymerization ($\overline{X}_n$), or weight-average degree of polymerization ($\overline{X}_w$). As in Eq. (3), dispersity of the degree of polymerization ($Đ_X$) can be determined by the relation $\overline{X}_w/\overline{X}_n$. It is worth mentioning that even though there is not a clearly defined boundary between chitin/chitosan oligomers and chitin/chitosan polymers, the boundaries proposed by the Professor Moerschbacher's research group can serve as a practical guide: molecules with $X < 10$ can be defined as true oligomers; macromolecules with $X > 100$ can be defined as true polymers; macromolecules with $10 \leq X \leq 100$ can be defined as large oligomers or small polymers depending on whether their X approach the minimum (10) or maximum (100) value, respectively (Cord-Landwehr et al., 2019, 2020; Giraldo et al., 2023; Naqvi & Moerschbacher, 2017).

In summary, actual samples of chitin or its derivatives are mixtures

of molecules that differ in their f_A, P_A, and X; i.e., they are not pure samples made up of only one molecular species. Therefore, when referring to pure samples, it is more appropriate to refer to a relative purity of the sample, e.g., chitin without traces of protein, minerals, pigments, or lipids. Accordingly, it may be more appropriate to refer to fully acetylated or fully deacetylated samples as poly-*N*-acetylglucosamine or poly-glucosamine samples, respectively (note that in that type of sample only varies the X parameter) (Cord-Landwehr et al., 2019, 2020; Giraldo & Rivas, 2020; Naqvi & Moerschbacher, 2017).

4. Conventional method

As chitin is insoluble in many organic and common inorganic solvents, it is not likely to be obtained from arthropod cuticles by solvent extraction processes, and thus deproteinization and demineralization processes are required to remove proteins and minerals. Subsequently, chitosan is obtained through a chitin deacetylation process (see Fig. 7).

The process of obtaining chitin and chitosan from shrimp, lobster, and crab waste is well defined. To avoid flesh residue decomposition and chitin degradation, exoskeletons (shellfish waste) require a temperature below −15 °C (reception of exoskeletons). Once the process begins, the first step is to clean the exoskeletons at room temperature, using a dilute NaOH solution as a corrosive agent (flesh residue removal). The second step is to increase the surface area of contact of the exoskeletons with the medium, decreasing their particle size (grinding and sieving). The third step is to remove the proteins that wrap around chitin fibers, using a NaOH solution at a high temperature for a short time (deproteinization). The fourth step is to remove minerals using an HCl solution at room temperature (demineralization), allowing for the obtention of chitin. The fifth and final step is to convert chitin to chitosan by removing acetyl groups from chitin, using a concentrated NaOH solution at a high temperature for a certain period of time (deacetylation). Decoloration, which is an optional stage, is commonly carried out using H_2O_2, while depolymerization is carried out using a strong acid such as HNO_3. The resulting polysaccharides must be dried and stored in containers to control humidity and its innocuousness.

According to different Life Cycle Assessments (LCA) of chitosan production through the conventional method reported in the literature, the hot spots (main environmental impacts produced) due to the chemical process can be grouped into three categories: (i) Human health, (ii) ecosystems, and (iii) resources. In the first category, the most relevant impacts are the human carcinogenic and non-carcinogenic toxicity associated with using NaOH and HCl, the production of wastewater, and the combustion of fossil fuels to generate energy. In the second category, the most relevant impacts are global warming, and the ecotoxicity of water bodies, provoked by CO_2 released at the demineralization step and in the combustion of fossil fuels to generate energy, and the wastewater discharge generated through the entire process, respectively. In the third category, the most relevant impacts are the high consumption of energy, water, biomass, and chemical reagents associated with high temperatures, low solid/aqueous-mediums relations, low yield of conversion biomass to chitin or chitosan, and high concentrations (mainly of NaOH at the deacetylation step), respectively (Beach et al., 2012; Berroci et al., 2022; Bonner & Kearney, 2020; Greca et al., 2024; Kusnadi et al., 2024; Leceta et al., 2013; Lizundia et al., 2022; Lopes et al., 2018; Meramo et al., 2022; Meramo-Hurtado et al., 2020; Mondal et al., 2020; Muñoz et al., 2018; Piekarska et al., 2023; Riofrio et al., 2021).

By the Eco-Point (using Eco-indicator 99), the conventional method's total environmental impact is considered moderate (Bonner & Kearney, 2020). Generally, the factors with a more significant effect are energy, water, NaOH, and HCl consumption. It is important to highlight that all the studies reported in the literature related to LCA of chitosan production through the conventional method present different configurations and geography locations (i.e., other supply chains and energy matrices) that imply that the normalized environmental impacts vary drastically between each situation. For this reason, there are no explicit numerical values in this section. Since this review is to ascertain what alternative methods are viable for the industrial-scale production of chitin, chitosan, and their oligomers, it is not the intention to go deeper into the conventional method. However, for those who want to delve into that method, Table 1 summarizes key literature to consider.

5. Biological methods

Other deproteinization and demineralization processes can be carried out by microbial fermentation and the use of proteolytic enzymes (Cai et al., 2006). Bacteria used in fermentation include *Serratia marcescens* (Visootsat et al., 2020), *Bacillus subtilis* (Zhang, Wang, et al., 2021), *Pseudomonas aeruginosa* (Ghorbel-Bellaaj et al., 2011), and combinations with *Lactobacillus plantarum* (Castro et al., 2018) to remove minerals, whereas enzymatic methods include proteolytic enzymes such as trypsin, papain, and pepsin (Duong & Nghia, 2014; Marzieh et al., 2019; Rohyami et al., 2018). Likewise, microorganisms and enzymes can be successfully used in deacetylation of chitin and depolymerization of chitin and chitosan. In recent years, enzymes such as chitin deacetylases, chitinases, chitosanases, β-*N*-acetylhexosaminidase, exo-1,4-β-D-glucosaminidase, and lytic polysaccharide

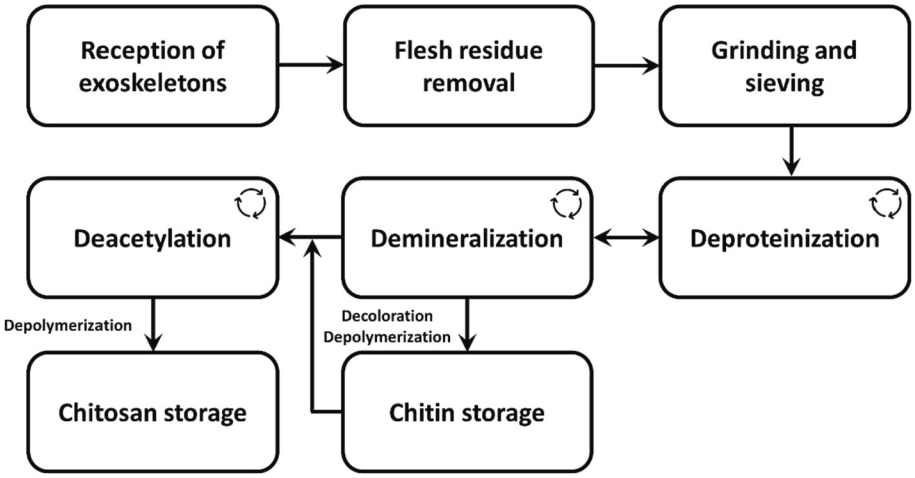


Fig. 7. Block diagram of an industrial process to obtain chitin and chitosan. The demineralization stage can be carried out prior to the deproteinization stage. Further, deproteinization, demineralization, and deacetylation stages can be carried out in more than one cycle. Decoloration and depolymerization are optional stages.

Table 1
Key literature related to the conventional method.

Title	Key content	Reference
Highly deacetylated chitosan and its properties	High deacetylation and molecular weight by intermittently washing the intermediate product in water two or more times during the alkali treatment.	(Mima et al., 1983)
Heterogeneous *N*-deacetylation of chitin in alkaline solution	The influence of alkaline concentration, temperature, and alkaline-solution/chitin ratio on deacetylation.	(Chang et al., 1997)
Response surface optimization and kinetics of isolating chitin from pink shrimp (*Solenocera melantho*) shell waste	Deproteinization kinetics and optimal conditions to get high yields of chemical deproteinization.	(Chang & Tsai, 1997)
On the influence of deacetylation process on the physicochemical characteristics of chitosan from squid chitin	Influence of deacetylation reaction conditions on chitosan characterization parameters.	(Tolaimate et al., 2000)
Controlling molecular weight and degree of deacetylation of chitosan by response surface methodology	Mathematical correlations that predict the change of molecular weight and degree of deacetylation as a function of deacetylation reaction conditions.	(Hwang et al., 2002)
Effect of chemical treatment on the characteristics of shrimp chitosan	Impact of deproteinization and demineralization, and vice versa, on the characterization parameters of chitosan.	(Lertsutthiwong et al., 2002)
Contribution to the preparation of chitins and chitosans with controlled physico-chemical properties	Comparative tables that related starting materials (shellfish waste) with arrival materials (chitin/chitosan) as a function of chemical process conditions.	(Tolaimate et al., 2003)
Heterogeneous *N*-deacetylation of squid chitin in alkaline solution	Mathematical correlation and kinetic of reaction to predict the degree of deacetylation of β-chitosan.	(Methacanon et al., 2003)
Optimization of chitin extraction from shrimp shells	Optimum conditions that allow obtaining chitin with high relative-purity.	(Percot et al., 2003a)
Characterization of shrimp shell deproteinization	A proposed mechanism that describes the deproteinization process.	(Percot et al., 2003b)
Comparative study of the first heterogeneous deacetylation of **α**- and **β**-chitins in a multistep process	Influence of crystallinity on the deacetylation of chitin and its relationship with acetylation patterns.	(Lamarque et al., 2004)
N-deacetylation and depolymerization reactions of chitin/chitosan: influence of the source of chitin	The composition of the raw material changes the quality of the obtained chitosan.	(Galed et al., 2005)
Thermodynamic aspects of the heterogeneous deacetylation of **β**-Chitin: reaction mechanisms	Identification of the most active species involved in the deacetylation reaction.	(Lamarque et al., 2007)
Multistage deacetylation of chitin: kinetics study	Benefits of a multistep deacetylation reaction.	(Yaghobi & Hormozi, 2010)
Use of a fractional factorial design to study the effects of experimental factors on the chitin deacetylation	Oxygen scavengers as protectors against chitosan degradation during the deacetylation reaction.	(Younes et al., 2014)
Comparison of process technologies for chitosan production from shrimp shell waste: a techno-economic approach using Aspen Plus	The chemical process for chitosan production could be more profitable if by-products such as protein, pigments, and minerals could be commercialized.	(Gómez-Ríos et al., 2017)
Optimization of chitin and chitosan production from shrimp wastes and characterization	Whiteness index is related to the process conditions to obtain chitin and chitosan from shell waste.	(Tokatlı & Demirdöven, 2018)
Chitin production from crustacean biomass: sustainability assessment of chemical and enzymatic processes	The economic viability of the enzymatic process is lower than the chemical process.	(Lopes et al., 2018)
Optimization of chitin extraction procedure from shrimp waste using Taguchi method and chitosan characterization	Mathematical correlation to predict the degree of deacetylation in a process where demineralization is followed by deproteinization.	(Pădureţu et al., 2019)
Evaluation of shrimp waste valorization combining computer-aided simulation and numerical descriptive inherent safety technique (NuDIST)	The conventional method is an inherently safe option to achieve shell waste valorization.	(Zuorro et al., 2020)

monooxygenases have attracted interest in the modification of chitin and chitosan. A schematic diagram of the bioproduction of chitin, chitosan, and their oligomers by the use of enzymes and microorganisms is shown in Fig. 8 (Jung & Park, 2014).

5.1. Biological demineralization – deproteinization

In biological methods, demineralization and deproteinization of chitin occur simultaneously but incompletely (Jung et al., 2007). Therefore, different biological methods such as microbial techniques have been proposed. The microbial treatment mainly uses lactic acid and miscellaneous fermentation as well as enzymatic processes, which use crude extracts or isolated enzymes (Deng et al., 2020; Liu, Xing, et al., 2020; Taser et al., 2021). For example, deproteinization by microorganisms is carried out by in-situ fermentation of shellfish waste with lactic acid bacteria (LAB) or sometimes with mixtures containing proteolytic microorganisms. In this process, LAB carry out the biosynthesis of biomass and lactic acid production using the sugars available in the medium. On the other hand, the accumulated lactic acid promotes solubility of inorganic salts in shellfish waste, carrying out the demineralization stage. Finally, complete mineral extraction occurs in the fermentation stage. Additionally, proteolytic enzymes allow bacteria to use proteins and peptides as N and C sources, carrying out deproteinization of the raw material (Aranday-García et al., 2019; Ríos, 2015).

Khanafari A. et al. carried out a study comparing the extraction of chitin and chitosan from shrimp waste by chemical and microbial methods and found that the latter is more effective for chitin recovery (Khanafari et al., 2008). However, this does not agree with other findings reported in the literature (Gómez-Ríos et al., 2017; Lopes et al., 2018). In fact, another study in which fermentation and chemical methods of chitin extraction were compared, it was found that, although the molar fraction of acetylation was similar (approx. 0.71), different crystallinity was obtained, with 73 % and 86 % for the biological and chemical method, respectively. This difference in crystallinity was explained by the differences in intermolecular hydrogen bonds and crystallite size (Taokaew et al., 2020).

In economic terms, Gómez-Ríos D. et al. evaluated the potential application of chitin bio-extraction on a pilot scale through lactic acid fermentation of Pacific white shrimp waste using the Aspen Plus software. After 50 h of reaction, the results indicated that it is possible to extract >90 % of the minerals and proteins from shrimp waste. In addition, it was found that the bio-extraction of chitin is profitable, as evidenced in the gross margin of the operation that varied between 42 and 52 %, depending on the composition of the raw material and the yield of the products (Gómez-Ríos et al., 2019).

Lactic acid fermentation has been combined with chemical methods to reduce the amounts of acid and alkali required in the chemical process, resulting in high yields of demineralization and deproteinization (Arbia et al., 2013; Cira et al., 2002). Other methods evaluated for chitin recovery are fermentation using non-lactic acid bacteria and co-fermentation (Zhang, Wang, et al., 2021). However, these procedures require long reaction times, reaching yields of up to 82 % after 5 days, preventing its implementation on an industrial scale. Detailed information on lactic acid and non-lactic acid fermentation methods can be found in the review carried out by Arabia W. et al. (Arbia et al., 2013). Another strategy used for biological demineralization and

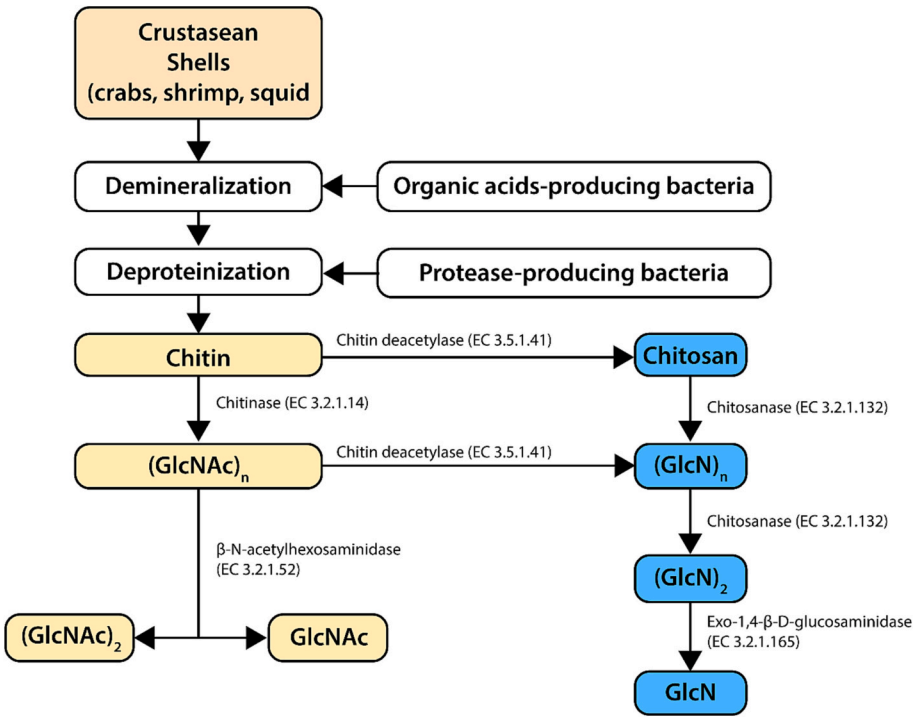


Fig. 8. Schematic diagram of the bioproduction of chitin, chitosan, and their oligosaccharides by biological methods. (Adapted with permission from (Jung & Park, 2014). Copyright (2014) MDPI).

deproteinization is the use of enzymes such as proteases, which is more specific and faster technique because an alkaline treatment is not required, while the use of energy and chemicals is reduced (Gagne & Simpson, 1993). Furthermore, enzymatic methods allow the recovery of high added value products such as protein hydrolysates and pigments (Lopes et al., 2018). Unlike the conventional method, the main advantages of these treatments are the mild conditions that they require (Hathwar et al., 2011), while the main disadvantage relies on the fact that protein and mineral removal is insufficient for industrial-scale applications. In this sense, combinations of enzymatic and chemical methods have shown promising results, demonstrating a highly efficient deproteinization (Deng et al., 2020; Dhanabalan et al., 2021). For example, Valdez-Peña et al. achieved the deproteinization of shrimp head using the Alcalase® enzyme and its subsequent microwave-assisted demineralization with lactic acid, reporting the recovery of high relative-purity chitin and removal of residual protein present in the enzymatically deproteinized shrimp head (Valdez-Peña et al., 2010). In addition, Lopes et al. carried out a comparative study between chemical and enzymatic deproteinization in terms of productivity and economic viability and found that two improvements are still necessary for enzymatic methods to be more sustainable than the chemical method: (i) increase chitin production yield, which has already been achieved with different substrates, and (ii) reduce environmental impacts and costs related to enzyme production (Lopes et al., 2018). In this sense, some environmental advantages of using biological methods include: no harsh conditions required; recovery of some fractions such as proteins and pigments from the shellfish waste that can be commercialized along with chitin and its derivatives; synthesis of chitin derivatives with a high control of their molecular structures, i.e., highly pure samples (almost conformed only by one molecular structure); and the use of solubilized minerals as for animal and human nutrition (Khor, 2014). However, an important limitation of the treatments with fungi and bacteria is the long fermentation time of 48 and 120 h for bacterial and fungal strains, respectively. Furthermore, as protein removal achieved by fermentation varies between 60 and 68 %, and the pigment content is reduced by about 50 %. Extra steps are required to complement the extraction of pigments and proteins with other methods (hardly ever chemicals) (Beaney et al., 2005; Gómez-Ríos et al., 2017).

5.2. *Enzymatic deacetylation*

The enzymatic method has emerged as a greener alternative to conventional chemical method. Apart from reducing energy consumption, the enzymatic method does not require the use of the large amounts of NaOH commonly used in the deacetylation step in the chemical method, while it allows achieving higher control over the molecular structure for the chitin and derivatives obtained, e.g., with other-than random pattern of acetylation (Sixto-Berrocal et al., 2023; Wattjes et al., 2020). Although the use of chemo-enzymatic methods, which use lysozyme and tri-*N*-acetylchitotriose derivatives as enzymes, has been suggested, the resulting products are mixtures with different P_A and X (Chang et al., 1997). For example, chitin de-*N*-acetylases (EC 3.5.1.41, CDA) to prepare chitosan and its oligomers emerge as an alternative that could potentially solve many of the current problems in the chemical method. The CDA enzymes work by hydrolyzing the bond between the acetyl group and the amine group in the *N*-acetylglucosamine residues of chitin, allowing the conversion mainly of chitin oligomers to chitosan oligomers with acetate as a by-product (see Fig. 9) (Jung & Park, 2014; Tsigos et al., 2000). To date, the chitin deacetylase enzyme with a reasonable activity on the surface of crystallin chitin as a substrate has not been identified. Therefore, soluble chitosans with high-f_A or chitin oligomers are used as starting materials to be converted into oligo-chitosans with low-f_A and a highly defined P_A (Aam et al., 2010; Grifoll-romero & Pascual, 2018; Zhao et al., 2010). Such limitation is cost inefficient since only obtaining a starting material that these enzymes can use to exhibit reasonable activity requires complete deacetylation of a chitin sample by the conventional method of alkaline heterogeneous deacetylation, and subsequent re-acetylate by the traditional homogeneous chemical method with acetic anhydride (Wattjes et al., 2019), which contradicts the principles of "Green Chemistry" (Doria Serrano, 2009; Kerton et al., 2013), and the rational idea of a viable industrial process.

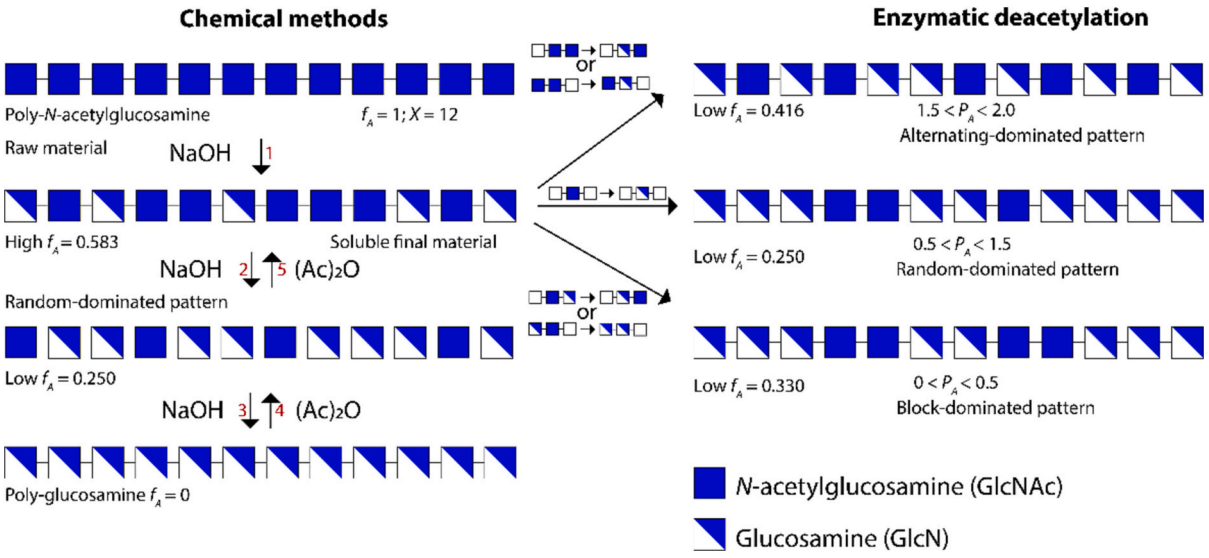


Fig. 9. Chemical and enzymatic methods for chitin deacetylation (in accordance to (Wattjes et al., 2020)).

Since CDAs were discovered, it has been suggested that they could be used as an environmentally friendly way to convert chitin to chitosan. However, different studies have shown that CDAs are not very active on crystallin chitin used as substrate, but activity has been observed when superfine chitin is used (Huang et al., 2022; Pareek et al., 2013). For example, Martinou et al. showed that when crystalline chitin is used as the substrate for a CDA in chitosan preparation, high molar fractions of acetylation are obtained (0.995–0.905) (Martinou et al., 1995). Furthermore, Harmsen et al. indicated that, although it is possible to eliminate up to 10 % of *N*-acetyl groups from two different chitin nanofibers (α and β), it is not possible to produce chitosan by using the CDA enzyme (Harmsen et al., 2019). Similar results have been reported in other studies using CDAs from different sources (Alfonso et al., 1995; Gao et al., 1995; Harmsen et al., 2019). In this respect, it has been hypothesized that this inability to convert crystalline chitin to chitosan occurs because the enzymatic action of CDAs is unable to overcome the thermodynamic penalty of removing single polymer chains from the crystal to access all available *N*-acetyl groups (Harmsen et al., 2019).

Enzymatic deacetylation is affected by the crystallinity, size, origin, and physical properties of the substrate, as well as the molar fraction of deacetylation (Blair et al., 2006). In this sense, the pretreatment of crystalline chitin substrates seems necessary before the enzyme is added in order to improve accessibility of the acetyl groups to the enzyme, and thus increase the rate of the deacetylation reaction (see Fig. 9) (Tsigos et al., 2000). CDAs have been shown to convert soluble chitosans with a high-f_A to low-f_A chitosans (Cord-Landwehr et al., 2016; Naqvi et al., 2016). The experimental results obtained in different studies have shown that CDAs act by partially deacetylating their substrates (Wattjes et al., 2019). Furthermore, it is known that each enzyme has a different deacetylation pattern, which could generate chitosan with different chemical and biological properties, depending on the type of CDA used in the deacetylation stage (Pawaskar et al., 2019).

CDA enzymes have also been used to produce partially acetylated chitosan oligomers (paCOS). It has been shown that if chitin oligomers with a defined degree of polymerization are used, the products will possess the same *X*. These enzymes also appear to have different regioselectivities, only partially deacetylating their substrates. In some cases, the paCOS produced are mixtures of oligomers (mixtures of molecules that differ in their f_A, P_A, and *X*), but the production of fully defined paCOS with deacetylations in specific units has also been reported (Li et al., 2007). For example, CDA-NodB from Rhizobium bacteria can specifically deacetylate the non-reducing end unit of a chitin oligomer, leading to mono-deacetylated paCOS (John et al., 1993). Furthermore, it has been observed that one CDA can act on the product of the other CDA and vice versa, maintaining its regio-selectivities. In this way, it is possible to access a wide variety of fully defined paCOS by using different CDAs and their combinations (Hembach et al., 2017).

Although enzymatic modification of chitin and chitosan has a high cost, it has important advantages as it allows for the production of specific molecular species, easy control of the deacetylation process, and no unwanted by-products (Kaczmarek et al., 2019a). However, the results of enzymatic deacetylation have only been obtained on a laboratory scale and are not commercially available.

5.3. Enzymatic depolymerization

As abovementioned, physicochemical behavior and biological activities are related to the molecular structure of chitin and its derivatives (Kulikov et al., 2014). For example, it has been described that chitosan oligomers have a lower antimicrobial activity than chitosan polymers (No et al., 2002). However, the plant strengthening activities of chitosan polymers are more commonly exerted by their oligomeric degradation products (Kaku et al., 2006). Although the production and characterization of specific and uniform paCOS remain a challenge, the development of enzymatic methods proposes a tool that allows the production of specific paCOS (Kaczmarek et al., 2019a).

To date, the production of chitosan oligomers with defined P_A has been approached by organic synthesis and chemical depolymerization. However, these methods are usually expensive, and while the former results in low yields, the latter requires the use of acids to finally obtain mixtures of paCOS or chitosan derivatives instead of chitosan oligomers (Aly et al., 2001; Vårum et al., 2001). An alternative for the hydrolysis of chitosan is the use hydrolases such as chitinases (EC 3.2.1.14) and chitosanases (EC 3.2.1.132), which can catalyze the hydrolysis of glycosidic bonds. However, they have different substrate specificity, hydrolyzing chitin and chitosan, respectively (see Fig. 10) (Jaworska, 2012). Chitinases are capable of hydrolyzing the GlcNAc-GlcNAc bonds. In the case of chitosanases, they have been classified into three main classes according to their substrate specificities. Class I corresponds to chitosanases that can hydrolyze GlcNAc-GlcN and GlcN-GlcN bonds; class II corresponds to those that can only hydrolyze GlcN-GlcN bonds; and class III corresponds to those that can degrade GlcN-GlcNAc and GlcN-GlcN bonds. More information on these enzymes and their mechanisms of action can be found in the review of W. Jung and R. Park (Jung & Park, 2014).

Although using enzymatic depolymerization is only possible to

obtain oligomers with random P_A, the ranges of X and f_A can vary by modifying the substrate and the enzyme used. For example, when using chitosanase in a digestion with low f_A polymers, relatively small oligomers are produced, whereas the obtained oligomers will be larger when digesting with high f_A polymers. However, the opposite occurs when using the chitinase enzyme. Low X chitinase products tend to have a higher f_A than high X products, while the opposite is observed for chitosanase (Cord-Landwehr et al., 2020).

Although the enzymatically produced paCOS are mixtures, even after chromatographic purifications in terms of X and f_A, they will also be moderately defined concerning their P_A. This occurs due to specificities of hydrolases when interacting with the GlcNAc or GlcN units on the chitosan polymer chain. As a result, the chitosan polymers will preferentially cleave at specific sequences (Heggset et al., 2009). However, these treatments may have a limitation because the range of fully defined paCOS produced is minimal (Munandar et al., 2023).

A new alternative for the enzymatic production of oligosaccharides is the use of hydrolytic enzymes acting in reverse. In some enzymes, muteins lacking hydrolytic activity have been modified and called glycol-synthases. These enzymes retain their capacity for transglycosylation in the presence of chemically activated transition state-mimicking substrates. The use of transglycosylating chitinases has been useful for obtaining chitin oligomers (Aam et al., 2010; Mallakuntla et al., 2017; Martinez et al., 2012). However, these enzymes have not been useful to obtain paCOS (Tanabe et al., 2003). Furthermore, the hydrolysis of chitin and chitosan has also been reported through various enzymes such as peptidases, pepsin, papain, lysozymes, lipases, and pronases, which act differently depending on the type of substrate (Muzzarelli et al., 1999; Poshina et al., 2018). However, their hydrolysis products have not been analyzed in detail in terms of its P_A (Cord-Landwehr et al., 2020).

These last enzymes have a chitosan hydrolyzing activity comparable to chitosanases. They are used mainly to obtain low-molecular-mass chitosans to decrease their viscosity. Some results show a decrease in viscosity of raw chitosan in 69 % (cellulase), 80 % (pepsin), 82 % (Lipase), 42 % (papain), and 61 % (bromelain), using these enzymes (Roncal et al., 2007). The hydrolysis by cellulase showed the most significant reduction of chitosan molecular weight from 371.5 kDa to <6 kDa, in contrast to chitinase (11.2 kDa) and lysozyme (22.2 kDa) (Lin et al., 2009). Enzyme mixtures such as pectinase, cellulase, and papain have also been used, obtaining chitosan fractions with a molecular weight of 34 and 14.6 kDa (Tishchenko et al., 2011). Cellulase has excellent hydrolysis capacity, making it a better option versus enzymes such as chitinases and chitosanases, which imply high costs and poor supply channels, limiting the transformation of raw chitosans to low-molecular-mass chitosans at an industrial level (Gonçalves et al., 2021).

The production of chitin oligomers is also widely studied due to its numerous applications. Chitin oligomers have shown more beneficial health effects than chitin due to their antioxidant, anti-inflammatory, antitumor, and antimicrobial properties (Kumar et al., 2020; Liu, Ma, et al., 2021; Zhang, Xiong, et al., 2021). By enzymatic methods it is possible to obtain two types of GlcNAc-based oligosaccharides from chitin: *N*-acetyl chitooligosaccharides (*N*-acetyl COSs) and lacto-*N*-triose II (GlcNAcβ1-3Lactose, LNT2). For this hydrolysis, proper selection of chitinolytic enzymes is critical for obtaining high yields. Enzymes such as cellulase, lysozyme, lipase, papain and pepsin have been reported in GlcNAc-based oligosaccharide production, however, the yields reported with these enzymes are very low (Guo et al., 2022). Chitinolytic enzymes are the major glycoside hydrolases (GHs), which degrade chitin to produce GlcNAc-based oligosaccharides, including chitinases and *β-N*-acetylhexosaminidases (Guo et al., 2022; Kaczmarek et al., 2019b). Obtaining *N*-acetyl COSs can be carried out from chitin by chitinases via hydrolysis activity, *N*-acetyl COSs can be used to produce LNT2 or *N*-acetyl COSs with higher X, this is achieved by the action of chitinolytic enzymes via transglycosylation activity.

Although the enzymatic production of these oligomers has shown good results due to the high crystallinity of chitin, the use of pretreatment methods has been shown to improve the efficiency of enzymatic production in chitin hydrolysis processes. The crystallinity of pretreated chitin decreases considerably with respect to untreated chitin, which improves the accessibility of the enzyme to the chitin substrate and the efficiency of hydrolysis. Different pretreatment techniques have given varied results in improving the hydrolysis rate. Ultrasound and chitinase treatment for chitin conversion showed a 27.9 % improvement in hydrolysis rate when operated at an intensity of 25 W/mL for 20 min (see Section 6.4) (Hou et al., 2020). In addition, the use of green solvents such as ionic liquids have been shown to be highly efficient in the pretreatment of chitin. A study reported by Xu, et al., double-chitinase hydrolysis of crab shell particles (CSP) pretreated by ionic liquids was investigated. Two types of chitinase from *P. pasadenensis* CS0611 and 8 ionic liquids were used in the study, with the pretreatment with [BMIm]Ac achieving a hydrolysis rate above 90 % (see Section 6.2) (Xu et al., 2019). Other reported methods for pretreatment of chitin with high hydrolysis efficiencies include ball milling (see Section 6.1), and

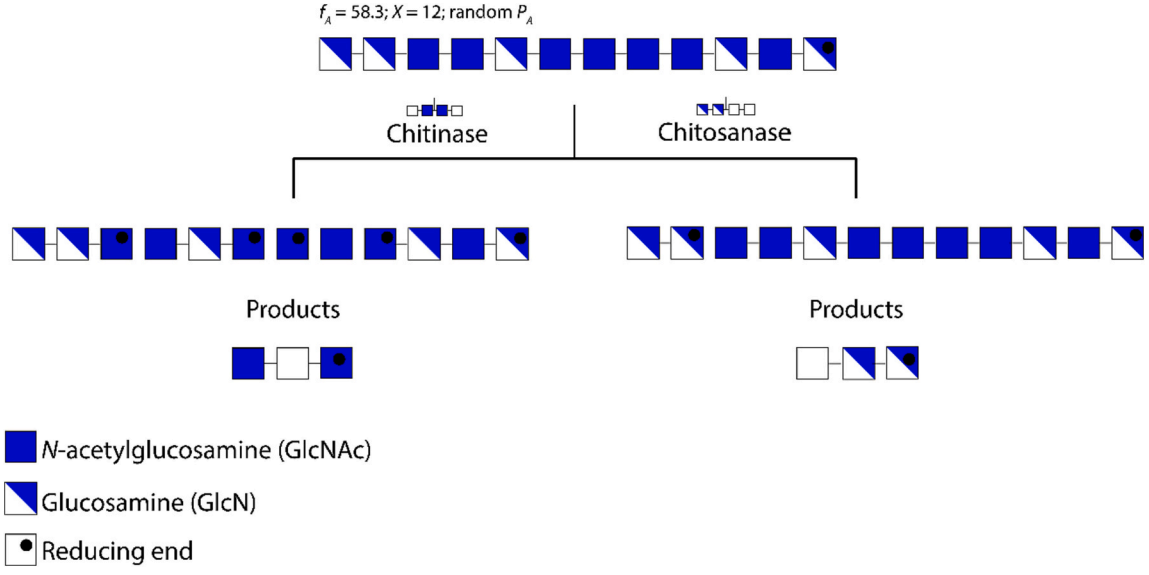


Fig. 10. Schematic diagram of the mechanism of action of chitinases and chitosanases (in accordance to (Cord-Landwehr et al., 2020)).

bacterial fermentation (Xing et al., 2023; Zhang, Wei, et al., 2018).

Enzymatic methods using chitinolytic enzymes (chitinases and *β-N*-acetylhexosaminidases) are preferred over chemical methods to produce GlcNAc-based oligosaccharides due to their mild conditions and high hydrolysis efficiencies. Chitinolytic enzymes with catalytic mechanisms are well known, especially chitinases of the GH 18 family and *β-N*-acetylhexosaminidases of the GH 20 family, which have been widely used to produce the two types of GlcNAc-based oligosaccharides, mainly through hydrolysis and transglycosylation activities, respectively (Adrangi & Faramarzi, 2013; Liu et al., 2023; Oyeleye & Normi, 2018). A more detailed review of the production of *N*-acetyl-*D*-glucosamine-based oligosaccharides from chitin is founded in the review by Liu, et al. (Liu et al., 2023).

6. Alternative processes

6.1. Mechanochemical method

Heinicke defines mechanochemistry as "the branch of chemistry dealing with the chemical and physic-chemical changes of matter due to the influence of mechanical energy" (Ranu & Stolle, 2015). Introducing mechanical energy through grinding bodies in the transformation processes of shellfish waste to obtain chitin or its derivatives can be the answer to a more efficient heterogeneous conventional method. This means amplifying the reactor concept to mills and talking about solid-state reactions. The most obvious advantage is the omission of solvents during the reaction, that in the case of the transformation of shellfish waste into chitin and its derivatives, implies a reduction of a 1/3 part (approx.) of the water used in the conventional method (Hajiali et al., 2022) — the water is only used in the filtration steps. On the other hand, there is an important reduction in the reagents consumed (e.g., $Bases_{(s)}$ or $Acids_{(s)}$) since the molar/mass ratio between raw-materials: reagents are lower than the conventional method (Akopova et al., 2010; Chen et al., 2017; Hajiali et al., 2022; Prut & Zelenetskii, 2001). In addition, energy consumption is considerably reduced because the bulk process is only carried out in one unit operation (the mill) without heating and in a shorter time. Based on the calculations of Di Nardo et al., there is a reduction of a 1/9 part of the energy consumed compared to the conventional method (Di Nardo et al., 2019).

Early reports related to the effect of mechanical forces induced by mixer/shaker mills, planetary mills, twin-screw extruders, and Bridgman anvils on chitin, and chitosan appeared in the mid-1990s (Akopova et al., 1996; Fukumori et al., 1998; Rogovina et al., 1998). The reports principally focused on the structural changes and size reduction suffered by these materials. Regarding structural changes, mechanochemical methods always provoke amorphization and depolymerization of the chitin/chitosan samples. If the process does not involve the addition of other reagents (e.g., acids, bases, clays, or enzymes), the *N*-acetyl and amine groups are retained on the chains. The main chemical change is the breaking of glycosidic bonds. As a particularity, when these amorphous materials are washed with water, recrystallization occurs (see Section 2.4), and other solvents (e.g., ethanol, methanol, etc.) are used when washing is required (Akopova et al., 1996; Chen et al., 2015; Margoutidis et al., 2018a; Mogilevskaya et al., 2006; Prut & Zelenetskii, 2001; Zhang, Zhang, Jiang, & Xia, 2012; Zhang, Zhang, & Xia, 2012). The primary purpose of amorphization and particle size reduction is to increase the reactivity of chitin/chitosan against reagents (e.g., acids, bases, clays, or enzymes). This gain in reactivity could be beneficial to achieve its subsequent chemical transformation in one-phase (solid) and two-phase (suspension) reaction systems. In this sense, a study conducted by Nakagawa et al. revealed that the increase in reactivity that follows amorphization results in the total degradation of chitin to GlcNAc, suspending amorphized chitin in a buffer solution of chitinase. The authors indicated that the degradation ratio of amorphized chitin was 10 times larger compared to that achieved with crystalline chitin (Nakagawa et al., 2011). In conclusion, smaller chitin particles allow a larger reaction surface area, while a lower crystallinity allows for better mixing at the molecular level, resulting in a higher degree of chemical conversion.

The mechanochemical transformation of shell-waste/chitin/chitosan varies according to: (i) chemical, (ii) technological, and (iii) process aspects. For example, the final degree of amorphization of milled chitin in a planetary ball mill at ambient temperature depends on the milling material (ii), e.g., zirconia, stainless steel, polytetrafluoroethylene, etc.; chitin filling fraction (ii); number of balls (ii); diameter of the balls (ii); frequency of rotation (iii); number of milling cycling (iii); and milling cycling time (iii). For deacetylation, the type of catalyzer (i) (e.g., bases, acids, and CDA enzymes), and catalyzer:chitin ratio (i) also need to be considered. Table 2 relates these aspects to the product characteristics for the most recent research.

Interestingly, the mechanochemistry approach offers a viable opportunity to obtain oligomers or small polymers of chitin or chitosan with more narrow distributions of their X and f_A (Chen et al., 2017; Rogovina et al., 1998). There is evidence that when middle to high milling energies (frequencies of rotation) are used, particle size and molar mass of the materials tend to be more homogeneous as milling time passes (Chen et al., 2017; Fukumori et al., 1998; Rogovina et al., 1998). At these levels of energy, the amorphization is enough for a mass transfer of the catalyzer (e.g., NaOH) at a molecular level, allowing a more homogeneous chemical transformation (e.g., deacetylation) of the chains (Mogilevskaya et al., 2006). Another advantage of the mechanochemical approach is that very low amount of water is required to transform shellfish waste, chitin, or chitosan. In fact, it has been demonstrated that this allows for higher enzymatic conversions than conventional bulk solution systems (Hammerer et al., 2020; Kaabel et al., 2020; Lee et al., 1996; Liu, Jiang, et al., 2020; Nakagawa et al., 2011; Therien et al., 2019; Yong-Hyun & Chan Park, 1999). A system of 200 mg chitin, 0.7 % w chitinase, 500 μL water, milled at 30 Hz for 30 min, followed by aging at 45 °C for 7 days, resulted in 6.1 mg GlcNAc compared with the 3.7 mg GlcNAc obtained in a bulk solvent system at the same chitin/enzyme ratio, reaction time, and temperature (Therien et al., 2019). Kaabel et al. have indicated that "this emergent mechanoenzymatic reaction strategy is able to take advantage of the stereospecificity, regio and stereoselectivity, as well as renewability of enzymes, while avoiding bulk solvents, offering the opportunity to control the direction of the reaction, bypassing reactant solubility issues, and enabling reactions with water-sensitive substrates or products (Kaabel et al., 2020)." In summary, the possibility to develop an "industrial processes that allow obtaining inexpensive and specific chitinous materials (with low dispersity in their X, f_A, and a non-random P_A) (Giraldo et al., 2023)."

6.2. Solvent extraction method

6.2.1. Ionic liquids

Ionic liquids (ILs) are organic salts consisting of a large organic cation and a smaller organic or inorganic anion. ILs are regarded as 'designer solvents' because of the numerous possible combinations of cations and anions (Tang et al., 2012), which allow adjusting their intrinsic characteristics such as viscosity, ionic conductivity, density, polarity, solvating power, hydrophilicity, and hydrophobicity (Hulsbosch et al., 2016; Shi & Wang, 2016; Silva et al., 2020), making them suitable solvents for a wide range of polar and non-polar compounds. The main advantages of ILs are their ease and safety of handling compared to mineral acids, higher thermal stability compared to the most commonly used organic solvents, a wide range of acidity and basicity, and solubility in the range of organic solvents (Morais et al., 2020; Singh, 2019). Apart for their unique physicochemical properties and versatile reactivity, the synthesis of ILs is relatively simple. However, their toxicity when exposed to the environment remains unclear (Ventura et al., 2014).

ILs can be used to extract different polysaccharides, including lignin

Table 2
Operation and response parameters in mechanochemical methods applied to chitin and chitosan.

Evaluated operation parameters	Mill type	Planetary ball mill (PM 100 CM; Retsch); Pulverisette P7 Premium Line Planetary Ball Mill (Fritsch) – PL11 PFI Ball Mill (PFI Mill)	Planetary ball mill (Fritsch, Pulverisette 6)	Pulverisette P7 Premium Line Planetary Ball Mill (Fritsch)	Ball mill SPEX SamplePrep 8000 M	Ball mill mixer mill MM 400	Retsch MM 400 Planetary Ball Mill	Makabe Giken Converging Ball Mill
	Grinding Bowl Material	Zirconium oxide (ZrO_2)	Alumina	Zirconium oxide (ZrO_2)	Stainless Steel 440C	Copper, aluminum, brass, tungsten carbide, zirconium, stainless steel, PTFE or poly (methyl methacrylate) (PMMA)	Polytetrafluoroethylene and stainless steel or zirconium	Zirconium
	Grinding vessel volume [mL]	125; 45	250	45	65		30	1000
	Grinding Balls Material	Zirconium oxide (ZrO_2)	Alumina	Zirconium oxide (ZrO_2)	Stainless Steel 440C	Copper, aluminum, brass, tungsten carbide, zirconium, stainless steel, PTFE or poly (methyl methacrylate) (PMMA)	Zirconium or steel	Zirconium
	Grinding ball diameter [mm]	10; 5	5	5	6.35–12.7	9.5	10	10
	Number of grinding balls	45		100	70–72		1	
	Filling degree of the grinding container				18 %			
	Ratio mass fed/mass of balls [g/g]		4.9/100	(0.4 g of chitin and 0.4 g of base) 1:1		0.2/2		20/271
	Rotational speed or operating frequency [rpm]	650–6000	500	700	5000	1770	1770	500–800
	Operation or reaction time [hours]	4	6	2	6	0.5	1.5	0.5–2
	Operation and rest cycles	Grinding for 20 min and rest for 10 min; Grinding for 10 min and resting for 5 min	Grinding for 10 min and rest for 10 min	Grinding for 10 min and resting for 5 min				
	Operation temperature [K]	323.95	298		338.15		323.15	
Response parameters	Crystallinity index [%]	28–89			51	51.4–62.3	52.4	
	Degree of acetylation and deacetylation [%]			70	75–85		98	
	Degree of depolymerization				1–5			
	Polydispersity or dispersion index			1.1				
	Reference	(Chen et al., 2015)	(Yabushita et al., 2015)	(Chen et al., 2017)	(Margoutidis et al., 2018b)	(Di Nardo & Moores, 2019)	(Di Nardo et al., 2019)	(Nakagawa et al., 2011)

from various types of lignocellulosic biomass (Kilpeläinen et al., 2007; Socha et al., 2014), cellulose, starch, hemicellulose, pectin (Morais et al., 2020), and marine polysaccharides such as agarose, chitin, carrageenan from their sources, residues or even wastes. ILs are being used with a sustainable approach that has been described as straightforward and highly efficient compared to conventional extraction methods (Mahmood & Moniruzzaman, 2019; Trivedi & Kumar, 2014). Research on the extraction of chitin from shellfish waste (crab or shrimp shells) using ILs has been led by Professor Robin D. Rogers' research group, achieving high yields. The team conducted a first study using 1-ethyl-3-methylimidazolium acetate ([C_2mim]OAc) and obtained a yield of 94 % by microwave heating at maximum power for two minutes (Qin et al., 2010). In a subsequent study, they achieved 100 % yield using hydroxylammonium acetate ([NH_3OH][OAc]), working at a temperature of 90 °C for two hours (Shamshina et al., 2016). The ILs used allowed the direct dissolution of chitin without deproteinization and demineralization (Yi et al., 2020). Furthermore, Rogers and his co-workers applied ILs on an industrial scale, which allowed them to found the company 525 Solutions (Morais et al., 2020). In addition, Tolesa et al. conducted a study on the production of chitin and chitosan from shrimp shells using 1-allyl-3-methylimidazolium bromide (AMIMBr) at 120 °C for 24 h and obtained yields of 12.6 % (Setoguchi et al., 2012). Additionally, Setoguchi et al. reported yields of 14.8 % using di-isopropyl-ethyl ammonium acetate ([DIPEA][Ac]) at 110 °C for 36 h (Tolesa et al., 2019). Both studies performed the demineralization and precipitation of chitin by treating the supernatant with citric acid.

ILs are also used to dissolve chitin or chitosan to obtain materials such as gels, films, hydrogels, sponges, nano and microspheres, or fibers, and these materials are mainly used for biomedical applications (Frantz et al., 2020; Singh, 2019). The following ILs were used for the solubilization of α-chitin or chitin extracted from marine wastes: [C_2mim]Br; [C_2mim]I; [C_2mim]OAc; [C_4mim]OAc; [C_2mim]Lac; [C_4mim]I, obtaining solubility percentages (w/w) of: 12 %; 11 % (Jaworska et al., 2017); 10 % (Mantz et al., 2007); 9 % (Jaworska et al., 2017) and 8 % (Jaworska et al., 2018), respectively.

6.2.2. Deep eutectic solvents

Deep eutectic solvents (DES) are arrangements made from a eutectic mixture of Lewis or Brønsted acids and bases that may contain a variety of anionic and/or cationic species. In contrast, ILs are formed from systems consisting of a discrete type of anion and cation (Smith et al., 2014). DES have physicochemical properties almost identical to those of traditional ionic liquids, e.g., low vapor pressure, high thermal and chemical stability, and low melting point (Zdanowicz et al., 2018), with advantages such as biodegradability, low toxicity, easier preparation, and relatively lower price. DES are usually formed by mixing a hydrogen bond donor (HBD) and a hydrogen bond acceptor (HBA) at a given temperature (Smith et al., 2014). The transparent solution formed has a significantly lower melting point than its components. DES are currently used to extract and dissolve chitin, as their strong intermolecular hydrogen bonds increase the possibility of breaking the robust network of hydrogen bonds within chitin (Vicente et al., 2020). For sustainability, natural deep eutectic solvents (NADES) have been designed. They consist of primary plant metabolites (sugars, carboxylic acids, amino acids) found in the tissues of living organisms and play an essential role in cellular processes (Duan et al., 2018). In addition, NADES can produce chitin in a one-step, fast, and environmentally friendly way to minimize the consumption of water and toxic chemicals in deproteinization and demineralization steps (Vicente et al., 2021).

DES or NADES have been used to extract chitin from marine residues. Bradić et al. used choline chloride and Lactic acid (1:1), with a reaction time of 6 h, temperature of 70 °C and a solid/NADES ratio of 1:50, and obtained a yield of about 19 %, a relative-purity of 98 %, a crystallinity index of 91 %, a $\overline{M}_w$ of 125 kDa and a molar fraction of acetylation of 0.89 (Bradić et al., 2019). In addition, Zhu et al. used choline chloride and malonic acid (1:2) in a 1:7 solid/DES ratio, reaction time of 2 h and temperature of 50 °C, achieving a chitin yield of 20.63 % (Zhu et al., 2017). Furthermore, Saravana et al. also worked with choline chloride and malonic acid (1:2), using the same reaction time, a higher temperature of 80 °C, and a higher solid/DES ratio of 1:25, achieving a yield of 19.41 %, a molar fraction of acetylation of 1.0778 (an overestimated value), a relative crystallinity index (CrI) of 87.59 % and an $\overline{M}_w$ of 79 kDa (Saravana et al., 2018). Another study conducted by Zhao et al. demonstrated the efficacy of obtaining chitin from shrimp shell using choline chloride and (ChCl)-glycerol (1:2) and citric acid (10 %), which was used in the demineralization stage. In this research, microwave heating was used for 9 min and a solid/DES ratio of 1:20, obtaining chitin with a f_A of 0.913, a $\overline{M}_w$ of 2.9×10^5 and a yield of 22.5 % (D. Zhao et al., 2019). On the other hand, Huang et al. used choline chloride and malic acids (1,1), with microwave heating with irradiation of 700 W for 9 min and a solid/NADES ratio of 1:20, obtaining chitin with a CrI of 70.91 % (Huang et al., 2018). It should be noted that the use of microwaves significantly reduces the time required to obtain the product, resulting in the same yields as the traditional method used with DES.

By using the methods described above, minerals can be recovered using NaOH (Bradić et al., 2019), while extracted proteins can be recovered by separations (Zhao et al., 2019). It has been determined that there can be a DES reuse of 3 cycles. After that, DES efficiency drops significantly. Vicente et al. used choline chloride-Lactic acid (1:1) for homogeneous deacetylation. For this, they solubilized the chitin in a reaction time of 2 h, temperature of 50 °C and a 1:10 ratio, and then applied a 30 % w concentration of NaOH at 80 °C for 18 h, resulting in chitosan with a f_A close to 0.2 and a CrI of 57.1 % (Vicente et al., 2020). Furthermore, a study with alkaline, neutral, and acidic DES evaluated temperature and reaction time conditions, in a solid/DES ratio of 1:50, and obtained the best results with choline chloride-malic acid, which showed chitin with a f_A of 0.6 and a CrI of 85 %, with a reaction time of 24 h at 120 °C (Vicente et al., 2021). It should be noted that the main applications of DES and NADES are dissolutions (Sharma et al., 2013; Vicente et al., 2020, 2021), obtaining chitin films or nano-fibers (Mukesh et al., 2014), and they have also been used to obtain chitin *O*-acylated directly from shrimp shells (Feng et al., 2019).

6.3. Microwave method

Microwave irradiation directly activates most of the molecules present in the reaction, being governed by two significant mechanisms, dipolar rotation or ionic conduction, and causing a rapid temperature rise. For this reason, this method can replace conventional heating (El Knidri et al., 2018). Advantages of microwave irradiation include high efficiency (Nguyen et al., 2017), low solvent consumption (Shams et al., 2015), reduction of extraction time (Ajavakom et al., 2012; Chen et al., 2007; Nguyen et al., 2017), higher yields, energy saving, and reduction of side reactions (El Knidri et al., 2019). These effects were previously observed when using ionic liquids, deep eutectic solvents, and enzymes.

Microwave heating is mainly used to obtain chitosan. In this sense, Cheng et al. compared extraction times to obtain chitosan by the conventional and microwave heating methods, using a concentration of 50 % w NaOH and a solid/liquid ratio of 1:30, and determined that the extraction time decreases by 1/3 when using microwave irradiation, in addition to obtaining chitosan with a f_A of 0.2614 (Cheng et al., 2020). On the other hand, Alishahi et al. compared chitosan extraction time using the autoclave and microwave techniques, and reported a f_A of 0.07 using autoclave for 3 h and a f_A of 0.05 using microwave irradiation for 35 min at 400 W (Alishahi et al., 2011). Likewise, a study conducted by Sagheer et al. used microwave irradiation and a NaOH concentration of 45 % w in the deacetylation stage, resulting in a f_A of 0.07 after 15 min of microwave heating (Sagheer et al., 2009). Furthermore, Neama et al. investigated the best conditions to obtain chitosan, analyzing NaOH concentration, microwave power, solid/liquid ratio, and reaction time.

The authors determined that the optimal conditions are a NaOH concentration of 50 % w, 800 W, 1:25 ratio solid/liquid, and a reaction time of 10 min. The results revealed a maximum yield of chitosan of 67.4 %, with a f_A of 0.07 (Omara et al., 2019). Similarly, Samar et al. obtained chitosan in a reaction time of 10 min with a power of 1400 W and a NaOH concentration of 50 % w. The authors also analyzed the influence of particle size of the chitin used and determined that a 60 mesh size was optimal to obtain a f_A of 0.0481 and a yield of 90.12 % (Samar et al., 2013). Finally, Sahu et al. obtained chitosan with a f_A of 0.147, applying a power of 900 W for 5.5 min (Sahu et al., 2009).

Other studies have focused on the microwave technique for the extraction of chitin and chitosan. For example, El Knidri et al. used shrimp waste for the demineralization process, using 3 mol/L HCl, in a solid-liquid ratio of 1/10, with a reaction time of 8 min and a power of 500 W. For deproteinization, they used a NaOH concentration of 10 % w and a solid-liquid ratio of 1/10, first heating for 5 min at 160 W and then for 3 min at 350 W, while deacetylation was carried out with a NaOH concentration of 50 % w and a solid-liquid ratio of 1/20, for a reaction time of 8 min at 350 W, obtaining a f_A of 0.1727 (El Knidri et al., 2016). The results revealed that chitosan from shrimp waste was obtained in approximately 24 min in total. Later, a scale-up study highlighted the usefulness and cost-effectiveness of scaling up chitosan production, using low NaOH concentrations, with a reaction time of approximately 12 min, obtaining a low molar fraction of acetylation (0.234), high molecular weight (165 kDa), and high crystallinity (El Knidri et al., 2019).

6.4. Ultrasonic method

Compared to the conventional method, advantages of the ultrasound method include high efficiency, capacity to process large quantities of raw materials, and easy access to equipment (Tsaih & Chen, 2003). This method is based on the shock of the ultrasonic wave, which generates small cavities that collapse and produce high temperatures (~5000 K) and pressures (~1000 bar), a phenomenon known as cavitation (Baxter et al., 2005). This can cause polymerization or depolymerization reactions by temporarily dispersing aggregates or permanently breaking covalent bonds in polymer chains (Kjartansson, Zivanovic, Kristbergsoon, & Weiss, 2006). Furthermore, cavitation enhances the penetration of the acidic or alkaline solution, resulting in high extraction quickly (Cui & Zhu, 2021).

Ultrasonication has been used for the extraction of different polysaccharides, such as starch (Wong et al., 2019), cellulose (Marim et al., 2021), pectin (Sengar et al., 2020), and chitin. In chitin extraction, ultrasound is used in the demineralization and deproteinization stages since it can eliminate non-chitinous material in half the time of that required by the conventional method (Suryawanshi et al., 2020). Singh et al. obtained chitin from squid pens (from *Loligo formosana*) using the ultrasonic method in the deproteinization stage, while demineralization was omitted because squid pens have low ash contents. The authors determined that the optimum conditions to obtain a chitin yield of 34 % was by using a 750 W ultrasound, operated at a frequency of 20 kHz, a sonication time of 41.46 min, with an amplitude of 69 %, a solid:solvent ratio of 1:18 with a NaOH concentration of 1.0 mol/L, and maintaining a temperature range between 30 and 40 °C (Singh et al., 2019). Amador-Mendoza et al. extracted chitin from shrimp shells (from *Penaeus sp*), applying ultrasonic waves at 750 W, 20 kHz, with a sonication time of 120 min, an amplitude of 40 %, and a solid:solvent ratio of 5:100 using water as the solvent, obtaining a chitin yield of 75.3 % (Amador-Mendoza et al., 2016). Kjartansson et al. extracted chitin from shrimp shells (from *Pandalus borealis*) (Kjartansson, Zivanovic, Kristbergsson, & Weiss, 2006), and freshwater prawn shells (*Macrobrachium rosenbergi*) (Kjartansson, Zivanovic, Kristbergsoon, & Weiss, 2006). In both studies, the ultrasonication method was used to demineralize and deproteinize the shells. For the demineralization, chitin shells were suspended in a 0.25 mol/L HCl solution in a solid: solvent of 1:40 ratio at 40 °C. Of the 4 h of reaction time, sonication was performed at 41 W/cm^2 for 1 h. In the deproteinization, the powders were suspended in a 0.25 mol/L NaOH solution in a solid:solvent 1:40 ratio at 40 °C, and sonicated for 1 h. The former study reported a chitin yield of 11.4 % (Kjartansson, Zivanovic, Kristbergsson, & Weiss, 2006), while the latter reported 5.02 % (Kjartansson, Zivanovic, Kristbergsoon, & Weiss, 2006). On the other hand, Vallejo-Domínguez et al. analyzed the effect of ultrasonication time in the deproteinization stage for chitin extraction. For a sonication time of 30 min, the authors obtained a chitin yield of 71.538 %; this chitin was subsequently deacetylated by the traditional method, reaching a chitosan yield of 11.074 %, with a f_A of 0 and a $\overline{M}_w$ of 9.68 g/mol. For a sonication time of 35 min, they reached a chitin yield of 53.494 %, a chitosan yield of 12.959 %, with a f_A of 0.06865 and a $\overline{M}_w$ of 60.51 g/mol (Vallejo-Domínguez et al., 2021).

Few studies have reported on the use of ultrasonic irradiation to obtain chitosan from α-chitin. For instance, Birolli et al. studied the production of chitosan by applying high intensity ultrasound to α-chitin suspended in 40 % w NaOH, at a power of 20 kHz, an amplitude between 75 and 95 %, and temperature between 100 and 105 °C (Birolli et al., 2016), and obtained a 100 % chitosan yield by submitting the chitin sample to irradiation and non-irradiation periods of 45 min and 30 min, respectively. The chitosan obtained presented a viscosity average molecular weight of 1.1×10^{-5} g/mol. When the system was initially irradiated for 5 min followed by 25 min of non-irradiation period, and then it was further irradiated for 60 min, a 99.9 % chitosan yield with a $\overline{M}_v$ of 1.3×10^{-5} g/mol was obtained (Birolli et al., 2016).

6.5. Electrochemical method

Electrochemical treatment of chitin sources was patented in 1996 by Maslova and Kuprina as an alternative to the conventional method within a large-scale technology (Maslova, Kuprina, & Bogeruk, 1996). Since then, this Russian research group has studied the optimal recovery of chitin and other value-added products from diverse raw materials through a complete electrochemical method involving controlled delipidation, deproteinization, demineralization, and decoloration stages. Shellfish waste biomass is smoothly disintegrated using a diluted aqueous NaCl solution, enabling the in-situ generation of an alkaline and acidic extractive medium inside an electrolytic cell. This method allows obtaining high-molecular-mass chitin due to the mild treatment conditions (Kuprina et al., 1999; Kuprina et al., 2001; Kuprina et al., 2008; Maslova, 2002).

The physicochemical principle of this gentle procedure is based on the Chlor-alkali process (O'Brien et al., 2005). Here, redox reactions evolve in a particular electrolytic cell, where anode and cathode are separated by a cation exchange membrane (see Fig. 11) (Seko, 1976). Water electrolysis occurs in the cathode cell producing H_2 and OH^-, also known as the catholyte solution, whereas Cl^- ions of NaCl are oxidized to produce Cl_2 in the anode cell. Subsequent chlorine electrolysis on the anode surface leads to the formation of H^+ and HClO, the anolyte solution (Gluhchev et al., 2015; Karlsson & Cornell, 2016).

Compared to other activation methods, such as ultrasound or microwave irradiation, electrochemical activation of aqueous solutions is more favorable because of the relatively long lifetime of water metastable states, causing reproducible variations on redox potential (ORP), pH, surface tension, electrical conductivity, dielectric constant, and other physicochemical parameters. In the anolyte solution, pH is lowered, and the ORP increases to extremely positive values (oxidizing acid). In catholyte, pH increases, and the ORP is lowered to extremely negative values (reducing base). In conventional chemical solutions, the combination between pH and ORP cannot be simulated thermodynamically. Therefore, anolyte and catholyte with identical pH characteristics to those of strong acids and alkalis do not show corrosive properties, representing a substantial reduction in the use of hazardous chemicals (Bakhir, 1992; Leonov et al., 1999; Prilutsky & Bakhir, 1997).

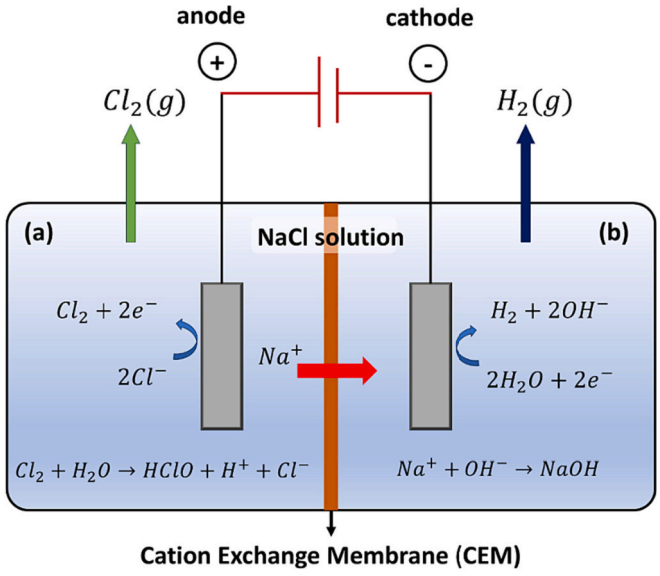


Fig. 11. Electrochemical activation of sodium chloride to prepare anolyte (oxidizing acid) and catholyte (reducing base) solutions. Conditions: a) ORP: +1100 mV, pH = 1–3, Cl_2 (*aq*): 400 ppm b) ORP: −800 mV, pH = 11–13, NaCl: 0.5–1 % w/v.

Depending on the nature of chitin sources, electrochemical parameters have been well established to ensure the elimination of proteins and minerals through catholyte and anolyte treatment, respectively, with minimal destruction of resulting products. In comparison with other methods, electrochemical decoloration is performed in an activated NaCl solution 1 % w/v and is achieved by highly active oxidants such as Cl_2, ClO_2, ClO^-, HClO, Cl, O_3, O, H_2O_2, which evolve in the anode chamber (Maslova, 2002).

On the other hand, electrochemical technology significantly increases the profitability of chitin production due to the accurate processing of raw materials and the possibility of recovering all its valuable components (Maslova, 1999; Maslova et al., 1995a, 1995b; Maslova et al., 1997, 1995a, 1995b). Maslova et al. developed and manufactured a module type of an original design for the first time, in which the anodic and cathodic treatment of wastes from cutting shell-containing marine and freshwater crustacean provides lipids, protein hydrolysates, nutrients, and mineralized residue along with high-quality chitin polymers in the same production cycle (see Table 3). An adaptation of the method also leads to the isolation of industrial value chitin complexes (Maslova, 2000; Maslova, 2000b, 2001). In a previous work, Kuprina et al. described a procedure for electrochemical preparation of *Gammarus pulex* shellfish in order to obtain a chitin-mineral complex (chisitel) with a high sorption capacity of heavy and transition metals, as well as chitin (chitinel) (Kuprina and Vodolazhskaya, 2002; Kuprina et al., 2002; Kuprina et al., 2006; Maslova, Krasavtsev and Bogeruk, 1996). The methodology involved the deproteinization of raw material in an aqueous salt suspension (particle size $d \leq 5$ mm) performed in a temperature-controlled catholyte at a density of 300–500 A/m^2 for 30–40 min and pH > 12. Afterward, the product was subjected to a second deproteinization stage with suitable degreasing by the effect of anionic surfactants (2 % w) under optimal conditions of time (30 min) and temperature (82 °C). The obtained chisitel material was then demineralized and decolorized in the anolyte for about 12–18 h at pH ≤ 2, giving chitin electrolyzed material (chitinel). Later, Vysotskaya et al. applied the original electrochemical procedure to recover chitin-glucan complex (CGC) from *Pleurotus ostreatus* Basidial Fungus (Vysotskaya et al., 2009). However, some modifications were required to ensure the complete deproteinization of raw material: a preliminary soaking in NaCl 1 % w/v and an increase of cycle-number ($n > 2$) for catholyte treatment without surfactants. Spectroscopic FTIR data of CGC product suggested the presence of amide groups of chitin and CHO groups in CGC structure, probably due to partial cleavage of glycosidic bonds of glucan or, to a lesser extent, the oxidation of some hydroxyl groups.

Gaseous products O_2 and H_2 are also formed during electrolysis in a non-explosive proportion. When NaCl is used as an electrolyte, gaseous halide products are captured by a typical spray scrubber. Nevertheless, one drawback of this method is the evolution of chlorine gas, which is highly toxic, corrosive, and out of proposals for a sustainable process (Tennakone, 1989). In this sense, Kuprina et al. evaluated in parallel the extractive power of diluted Na_2SO_4 solutions, obtaining the same results for all the stages.

Recently, Nowacki et al. developed a novel electrochemical procedure to isolate chitinous 3D scaffolds from *Aplysina aerophoba* marine demosponge (Nowacki et al., 2022; Nowacki, Stępniak, Machałowski, et al., 2020). The approach improved time efficiency and reduced environmental impact by using Na_2SO_4 aqueous solution to convert NaOH and H_2SO_4 (Holze et al., 1994; Jörissen & Simmrock, 1991). The initial electrolyte concentration (Na_2SO_4) for every step was 1.9 mol/L, and the initial temperature for both anolyte and catholyte was 40 °C. Furthermore, the following alternating steps between the catholyte and anolyte treatment were set: (i) decellularization (cells lysis and lipids degradation), (ii) decalcification (demineralization), and (iii) main

Table 3
Comparison of conventional and alternative methods for chitin/chitosan preparation from shellfish waste sources.

General aspects		Methods						
		Chemical	Biological	Solvent	Microwave	Ultrasound	Electrochemical	Mechanochemical
Allow additional value-added products	Lipids	No	Yes	Yes	No	No	Yes	No
	Minerals	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	Proteins	No	Yes	Yes	No	No	Yes	No
	Pigments	No	Yes	Yes	No	No	Yes	No
Critical operational considerations	Pretreatment	No	Yes	Yes	No	No	No	No
	Energy consumption	High	High	Low/ Medium	Medium	Medium	Low/Medium	Low
	Condition	Harsh	Sterile/Soft	Soft	Harsh	Harsh	Soft/Medium	Soft/Medium
	Wastewater	High/ Corrosive	High/Neutral	Low/ Neutral	Medium/ Corrosive	Medium/Corrosive	Low/Corrosive	Low/Corrosive
	Production cycle	Hours	Days-Weeks	Minutes-Hours	Minutes	Minutes	Hours-Days	Minutes-Hours
	Yields	High-Medium	Medium-Low	Low	High	High	High-Medium	High
	Cost	Medium	High	High	High	High	Low	Low
References		(Berroci et al., 2022; Bonner & Kearney, 2020)	(Gómez-Ríos et al., 2019; Jung & Park, 2014)	(Vicente et al., 2020; Yi et al., 2020)	(El Knidri et al., 2016; El Knidri et al., 2019)	(Suryawanshi et al., 2020; Vallejo-Domínguez et al., 2021)	(Maslova, 2002; Nowacki et al., 2022)	(Di Nardo et al., 2019; Kaabel et al., 2020)

deproteinization, desilicification, and depigmentation. In some cases, the cycle between step (i) and step (iii) were repeated over 10 times (Nowacki et al., 2022).

The Na_2SO_4 electrolytic method is also appropriate for chitinous biomass with low mineral content. Two-successive catholyte treatments were reported by Nowacki et al. for the isolation of chitin from the skeleton of the black coral *Cirrhipathes* sp. (Nowacki, Stępniak, Langer, et al., 2020). This suggested that demineralization of the sample was indeed a desilicification. OH^- ions removed the coral lipids and proteins in this step and eventually dissolved the residual silica and pigments. Based on data available in the literature, it was inferred that the extracted black pigment corresponds to a catechol-related compound, which is soluble in a highly alkaline environment.

The characteristics of isolated chitin products are mainly influenced by treatment conditions (number of cycles, time of treatment in the anode or cathode chambers, subsequent holding in the anolyte, etc.) rather than by the source of raw material (Maslova, 2002). In general, the chitin obtained by electrochemical method (chitinel) is a high molecular weight biopolymer in the form of flakes of up to 2 mm in size or a white powder without taste and smell with an average content of: moisture 8–10 % w, ash 0.3–0.5 % w, total nitrogen 6.2–6.4 % w, main substance 85–90 % w (Maslova, 2002). Until now, there is no spectroscopic evidence of chitosan formation as a possible product of chitin deacetylation for any studied chitin source.

Chitinel is intended for use as a semi-finished product in the manufacture of chitosan and other chitin derivatives. In a low molecular weight format, chitinel has been widely used in cosmetology, medicine, and agriculture (Maslova, 2000a; Maslova, Kuprina, & Bogeruk, 1996). Regarding value-added products, protein hydrolysates and concentrates have a tremendous nutritional value and have been recommended for use in public nutrition as food additives, both in liquid and dry formulations. Reducing agents formed due to water electrolysis (H^+, H_2) can cause the breakdown of -S-S- disulfide bonds and form –SH groups in proteins. This constitutes the advantage of the electrochemical method over the traditional alkaline treatment (NaOH 1 N), which mainly breaks -C-S- links in proteins and produces side impurities in the product, which prevents further use (Kuprina, Timofeeva, et al., 2003; Kuprina, Vodolazhskaya, & Nyanikova, 2003; Nyanikova et al., 2003; Vodolazhskaya, 2001).

Although electrolysis-assisted isolation of chitin has not received much attention, it seems to be a promising sustainable alternative over conventional extraction procedures (Roberts, 2008; Younes & Rinaudo, 2015). The prototype module (4.0 × 2.2 × 2.5 m, 20 m^2 occupied area) has a production capacity of 1.5 t of chitin per year with an electric power consumption of 20 kW/h (220/380 V, 50 Hz). Additionally, a container version has been used to implement the production process in a very short time, which in turn allows the production of chitin in any region and especially in places of crustacean processing (Maslova, 2002).

Some advantages of the electrochemical method are to decrease the factor of ecological risk due to elimination of aggressive media (usage of acids and alkali), waste water neutralization, and reduction of water consumption; to increase chitin productivity using an all-in-one operational unit in a very short time; to recover chitin-complex, lipids, proteins, minerals and pigments of high industrial value; and to drive a closed-economic cycle with the self-hydrogen generation as a potential biofuel (Kuprina, Timofeeva, et al., 2003; Yang & Yan, 2018).

7. An inverse route

Chitooligosaccharides (COS) can be obtained by organic synthesis reactions from monomers of *N*-acetylglucosamine and glucosamine. Even though this process does not allow the direct exploitation of shellfish waste, samples of oligomers can be obtained without dispersion in their characterization parameters. This type of samples are a sine qua non condition for the identification of molecular configurations with biological activity (Giraldo et al., 2023). This section reviews the literature on the organic synthesis of chitooligosaccharides in a more global language (easy to understand), intending that the readers compare this route with the previously proposed ones, and they can visualize a possible complementarity between them.

7.1. Organic synthesis of chitooligosaccharides

The synthesis of COS has been increasingly studied in the last 30 years, representing a challenge of organic synthesis (Demchenko, 2008a; Kuyama et al., 1993). Their synthesis is based on "glycosylation" reaction, which involves nucleophilic displacement of the leaving group (LG) of the glycosyl donor with the hydroxyl moiety of the glycosyl acceptor, being the other groups partially or fully protected (OP). The reaction proceeds in the presence of a catalyst as a reaction promoter (see Fig. 12). Some of the drawbacks when designing the synthesis are the low reactivity of the hydroxyl group located at carbon 4 of glucosamine/*N*-acetylglucosamine monomer or the use of protecting groups, e.g. to regulate the degree of polymerization, resulting in several steps and consequently diminishing yields (Crich & Dudkin, 2001; Fittolani et al., 2021; Kim, 2022).

For COS production, the reaction occurs at the weak nucleophile's fourth carbon atom (sugar acceptors), and it often follows an S_N1 mechanism (Demchenko, 2008a). In terms of glycosyl donor groups, there are two types, those that do not participate and those that participate. Depending on the molar fraction of acetylation, the functional group (amino or acetamido group) of COS at the end of the molecule would participate in the mechanism (Demchenko, 2008a; Fittolani et al., 2021).

Fig. 13 shows the carbocation formation after interacting the leaving group with the catalyst. This secondary carbocation can be stabilized by the electrons of the oxygen ring or the oxygen of the acetamido group. However, the formation of stable derivatives (1,2-O, *N*-oxazoline) is the major problem to obtain glycoside bonds. This is why the use of an *N*-protecting group is required (Demchenko, 2008a; Yang & Yu, 2014). In this respect, the choice of the protecting group is essential to promote the formation of β-glycosidic bonds. Considering the poor donor properties of *N*-acetylated derivatives, groups with a solid electron-withdrawing nature are adequate. These include *N*-phthaloyl (*N*-Phth), *N*-trichloroethoxycarbonyl (*N*-Troc) (Enugala et al., 2012; Yang & Yu, 2014), *N*-trichloroacetyl (*N*-TCA) (Enugala et al., 2012; Yang & Yu, 2014), *N*-trifluoroacetyl (*N*-TFA), and azide N_3 (Enugala et al., 2012; Yang & Yu, 2014; Zhong & Boons, 2008a).

The glycosylation method features a new orthogonal glycosylation procedure, which consists of using two chemically different orthogonal glycosyl donors (X and Y) and two activation conditions (a and b) (see Fig. 14). A sine qua non condition of this strategy is that X must be unaffected under the activation condition required to activate the other donor (Y) and vice versa. Furthermore, X and Y must be compatible with subsequent manipulations on temporary protecting groups (Kanie et al., 1994). This synthetic route allows the obtention of well-defined COS, samples composed of molecules with only one molecular structure, i.e., with the same X, f_A, and P_A (Giraldo et al., 2023).

The manipulation of P_A was first controlled by Kawada and Yoneda (2009a, 2009b) using azido and phthalimido as salient groups; the azido group is known to be stable under several conditions. Moreover, the phthaloyl group is stable under various conditions and can usually be quickly introduced and cleaved (Fittolani et al., 2021). Therefore, both azido and phthalimido groups can be converted into free amino or acetamido groups. However, the formation of disaccharides has a low yield (26 %) (Kawada & Yoneda, 2009a). Subsequently, the disaccharide was converted to the trichloroacetimidate donor to increase the

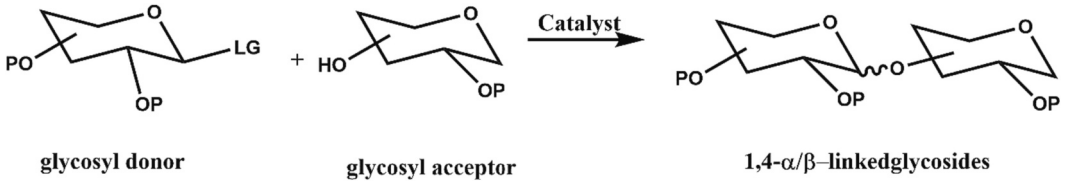


Fig. 12. Schematic representation of the general glycosylation reaction, where OP represents the protected hydroxyl group, and LG is the leaving group (Reprinted with permission from (Liu, Qin, & Ye, 2021) Copyright (2021) John Wiley & Sons).

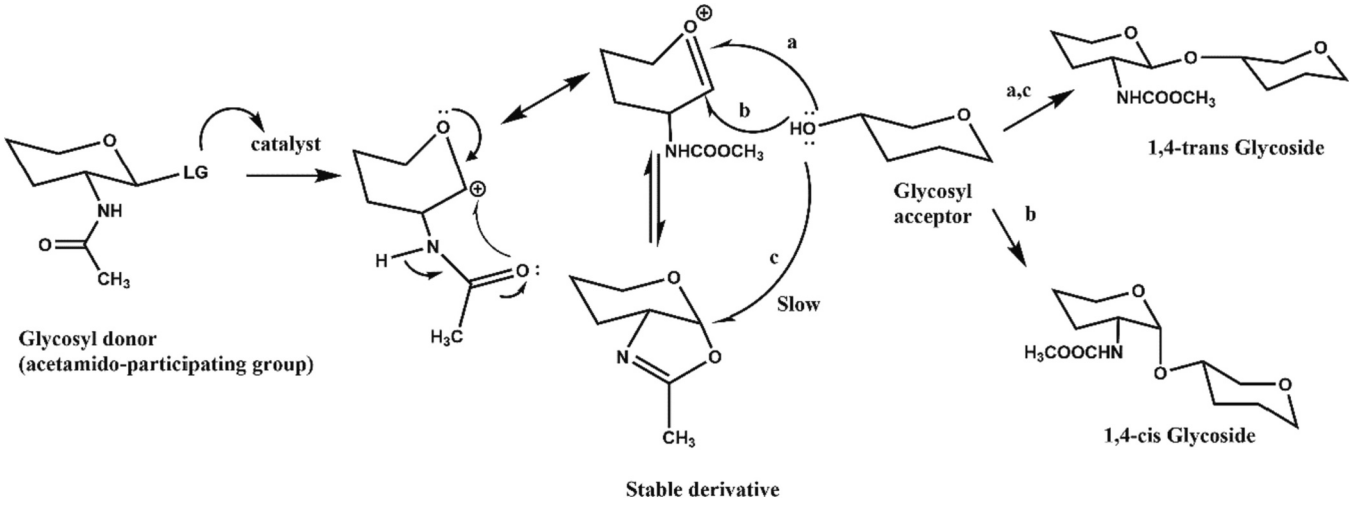


Fig. 13. Schematic diagram showing the possible mechanism of glycosylation; a,c indicates the process of formation of the 1,4-trans glycoside; b indicates the formation of 1,4-cis glycoside (Reprinted with permission from (Demchenko, 2008b) Copyright (2008) John Wiley & Sons).

reactivity and used in a [2 + 2] glycosylation, giving the tetramer an alternating *N*-acetyl pattern (yield: 74 %) (Kawada & Yoneda, 2009b).

Another strategy to prepare well-defined COS is the use of automated glycans assembly (AGA), developed by Seeberger's group (see Fig. 15) (Seeberger et al., 2022). This strategy consists of performing the glycosylation reaction between a monosaccharide and a linker attached to a solid support. Subsequently, the unreacted groups are protected to avoid unexpected reactions, and the temporary protecting group is removed to generate a hydroxyl group that acts as a glycosyl acceptor in the next step. Then, glycosylation using another glycosyl donor and protection of unreacted groups on the acceptor initiates the elongation process. The cycle of deprotection and protection is repeated until the target molecule is generated as expected. Finally, the glycan is cleaved from the solid support, and all protecting groups are removed to obtain the desired glycan. Unlike typical solution-phase glycan assembly, purification is not performed after each glycosylation on AGA. Accordingly, it is assumed that all reactions are complete with high yields and excellent stereoselectivity (Liu, Qin, & Ye, 2021).

Linkers are essential in attaching the growing glycan to the solid support. These are considered inert to the conditions used in glycosylation and deprotection. Furthermore, the glycan must be released from the linker through a chemoselective reaction after complete assembly without affecting the glycan attachment. Most of the linkers used in the AGA strategy are bifunctional structures. One end connects to the reducing end of the glycan through an ether bond, and the other connects to the solid support through an ester amide bond or carbamate (Liu, Qin, & Ye, 2021).

AGA along with orthogonality has allowed obtaining well-defined COS in *X* and P_A, but with yields between 10 and 34 % (Fittolani et al., 2021). NMR analysis and MD simulations reveal the importance of deacetylated residues because the free amino group stabilizes new geometries through protonation, promoting intramolecular interactions. Therefore, the acetylation pattern of COS is crucial because these interactions exist only in the presence of a deacetylated units GlcN (NH_3^+). Therefore, knowing the position of the deacetylated residue in the polymer is crucial to describing COS conformation (Delbianco et al., 2018; Fittolani et al., 2021; Tyrikos-ergas, Bordoni, Fittolani, & Chaube, 2021; Tyrikos-Ergas, Bordoni, Fittolani, Chaube, Grafmüller, et al., 2021; Yu et al., 2019a).

The main disadvantages of the synthetic method include the large number of steps required, low yields, and poor reproducibility, which prevents implementation on an industrial scale. Recent reports indicate that the lack of reproducibility may be associated with the different reaction temperature conditions. Seeberger et al. (2022) propose a novel AGA, carrying out reactions at different temperatures and relate it to the reactivity using thioglycosides (Seeberger et al., 2022). In general, thioglycosides are an excellent protecting group for the anomeric center and chemoselective, and they can act as a donor or acceptor in glycosylation reactions (Zhong & Boons, 2008b).

Seeberger et al. (2022) have indicated that glycosylation reactions proceed in seconds, and parallel reactions proceed when there is an increase in temperature. In addition, other studies available in the literature describe that activation energy, selectivity, and complex rate mechanisms involved in forming glycosidic bonds are related and depend on temperature (Delbianco et al., 2018; Joseph et al., 2020; Yu et al., 2019b). Therefore, experiments with better temperature control would improve reproducibility (Seeberger et al., 2022). In this sense, several factors are needed to prepare COS. The factors are structural, stereochemical, chain length, and strategy choice. Given the significant specificity of the molecules to be prepared, the complexity of the synthesis increases. Table 4 summarizes some syntheses carried out and their respective yields.

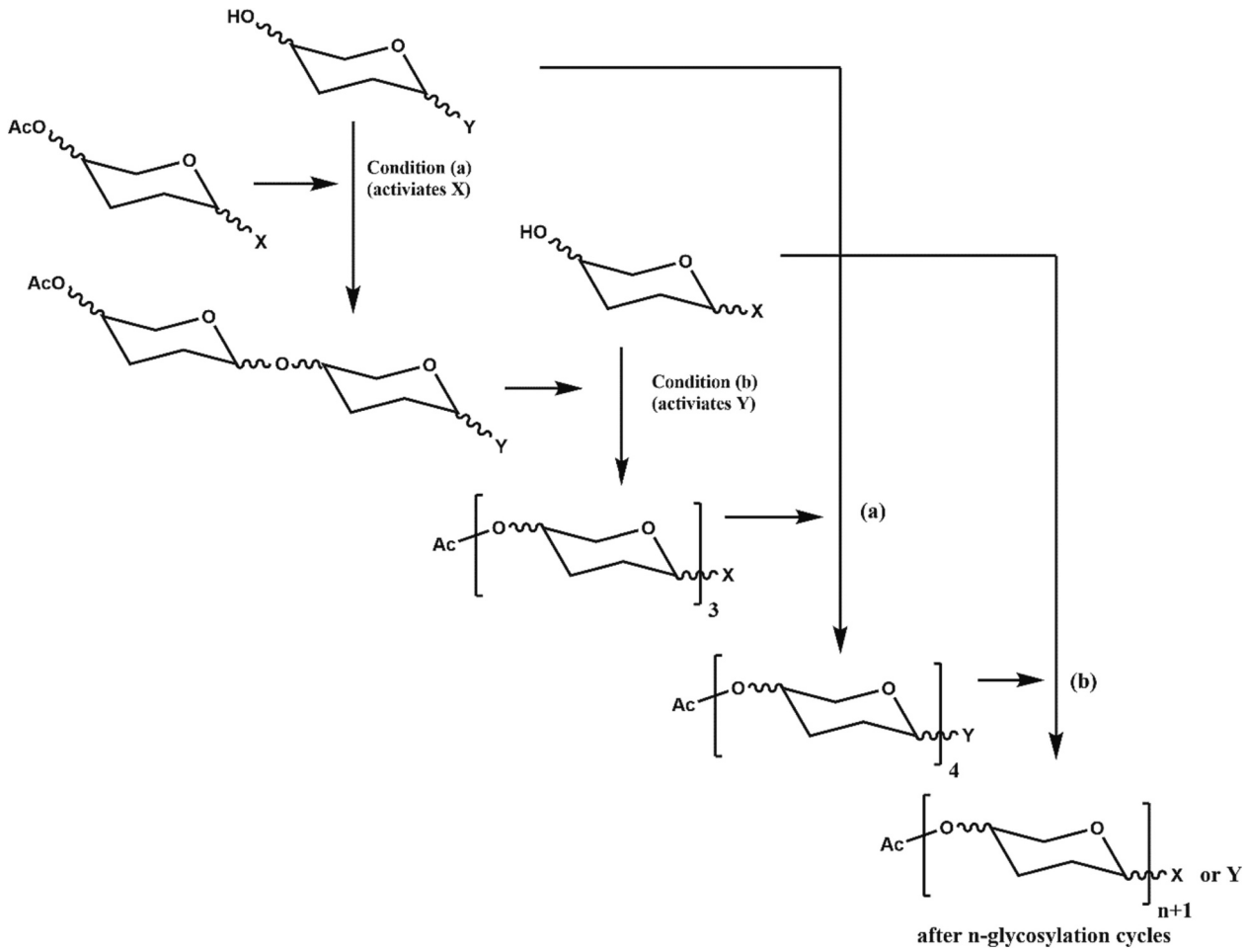


Fig. 14. Schematic representation of the orthogonal glycosylation strategy (in accordance to (Kanie et al., 1994)).

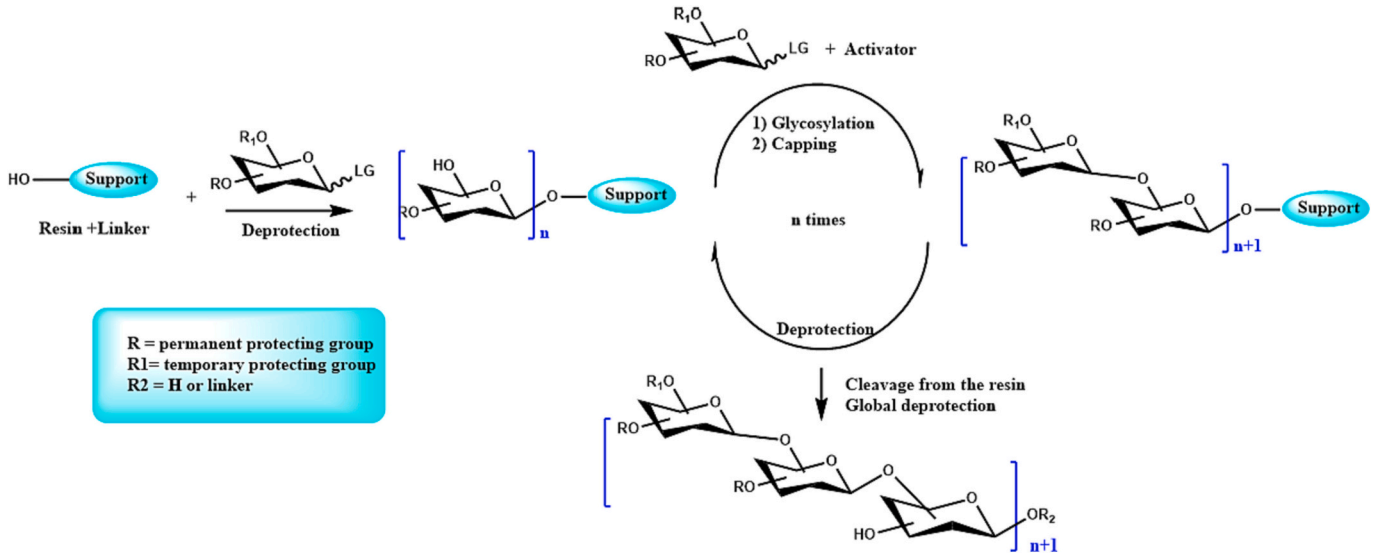
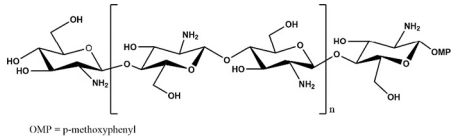
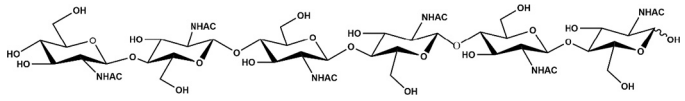
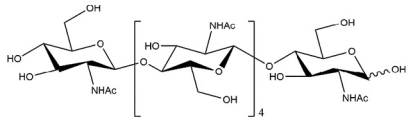
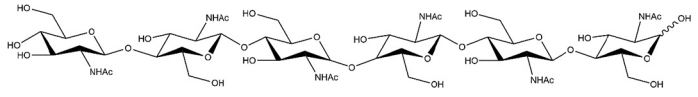
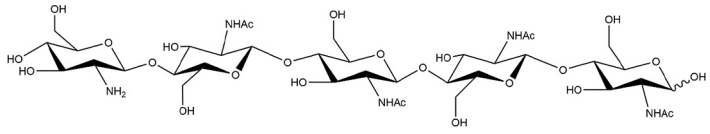
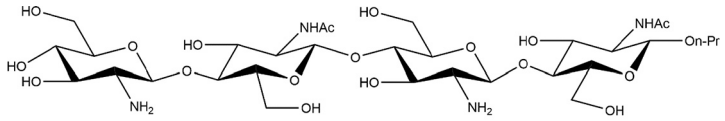
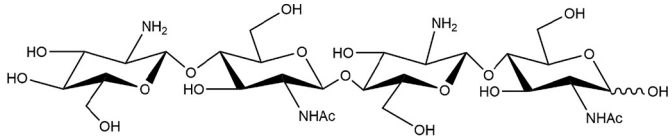


Fig. 15. Schematic representation of automated assembly of glycans (Reprinted with permission from (Liu, Qin, & Ye, 2021) Copyright (2021) John Wiley & Sons).

8. Perspective: Combined methods

According to the scientific literature volume (López-Muñoz et al., 2023), market reports, and commercial products that involve the words chitin and/or chitosan (Lárez-Velásquez, 2023), it is clear that these biomaterials already represent an important commodity in the global market. The Grand View Research consulting company informed a chitosan market size of USD 10.88 billion in 2022 and a compound annual growth rate (CAGR) of 20.1 % from 2023 to 2030. Its demand is mainly derived from the sectors of waste-water treatment,

Table 4
COS synthesized by organic synthesis methods.

Chitooligosaccharides (COS)	*X*	f_A	P_A	Yield[a]/ Steps[b]	Reference
 OMP = p-methoxyphenyl	1 to 5	0	–	60 to 85 % 10 steps	(Kuyama et al., 1993)
	6	1	–	70 % 14 steps	(Aly et al., 2001)
	6	1	–	52 % 8 steps	(Yang & Yu, 2014)
	6	1	–	34 % AGA*x*6[c]	(Fittolani et al., 2021; Tyrikos-ergas, Bordoni, Fittolani, & Chaube, 2021; Tyrikos-Ergas, Bordoni, Fittolani, Chaube, Grafmüller, et al., 2021)
	5	0.8	0	67 % 6 steps	(Despras et al., 2014)
 n-Pr = n- propyl	4	0.5	2	55 % 6 steps	(Bedini et al., 2013)
	4	0.5	2	72 % 6 steps	(Fittolani et al., 2021; Kawada & Yoneda, 2009b)

[a] The yield corresponds to the last step (the current global yield is low).
[b] Number of steps to prepare COS.
[c] Number of cycles of AGA.

pharmaceutics, cosmetics, biomedical, food & beverage, and others (e.g., agriculture) (Research, G. V, 2024). This scenario implies that these materials have applications in almost all the most critical sectors of the global economy.

The next step to consolidate the presence of these chitinous materials in the market is to transit to more specific products (i.e., biomaterials with a low dispersity in their

X and f_A, and a non-random P_A). According to recent studies, two approaches could conduce to achieve this goal: (i) the *in vivo* synthesis of defined and pure oligomers through microbial production (Weyer et al., 2022), and (ii) the precise transformation (highly controlled) of chitinous materials from shellfish waste through alternative methods. In the authors' opinion, the first approach will happen in the long term at an industrial level and potentially be a disruptive innovation. However, the alternative methods reviewed have significant advantages that make them very attractive in both economic and environmental aspects in the short and middle term (Islam et al., 2023; Junceda-Mena et al., 2023; Lv et al., 2023; Mohan et al., 2022, 2020; Yadav et al., 2019). Its combination could answer the "sustainable consumption and production goal (SDG 12)" proposed by the United Nations by valorizing shellfish waste more efficiently, decreasing the consumption of energy, water, and reagents (Giraldo et al., 2023). One example of that is the postulated process in Fig. 16, which is born from identifying the advantages and disadvantages of chemical, biological, and alternative methods.

The combination of electrochemical and mechanochemical methods is expected to reduce the amounts of reagents (NaOH and HCl), energy, and water consumed in 1/10, 1/4, and 1/3 parts compared to the chemical method, respectively (Di Nardo et al., 2019; Hajiali et al., 2022; Kuprina et al., 2002). Furthermore, it is expected that amorphization of the waste before electrochemical extraction will facilitate the processes of deproteinization, decoloration, and demineralization. On the other hand, there is evidence (Di Nardo et al., 2019; Di Nardo &

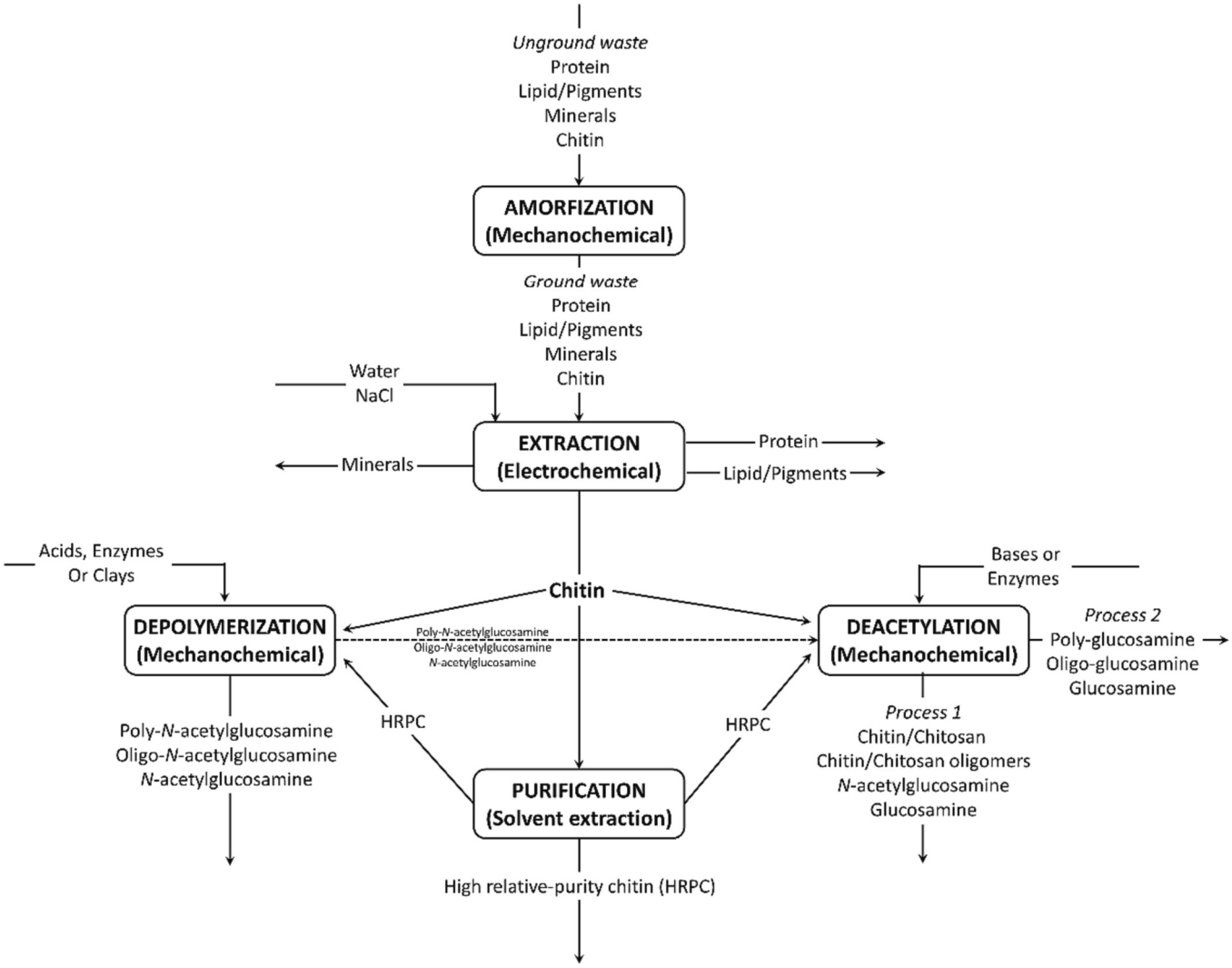


Fig. 16. Schematic diagram showing a base process combining alternative methods for extracting and modifying shellfish waste components. Processes 1 and 2 refer to partial or total deacetylation, respectively.

Moores, 2019; Margoutidis et al., 2018a) that depolymerization and/or deacetylation of chitin through the mechanochemical method results in chitins and/or chitosans with greater monodispersity in their molar fraction of acetylation and degree of polymerization, as well as non-random acetylation patterns. The latter would allow obtaining macromolecules or oligomers with defined biological activities or known modes of action in cells depending on their chemical structure (Basa et al., 2020; Cord-Landwehr et al., 2020; Dey & Ghosh, 2020; Wattjes et al., 2019, 2020). In short, materials of third generation are obtained (Nano3Bio, 2017), i.e., highly defined materials in terms of their properties and functionalities, which allow refinement of existing products and the creation of new market opportunities.

Based on everything described above, the proposed process would result in greater profitability compared to that of the conventional method since it is possible to obtain additional marketable products such as proteins, lipids/pigments, and minerals and add greater commercial value to the chitinous products obtained due to their high specificity. Additionally, this process would allow obtaining chitinous products of technical or analytical grade (HRPC) that can be used in many fields, including pharmaceutical, food, agricultural, water treatment, etc. Major profitability will result in greater competitiveness of the chitinous materials-based products, which is necessary to compete against synthetic-based products, almost all related to non-renewable resources. This will allow faster transit to a more sustainable global market of commodities (Giraldo et al., 2023).

The sustainable use of chitinous materials from shellfish waste will depend on reducing energy, water, and reagent consumption in their process production. The reduction in these items will result in a lower impact on (i) human health, (ii) ecosystems, and (iii) resources (see Section 4). Additionally, it will also depend on an integrated quantitative analysis of their environmental (LCA), social (S-LCA), and economic (*E*-LCA) impacts (Kusnadi et al., 2024).

Readers can consult other promising alternative processes in the literature (Borić et al., 2018; Devi & Dhamodharan, 2018; Lee et al., 1996; Yang et al., 2019; Zhang, Feng, et al., 2018).

CRediT authorship contribution statement

Juan D. Giraldo: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Data curation, Conceptualization. **Yadiris García:** Writing – original draft, Data curation, Conceptualization. **Myleidi Vera:** Writing – original draft, Data curation, Conceptualization. **Karla A. Garrido-Miranda:** Writing – original draft, Data curation, Conceptualization. **Daniela Andrade-Acuña:** Writing – original draft, Data curation, Conceptualization. **Kelly P. Marrugo:** Writing – original draft, Data curation, Conceptualization. **Bernabé L. Rivas:** Data curation, Conceptualization. **Mauricio Schoebitz:** Writing – review & editing, Data curation, Conceptualization.

Declaration of competing interest

The authors declare no conflicts of interest.

Data availability

Data will be made available on request.

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