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*Perspective*

# Green Routes for the Development of Chitin/Chitosan Sustainable Hydrogels

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**Abstract:** The eco-sustainable use of materials derived from agricultural and food processing waste will represent one of the most stimulating challenges shortly. Chitin and chitosan are two remarkable examples of how molecules with high added value can be extracted from food waste, such as crustaceans' shells, fungi, mollusks, etc. This Perspective summarizes the current state of knowledge about chitin extraction, chitosan production, and hydrogel formation, highlighting the environmental critical steps in the common route (use of strong acids and basis, toxic solvents, and not eco-friendly crosslinkers). At the same time, promising green alternatives are described and analyzed. Examples are the employment of NADESs or DESs (such as choline chloride: urea or choline chloride: organic acids mixtures) for chitin extraction and dissolution, use of citric acid both in chitin extraction and hydrogel formation or utilization of natural extracts, like genipin, as green cross-linkers under mild conditions (heating at 37 °C for 12 h). In particular, this perspective aims to provide a stimulating basis for the development of processes based on the recycling and reusing of chemicals, during the different preparation steps, in line with "system chemistry" and "circular economy" principles.

**Keywords:** hydrogels; food-waste recycling; chitin; chitosan; green processes

## 1. Introduction

One of the consequences of the increasing world population is food waste increment. A report from the Food and Agriculture Organization (FAO) estimated that 1/3 of agriculture production is discarded [1], corresponding to 1.6 Gtonnes of "primary product equivalents" and 1.3 Gtonnes of the edible part of the food. This is a major problem in terms of environmental impact, not only for the waste of valuable resources but also for greenhouse gas emissions (measured in tons of $CO_2$ equivalent) linked to product disposal at the end of their life [2].

For this reason, searching for new routes for minimizing food waste is a paramount goal for scientists and policymakers. In 2008, the Environmental Protection Agency (EPA) presented a hierarchical pyramid showing the preferable strategies that should be actuated to reduce food waste [3] (Figure 1).

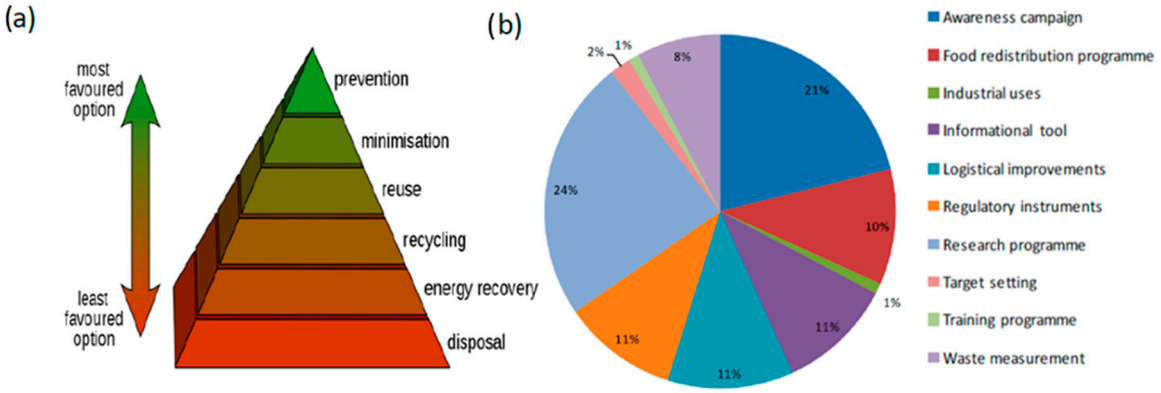


**Figure 1.** (**a**) Environmental Protection Agency (EPA) food waste recovery hierarchical pyramid; (**b**) types of strategies used to prevent food waste. Adapted and reprinted with permission from Ref. [2,3].

At present, industrial use represents only 1% of residue reduction [2].

However, in the last few years, scientific interest seems to move precisely in this direction. The so-called "Food Supply Chain Waste" (FSCW), which includes agricultural and processing residues generated in the food production/utilization chain, is considered as an exciting new frontier for recycling. Even though most of the research in this area has been focused so far on the production of biofuels, fertilizers, animal feed, and packaging, food waste can be an endless source of molecules, including proteins, polysaccharides, lipids, and so on, which can be exploited for fabricating a variety of functional materials.

For example, vegetable and fruit waste is an extremely rich source of functional molecules, including carboxylic acids (e.g., ascorbic, citric, arachidic acids), biogenic amines, phenols and polyphenols, flavonoids, carotenoids, limonene, as well as starch, pectin, cellulose, and other biopolymers [4]. In addition to calcium carbonate, eggshells contain key structural proteins (ovocleidins, ovolayxins, osteopontins), uronic, hyaluronic, and sialic acids [5]. The surplus in milk production can open the door to a viable extraction of casein and amyloid proteins [6].

A rational combination of those molecules has driven recent research towards new types of applications. In particular, environmental monitoring and remediation, which are strategic areas in sustainable development, can have major benefits [7].

For example, many toxic heavy metal cations [8] and oxoanions (chromate, arsenite/arsenate) have been captured and immobilized on either cellulose or eggshell-based membranes functionalized with carboxylic acids that can be easily extracted from vegetable and fruit waste [9]. β-lactoglobulin amyloid proteins extracted from milk have been successfully utilized to fabricate membranes that absorb arsenic ions from water [10].

Another major breakthrough in developing value-added products from FSCW is represented by food-based electronics [11]. This approach entails the selection and functional characterization of food waste, to create versatile toolkits for producing electronic components (antennas, resistors, capacitors, inductors, sensing elements, conductive and insulating substrates, etc.) that can be assembled into functional devices. A good example is described in a very recent patent [12] in which a chitin hydrogel is used into supercapacitors as electrolyte membrane.

In this perspective, we will focus our attention on chitin and chitosan, two closely-related polysaccharides, that are finding a number of applications in several fields of materials science and bio-engineering (Figure 2). In particular, we will focus on their use in the production of food waste-based hydrogels, which is one of the hottest topics in the field of food recycling for technology applications [13]. Chitin can be extracted from the exoskeleton of insects, crustaceans (more generally, arthropods) [14], from mollusks, and it is also present in the fungi cell wall [15,16] (Figure 2).

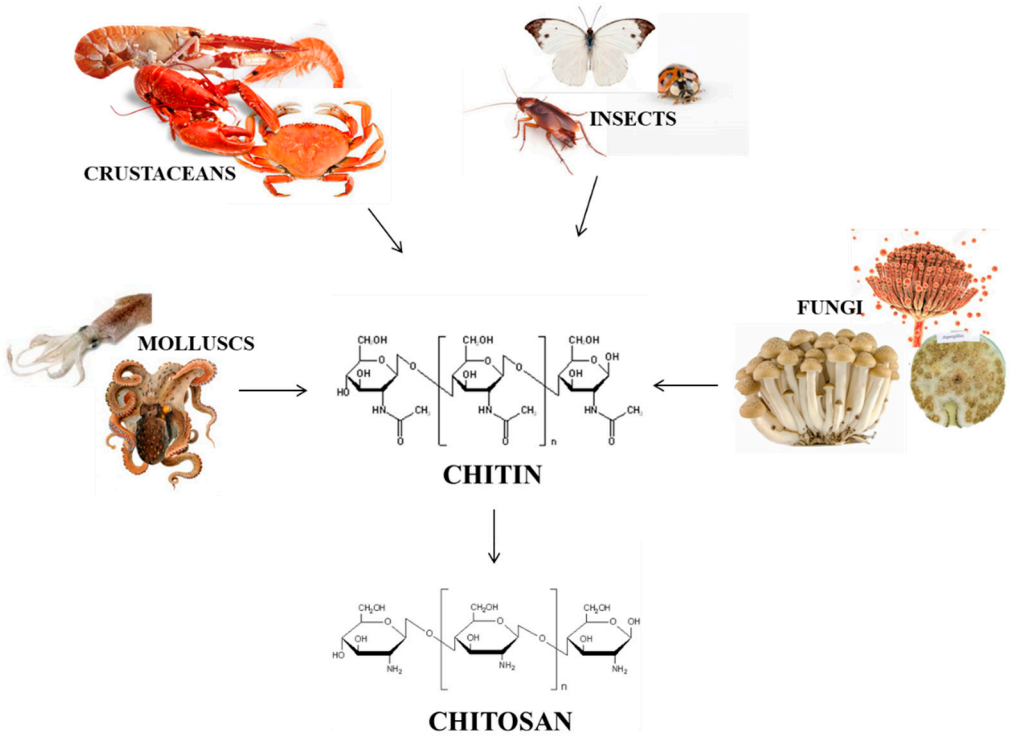


**Figure 2.** Examples of chitin and chitosan sources.

Extractions from crustaceans and fungi are the most common ones thanks to the large availability of raw materials in food waste and due to the satisfactory percentages of extraction. It is estimated that about $2.0 \times 10^{10}$ kg of chitin is produced in a year by freshwater arthropods and about $1.3 \times 10^{12}$ kg by marine arthropods [17,18].

However, this polysaccharide is insoluble in water, and this fact seriously limits its use.

On the contrary, chitosan, which can be obtained by deacetylation of chitin and is present in fungi cell walls too, is water-soluble and is protonated [19] in neutral water solutions.

The advantages of using chitin and its derivatives are linked not only to their natural abundance but also to renewability, biodegradability, and non-toxicity. For these reasons, they find application in a large variety of fields, for example as anti-bacterial and anti-tumoral agents [20] in medicine, as constituents of artificial bones and skin [21] or for drug delivery in the biomedical field [22,23] and the detection and removal of organic and inorganic pollutants for environmental remediation [24–26]. However, conventional extraction and processing of chitin and chitosan pass through the use of chemicals with significant environmental impacts, such as highly concentrated acidic and basic solutions and toxic chemical cross-linkers (Figure 3). Even if in literature it is already possible to find different reviews regarding chitin, chitosan, and their derivatives, especially as regards the analysis of their properties and their possible applications [7,27–33], in this perspective we want to bring to light the critical points that can be encountered during the process of conversion of chitin-rich raw materials into functional advanced systems. In particular, we will compare conventional routes for extraction/processing of chitin and chitosan with the most recent green alternative processes, highlighting from the point of view of environmental sustainability the drawbacks and limitations encountered during the production process following conventional well-established routes. We will propose some alternative environmentally-friendly solutions that have been already proposed in the literature and we will discuss the challenges that remain on the road towards the development of a sustainable production chain of functional hydrogels.

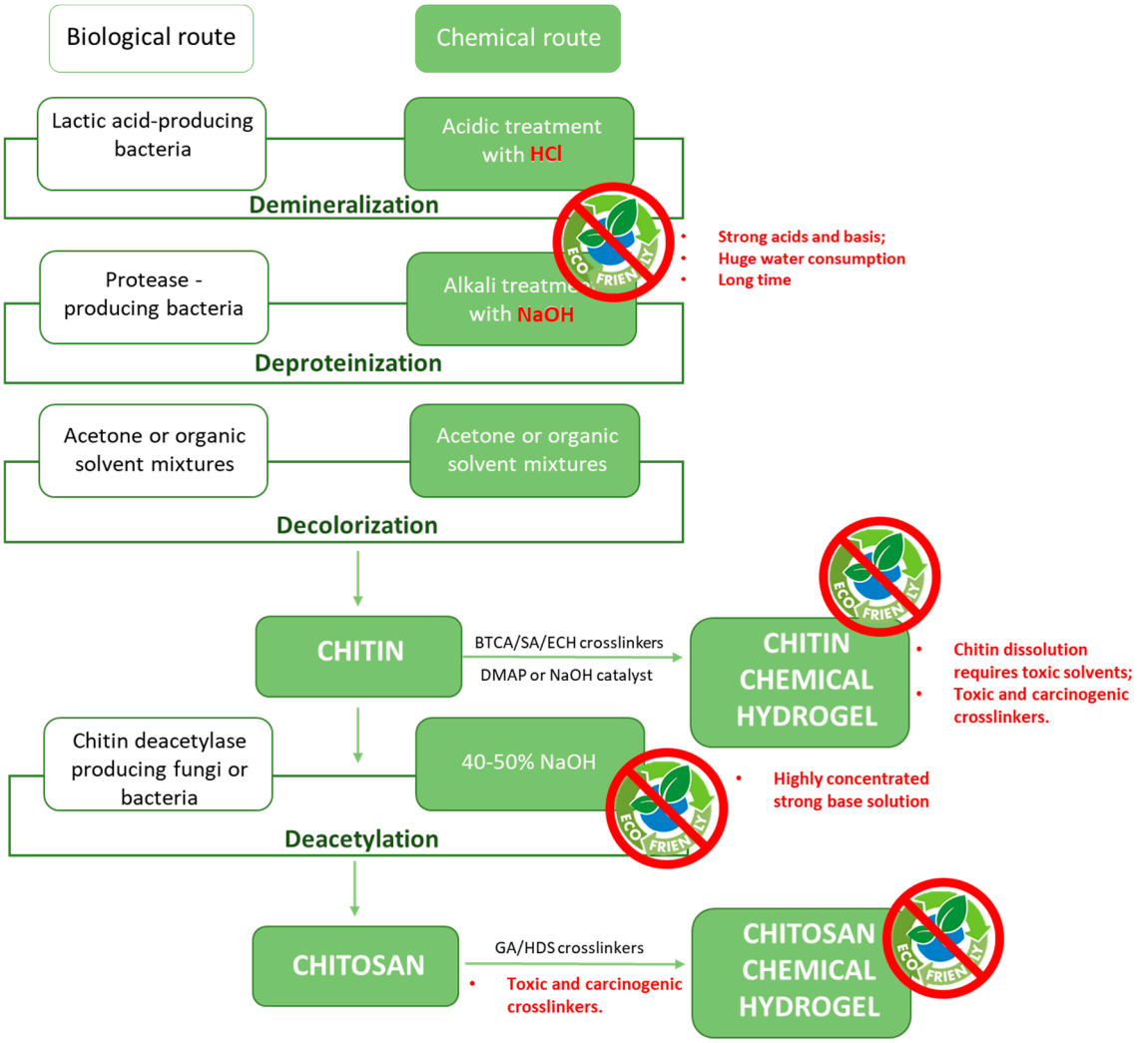


**Figure 3.** Conventional steps for chitin and chitosan extraction from crustacean shells to fabricate functional hydrogels.

## 2. Chitin Extraction

Chitin is the most abundant natural polymer after cellulose, discovered for the first time by the French pharmacist Henri Braconnot [34] in 1811. In 1843 the chemist Jean Louis Lassaigne, working on the possibility to qualitatively determine heterogeneous elements in organic compounds, demonstrated the presence of N in chitin. Nowadays, we know that chitin consists of multiple units of *N*-acetyl-D-glucos-2-amine bonded together by a β-1,4 bond. The units are the same as cellulose with the difference that chitin has an acetylamine group instead of hydroxyl.

Chitin occurs in nature as ordered crystalline microfibrils. X-ray diffraction and solid-state NMR spectroscopy revealed two main crystalline forms, namely α and β [35]. The α structure is the most common and stable form. It occurs in fungi cell walls, shrimp cells, and insect cuticle. The chains in α-chitin are arranged in anti-parallel strands, responsible for the characteristic resistance of insect and crustacean exoskeleton.

The less stable and rarer β structure is found in squid pens [36], spines and annelids; the chains are parallelly distributed, which justifies its higher degree of softness.

Finally, an additional allomorph is known, ϒ-chitin, which is probably just a variant of α-structure [37].

In its stable form, chitin presents itself as white or pale-pink, odorless, and tasteless solid. The strong intra- and intermolecular hydrogen bonds make chitin extremely aggregated, which makes it insoluble in common solvents such as water, organic solvents, and mild acidic or basic solutions [38]. This drawback represents a major limitation for many applications. Conversely, it can be solubilized in strongly polar solvents, such as hexafluoroisopropyl alcohol and hexafluoracetone, lithium thiocyanate [39], dimethylacetamide (DMA)/LiCl mixture [40], etc.

As many of these solvents are toxic or mutagenic, seeking safer alternatives is vital for this field of research. An example of a good "green" alternative for chitin dissolution is reported in the patent "Dissolution method of chitin" [41]. The protocol foresees the use of a mixture of NaOH and carbamide where chitin is held for 3–10 h. The solution is stirred at room temperature after a freeze of 7 days at −18 °C/−10 °C. The great advantage of this method is that the waste liquid can be reused more times by reducing toxic by-products, but the required dissolution time (10 h + 7 days) is too high and remains an open issue.

### *2.1. Conventional Chitin Extraction from Crustaceans*

Thanks to the abundance of waste crustacean exoskeletons (6–8 million tons are produced globally per year) [17], the extraction from crustaceans are the most common procedure, and two routes can be followed: biological and chemical methods.

Both of them involve two fundamental steps: demineralization and deproteinization.

In the biological method, demineralization occurs thanks to lactic acid produced by lactic acid bacteria. Lactic acid reacts with calcium carbonate in the crustacean exoskeleton and induces the formation of calcium lactate, which precipitates and can be removed. Moreover, lactic acid creates the necessary low pH to activate the protease present in the biowaste that is responsible for the deproteinization step [42].

Even though the biological method is eco-friendly, the use of lactic acid bacteria (for demineralization) and protease (for deproteinization) make it a time-consuming route (an average of 6 to 7 days, up to a maximum of 14 days for *Parapenaeus longirostris* [42]).

As a result, the chemical method is still the most commonly used.

The chemical method consists of different steps. In the beginning, the powdered raw material is processed with an acidic solution (generally HCl 1–2 M at 100 °C) for up to 48 h. The purpose is to remove exoskeleton mineral constituents (calcium carbonate and calcium phosphate). Subsequent alkali treatment is performed for achieving a deproteinization of the demineralized shells. Proteins are removed with NaOH (typically 1 M; 3–6 h at 65–100 °C), leading to the production of chitin [42]. An additional decolorization step can be performed, if a colorless product is wanted. Acetone, 10% $H_2O_2$ solution, NaClO, or organic solvent mixtures are used to remove the pigments. All these steps can be performed consecutively, by interposing washing steps with fresh water to achieve neutralization. Unfortunately, these washing steps require huge amounts of water, which further limits the sustainability of the whole process (Figure 3).

### *2.2. Conventional Chitin Extraction from Fungi*

Fungi could be valid alternative sources of chitin [18]. Unlike crustaceans, in this case, the demineralization step is not necessary [43,44]. The starting materials are mycelial biomass that, after a washing step, can be homogenized in a common kitchen blender and directly subjected to deproteinization.

This step is the same as that previously described for crustaceans and it can be done with NaOH 1 M.

This way provides an alternative to crustacean chemical methods characterized by several advantages: for example, unlike crustaceans, fungi are not subject to seasonal or regional variations. Moreover, fungi do not need HCl treatment, which is otherwise requested by crustaceans to achieve demineralization (Figure 4).

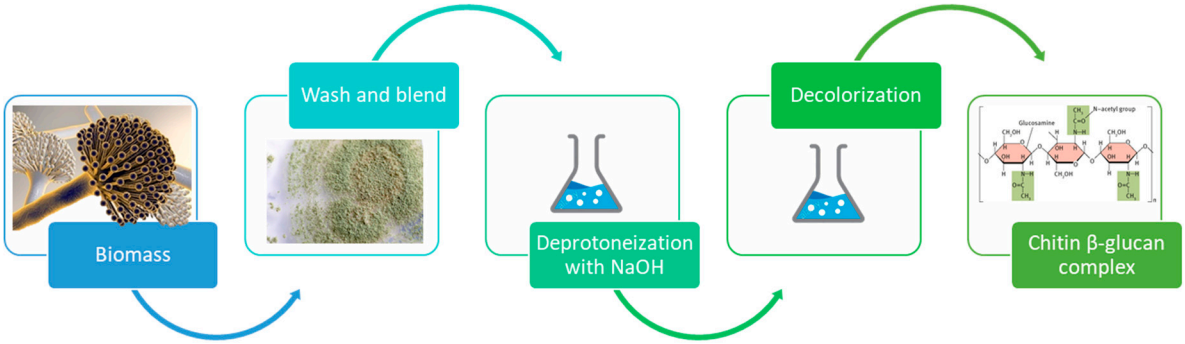


**Figure 4.** Conventional steps for chitin extraction from fungi.

However, the lower chitin content (from 9 to 42% for fungi, compared to 30–70% in crustaceans [14]) and the fact that fungal chitin sources yield to a chitin-β-glucan complex [14] rather than pure chitin, makes this way unpractical for industrial and commercial purposes.

Pure chitin could be derived from the chitin-β-glucan complex introducing an acidic treatment to degrade glucan [16], but this would mean to waste one of the main advantages taken from fungi extraction.

### *2.3. Green Extraction Methods*

The employment of strong acids or alkali solutions, huge amounts of water for neutralization steps between acid and alkali treatments, and the lengthy times are the main limitations for the reported conventional extraction protocols. In the last few years, the need to find a valid eco-friendly alternative for chitin extraction, adopting a circular economy approach, became an unavoidable challenge.

Deep Eutectic Solvents (DESs) and Natural Deep Eutectic Solvents (NADESs), in particular, have aroused scientific interest for their ability to dissolve and extracting materials from natural sources [45,46].

DESs are a mixture formed by a hydrogen bond donor (HBD) and a hydrogen bond acceptor (HBA), characterized by a melting temperature lower than the melting temperatures of each pure component, thanks to the charge delocalization between HBD and HBA [47].

For their physical properties, they can be defined as a new class of ionic liquids with the advantage of easy preparation and availability from low-cost components.

Nowadays the use of DESs in a wide range of fields is promoted by their low price, non-toxicity, low flammability, and biodegradability. DESs are already used in catalysis and organic synthesis [48,49], in electrochemistry, analytical chemistry [50], and they are getting space in dissolution and extraction procedures [51,52].

In particular, Zhao et al. reported an interesting two-steps method for chitin extraction using different DESs and citric acid, in combination with microwave heating [53] (Figure 5).

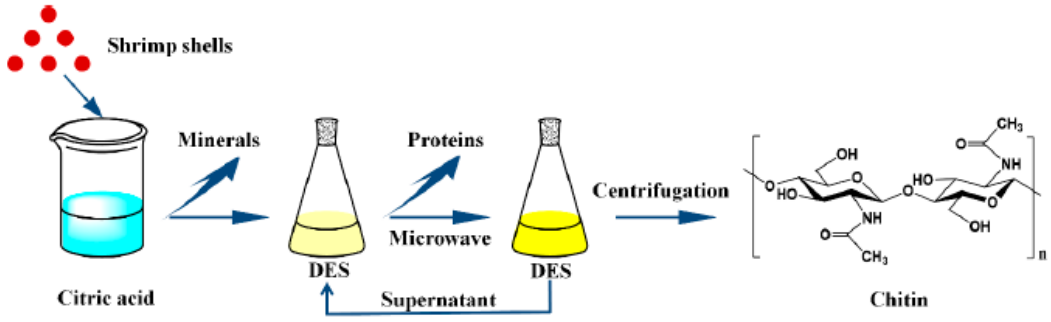


**Figure 5.** Green chitin extraction process with citric acid and Deep Eutectic Solvents (DES). Adapted and reprinted with permission from Ref. [53].

In this extraction procedure, shrimp shells, properly washed, dried, and ground, are treated with 10% citric acid for demineralization. In this case, citric acid (an edible, weak acid that can be extracted from natural sources) takes the place of HCl in the classic chemical extraction.

The step of deproteinization is performed by suspending the pretreated sample in different DES solutions and heating by means of microwave irradiation. Finally, simple centrifugation allows for the separation of chitin from DES.

Betaine hydrochloride-urea, choline chloride (ChCl)-urea, ChCl-ethylene glycol, and ChCl-glycerol mixtures have been tested as DESs and the best results in terms of yield and chitin purity are obtained in the case of ChCl:urea 1:2 mixture.

The recovered DES can be reused without purification until five times. This protocol provides a double advantage: (i) the utilization of environmental-friendly solvent (DES mixture), which, combined with microwaves, avoids the use of concentrated NaOH solution, with the possibility of recycling and reusing the extracting solvent; (ii) in the preliminary demineralization step, HCl has been substituted by citric acid, which is more eco-friendly. It can be naturally extracted from a variety of fruits and vegetables [54,55]; moreover, it can be biochemically produced by fungi like *Aspergillus niger* [56]. In this way, fungi can be at the same time source of chitin, chitosan, and citric acid.

However, it is necessary to point out that the performance of DESs during deproteinization decreases from the third cycle.

In this regard, Bradić et al. [57] developed a zero-waste method to convert shrimp shell waste into chitin. In this case, shrimp shells are solubilized in NADESs rather than in DESs. Natural DESs (NADESs) are deep eutectic solvents that are composed of two or more compounds that are generally primary metabolites of plants, i.e., organic acids, sugars, alcohols, amines, and amino acids. In this process, chitin can be separated from minerals and proteins in one step at elevated temperatures (60–90 °C). Four different NADES have been tested: ChCl:lactic acid, ChCl:malonic acid, ChCl:citric acid, and ChCl:urea, and the best results are obtained in the case of ChCl:lactic acid mixture, combined with a heating treatment at 70 °C. Once again, minerals and protein separations are achieved and NADES can be recycled (Figure 6).

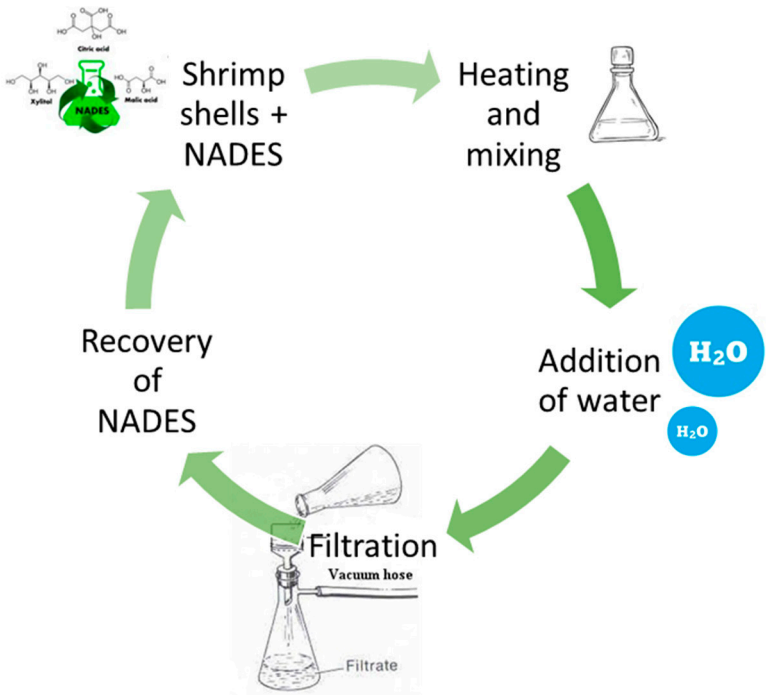


**Figure 6.** Green chitin extraction process with Natural Deep Eutetic Solvents (NADES). See text and Ref. [57] for details.

The great potential of this method compared to the classical one is that not only chitin but also the chemicals (NADESs) used during extraction can be recovered from food waste, vegetables, and natural sources.

Moreover, the possibility to recycle NADESs more times makes this approach in line with the principles of the circular economy.

Other examples of chitin extraction with DES can be found in the literature. For example, Zhu et al. [51] reported the possibility to extract chitin from lobster shell using ChCl:urea, ChCl:thiourea, and ChCl:malonic acid. Interestingly, in the case of ChCl:malonic acid (1:2) mixture mild working conditions (heating at 50 °C for 2 h) are enough, which represents the most sustainable protocol for this type of extractions. A comprehensive summary of chitin extraction procedures reported in the literature can be found in Table 1.

**Table 1.** Synoptic summary of chitin extraction methods discussed in the text.

| | Extraction Routes | Chemicals and Conditions | Open Issues |
|---|---|---|---|
| **Chitin Extraction** | Biological route [42] | 1. Lactic acid bacteria (demineralization);<br>2. Protease (deproteinization). | Time-consuming (6/7 days up to a maximum of 14 days). |
| | Chemical route from crustaceans [42] | 1. HCl 1–2 M, 100 °C, 48 h (demineralization);<br>2. NaOH 1 M, 65–100 °C, 3–6 h (deproteinization);<br>3. Optional: Acetone/10% $H_2O_2$ solution/NaClO/organic solvent mixtures (decolorization) | • Use of strong acids and bases;<br>• Use of large amount of water for washing steps;<br>• Use of harmful chemicals for the decolorization step. |
| | Chemical route from fungi [4,6,10,36,37] | 1. NaOH 1 M, 65–100 °C, 3–6 h (deproteinization);<br>2. Optional: Acetone/10% $H_2O_2$ solution/NaClO/organic solvent mixtures (decolorization). | • Lower chitin content (9 to 42% for fungi, compared to 30–70% in crustaceans);<br>• chitin-β-glucan complex achieved. |
| | "Green" route: DES [46] | 1. 10% citric acid, 6 h, 70 °C (demineralization);<br>2. Betaine hydrochloride:urea, choline chloride (ChCl):urea, ChCl:ethylene glycol and ChCl:glycerol, microwaves irradiation, 2 to 3 s pulses, 1–9 min. (deproteinization). | • Poor literature on "green" chitin extraction |
| | "Green" route: NADES [50] | 1. ChCl:lactic acid, ChCl:malonic acid, ChCl:citric acid and ChCl:urea (demineralization and deproteinization), 60–90 °C, 3–6 h. | |

## 3. Chitin Conversion into Chitosan

The most important derivative of chitin is chitosan. Chitosan is a linear polysaccharide composed of randomly distributed β-(1 → 4)-linked D-glucosamine and *N*-acetyl-D-glucosamine.

Chitosan was discovered in 1859 when the physiologist Charles Rouget discovered that by boiling chitin in KOH under reflux, it was possible to get a new product soluble in aqueous acidic media [27]. Its solubility is linked to the presence of positive charges on the amino groups in the C-2 position in D-glucosamine.

In the solid-state, this biopolymer is semi-crystalline with a wide range of polymorphs depending on formation conditions [58].

Chitosan is obtained by deacetylation of chitin when the degree of acetylation (the ratio between glucosamine and *N*-acetyl glucosamine) is lower than 50%.

In Table 2, different chitosan production methods reported in the literature are summarized and compared. The common method to deacetylate chitin requires treatment with highly concentrated (40–50%) NaOH solution for 6 h at 107 °C [14].

The concentrated alkali treatment makes this process with high environmental impact and with high cost, not to mention that it is necessarily a huge amount of water in the neutralization step. All these drawbacks limit chitosan scale-up and industrial applications.

For this reason, environmentally sustainable, inexpensive alternatives have been investigated.

An enzymatic method could seem a valid option. In this case, the deacetylation occurs thanks to the Chitin Deacetylase (CD) enzyme produced by fungi or bacteria [59]. CD can catalyze the hydrolysis of *N*-acetamido bonds in chitin. This option is eco-friendly also for mild reaction conditions. Kim et al. [60] managed to obtain chitosan at 60 °C and pH 5.5 using extracellular CD.

However, the enzymatic way might not be affordable for industrial use due to the high cost of CD and too long reaction time [61].

**Table 2.** Synoptic summary of chitosan production methods discussed in the text.

| | Extraction Routes | Chemicals and Conditions | Open Issues |
|---|---|---|---|
| **Chitosan Production** | Biological route [4] | Chitin Deacetylase (CD), 60 °C, pH = 5.5 | • High cost of CD;<br>• Time-consuming. |
| | Chemical traditional route [14,54] | 40–50% NaOH. 6 h, 107 °C | • Use of strong concentrated bases. |
| | Green route [62] | NaOH 30%, glycerol, solid:liquid ratio 1:40, 180 °C, 12 h | • High temperature for a long time. |

Liu et al. [62] tried to develop an efficient and green chemical process by using glycerol as a reaction solvent at 180 °C, enabling to lower the NaOH concentration needed for the deacetylation reaction. Glycerol is a recyclable, stable, green solvent that can be obtained as a by-product of biodiesel (Figure 7). Thus, the idea to use it for producing chitosan offers the opportunity to show a perfect example of circular economy and by-product recycling.

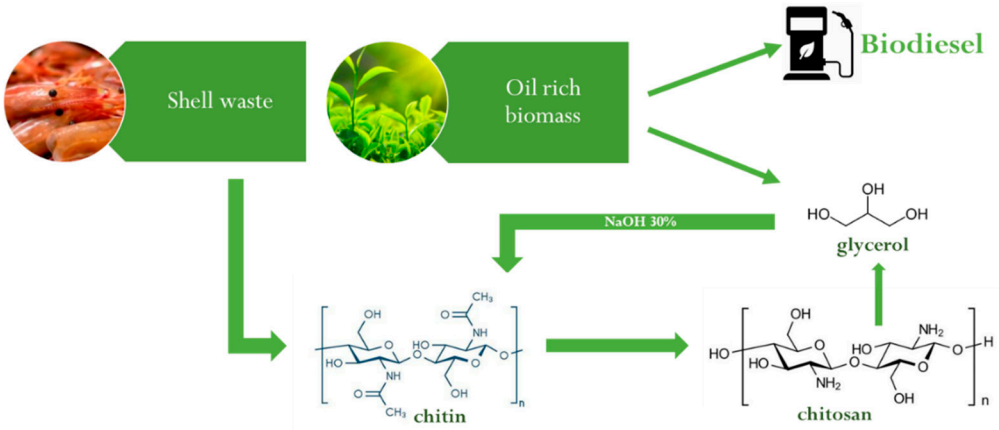


**Figure 7.** Optimized green procedure to deacetylate chitin in chitosan. See text and Ref. [62] for details.

The optimized procedure involves the treatment of chitin with 30% NaOH and glycerol, keeping a 1:40 chitin/glycerol ratio. During the process, 1% of water was added to avoid the polymerization of glycerol catalyzed in an alkaline environment.

This new deacetylation route allows, at the same time, to obtain chitosan with lower NaOH concentration and to employ a by-product of another fundamental chemical process. Moreover, glycerol and NaOH can be recovered and reused for another deacetylation reaction.

Again, the concepts of "eco-sustainability", "reuse" and "recycling" become the basis of the research.

## 4. Production of Chitin- and Chitosan-Based Hydrogels

Hydrogels are 3D hydrophilic polymeric networks that do not dissolve but can swell, in water.

In the last few years, there is increasing interest in these materials thanks to their solid and liquid-like properties, high biocompatibility, easy preparation, and versatile applications.

Hydrogels can be classified depending on their sources, pore size, nature of swelling, ionic charge, chain composition, and type of cross-linking [63,64]. In particular, according to their inter-chain interactions, they can be divided into physical and chemical hydrogels.

Physical hydrogels are cross-linked by physical interactions, such as entangled chains, hydrophobic or electronic interactions, van der Waals forces, or hydrogen bonds, which can be reversibly created and destroyed [65], setting the basis for stimuli-responsive "smart" materials.

Despite physical hydrogel can adsorb water, it is not uncommon finding some little defects in their network due to the presence of free chains [66,67].

Sometimes, to guarantee a stable structure and effective swelling, a covalent bond between polymeric chains, achieved by adding chemical cross-linkers, is required. These types of materials are referred to as chemical hydrogels [65].

Hydrogel formation is one of the most appreciated applications for chitin and chitosan [68–70]. They are classified as natural gels according to sources and have a great potential in various fields. Chitin and chitosan hydrogels, for example, are used in drug delivery [71,72] where the small pore in chitin, is exploited for a slow release of drug [73]. Tissue engineering wound dressing [74] and water purification [75] are other interesting applications, that make these hydrogels increasingly studied.

### *4.1. Common Chitin and Chitosan Physical Hydrogel*

Most hydrogels based on chitosan and chitin required a two-step process: polymer dissolution and cross-linking (or gelation).

In both cases, the starting materials are chitin and chitosan powders.

While for chitosan the dissolution step is relatively easy and commonly takes place with an acidic aqueous solution (generally, acetic acid 5%) at room temperature, for chitin it is more complicated, due to its insolubility in water and the high number of inter- and intramolecular interactions between polymeric chains.

Therefore, in this step, finding a good solvent for this polymer is mandatory. The commonly used solvents include lithium chloride/dimethylacetamide (LiCl/DMAc) [76,77], Lithium chloride/*N*-Methyl-2-pyrolidone (LiCl/NMP) [78] and $CaCl_2 \cdot 2H_2O$/MeOH [79].

In these cases, the gelation begins when the chitin concentration exceeds a certain threshold value. The polymer increasing concentration leads to an increase of entanglements between chains and, therefore, to a passage from a liquid isotropic structure to a solid gel with an anisotropic structure.

By pouring the obtained gel in a specific mold or in vials at a temperature between 5–60 °C for a certain time it is possible to facilitate the interaction between chains and increase the hydrogel stability. A similar effect can be obtained thanks to a process of coagulation, which consists of immerging gels in anti-solvents (water, methanol, ethanol, etc.) [77–80]. It is worth remembering that despite the fact that these solvents are still commonly used, they are toxic and not eco-friendly. In Table 3, a summary of different methods conventionally used for the preparation of hydrogels from chitin is reported.

**Table 3.** Synoptic summary of common methods for the preparation of chitin-based hydrogels.

| | Solvents | Dissolution | Gelation | Open Issues |
|---|---|---|---|---|
| **Chitin Physical Hydrogel** | LiCl/DMAc | 48 h, RT under stirring | 0.3–1.5% *w/v* (antisolvents: water, ethanol or acetone) mold/bead [77] | Use of toxic and not eco-friendly solvents |
| | LiCl/NMP | | 0.5–5% (water vapor as non-solvent) mold/bead [77,78] | |
| | $CaCl_2 \cdot 2H_2O$/MeOH | 100 °C (several hours-time non clearly specified) | 1.96%, coagulate, dialyze/filter [79] | |

Chitosan-based physical hydrogels (as summarized in Table 4) are normally obtained by combining its acidic solution with an alkaline medium containing a physical cross-linker, such as Sodium Citrate (SC), Tripolyphosphate (TPP), and β-Glycerophosphate (β-GP) [81–89].

**Table 4.** Overview of chitosan physical hydrogel production methods.

| | Type | Cross-Linker | Chemicals and Conditions | Open Issues |
|---|---|---|---|---|
| **Chitosan Physical Hydrogel** | Electrostatic interaction | SC | Soak in 1.0–10.0% *w/v* sodium citrate (4 °C; 0.5–4 h); cure (37 °C-48 h) [81] | Hydrogel low stability |
| | | TPP | Drop in 10 wt% TPP (RT)/Cure (RT, 12 h) [82] | |
| | Hydrophobic Interaction | β-GP | Thermogelate [88,89] | |

In particular, SC and TPP are negatively charged (SC contains $COO^-$ groups, while TPP contains $P_3O_{10}{}^{5-}$ units) and can establish electronic interactions with $-NH_3{}^+$ units of chitosan in alkaline media, while β-GP is defined "hydrophobic cross-linker" and promotes a favorable environment to form hydrogel thanks to GP-polymer electrostatic interactions and polymer-polymer hydrophobic interactions. Despite the fact that these physical hydrogels are obtained with a greener approach thanks to the use of a non-toxic cross-linker, their application is still quite limited due to their low stability.

### *4.2. Common Routes for Chitin and Chitosan Chemical Hydrogels*

In chemical hydrogels, the gelation step requires a chemical cross-linker that enables to achieve covalent bonds between polymeric chains.

As summarized in Table 5, for chitin, cross-linkers can be classified into two groups: those which lead to esterification reactions and those which lead to etherification reactions.

**Table 5.** Overview of common chitin chemical hydrogel production methods.

| | Type | Cross-Linkers | Chemicals and Conditions | Open Issues |
|---|---|---|---|---|
| **Chitin Chemical Hydrogel** | Esterification [84,86,87] | BTCA | DMAP, cure/coagulate (RT, 24 h) | • Use of toxic cross-linker;<br>• Use of toxic catalyst (DMAP). |
| | | SA | | |
| | Etherification [88,89] | Epichlorohydrin | NaOH (50–60 °C, 1–20 h) | • Use of toxic cross-linker;<br>• Use of strong bases. |

The first ones, including butane tetracarboxylic dianhydride (BTCA) and succinic anhydride (SA) [90–92], create an ester bond with the chitin hydroxyl group.

Curing or coagulation are required, alongside the presence of a nucleophilic catalyst, such as dimethyl aminopyridine (DMAP) [93].

In the case of etherification, epichlorohydrin is commonly used [94,95]. In this case, nucleophilic catalysis induced by DMAP is not necessary, but alkali catalysis induced by solvents, such as NaOH or urea, is required.

For chitosan (Table 6), instead, gelation generally is obtained by using chemical cross-linkers glutaraldehyde (GA) and hexamethylene-1,6-di (aminocaroxysulfonate) (HDS) [96–98]. In these cases, nucleophilic or alkali catalysis is not required, but the cross-linkers can be added during or after curing. Alternatively, a gel solution can be soaked in the cross-linker solution through the use of a syringe.

**Table 6.** Overview of common chitosan chemical hydrogel production methods.

| | Cross-Linkers | Chemicals and Conditions | Open Issues |
|---|---|---|---|
| **Chitosan Chemical Hydrogel** | GA | Cast GA 1 wt% with Chitosan 1% (*w*/*v*) in acetic acid, dry at 37 °C [96] | Use of toxic cross-linker. |
| | HDS | Mold, chitosan 4.55 wt% 60 °C, 48 h [97] | |

### *4.3. Green Hydrogels*

Both physical and chemical illustrated processes required the use of solvents and cross-linkers that are not environmentally or human health-friendly.

This perspective aims to show a valid sustainable alternative and, where possible, tries to recycle by-products of chitin and chitosan extraction.

For the preparation of physical chitin hydrogels, the use of the cited solvents in the dissolution steps is the main concern, due to their toxicity. For this reason, finding eco-substitutes is a primary issue.

Chen et al. [99] show a new dissolution process based on the simple treatment of chitin powder with low-concentrated NaOH solution at low temperature.

The protocol provides for the dispersion of chitin powder in a 20% NaOH aqueous solution under stirring. This NaOH concentration is not enough to deacetylate chitin, but after soaking the chitin/NaOH solution in a refrigerator at −18 °C, chitin undergoes dissolution. In these conditions, in fact, NaOH uptake is maximized, which promotes the formation of hydrogen bonds with chitin, destroying its original structure. After 12 h, the chitin solution can be recovered and heated at 60 °C. At this temperature, polymer chains establish hydrogen bonds forming entanglements that lead to the formation of the desired gel.

Valid alternatives for complete chitin dissolution are also other alkali solutions, such as 2.5 M KOH/0.67 M urea, 2.5 M NaOH/0.7 M urea, and 2.5 M LiOH/0.l67 M urea [100,101]. In these cases, the suspensions are frozen at −30 °C overnight and, then, cured at 0 °C.

Other interesting methods to dissolve chitin with green solvents have been reported by Sharma et al. [102] and Vicente et al. [103]. In these cases, DESs are used as solvents. In particular, ChCl:urea 1:2 dissolves chitin at 100 °C in 10 h, while ChCl:thiourea 1:2 dissolves the biopolymer at the same

temperature in 6 h. In both cases, it is possible to reduce the required temperature for dissolution from 100 °C to 80 °C through ultrasonication for 1 h or microwaves irradiation for 2 h. ChCl: lactic acid 1:1 mixture (2 h) is evaluated as a further valid alternative.

As reported in Section 2.3, it is possible to extract chitin from crustaceans by using the same DESs, and these DESs can be recovered and reused a certain number of times. Therefore, the potential of these last methods consists in the employment of the same green chemicals used for chitin extraction and the recycling of subsequent chitin dissolution. This is a good example of a circular process.

A summary of different methods that can be used to achieve chitin-based hydrogels following green protocols can be found in Table 7.

**Table 7.** Overview of chitin green hydrogel production methods.

| | Solvents | Dissolution | Chemicals and Conditions | Open Issues |
|---|---|---|---|---|
| **Chitin Green Hydrogel** | NaOH | 20 wt% NaOH, −18 °C, 12 h [93]. | Cure at 60 °C | Use of strong bases |
| | Alkali/urea [101,104] | • KaOH/urea (−30 °C, overnight);<br>• LiOH/urea (−30 °C, overnight);<br>• NaOH/urea (−30 °C, overnight) [94]. | Cure at 0 °C | |
| | DESs | • ChCl:urea 1:2 (100 °C; 10 h/80 °C with 1 h ultrasonication/80 °C with 2 h microwave irradiation);<br>• ChCl:thiurea (100 °C; 6 h/80 °C with 1 h ultrasonication/80 °C with 2 h microwave irradiation);<br>• ChCl:lactic acid 1:1 (2 h) [95]. | Cure | • Use of high temperature for several hours<br>• Poor literature on self-assembly of chitin in such solvents |

In human health and the environment, the aforementioned glutaraldehyde, for example, can be toxic for humans, environment, and some species of animals [105–107] in certain conditions, so green alternatives are appreciated (summarized in Table 8).

Agu et al. [108] demonstrated that citric acid can be used as a cross-linker for chitosan at room temperature, stirring the obtained chitosan dispersion for 40 min at 400 rpm.

Again, it is worthy of noticing that citric acid can be extracted by fungi [56], which can be at the same time source of chitin and hence of chitosan.

Genipin can replace more aggressive chemical cross-linkers [109–112]. Genipin is a natural chemical compound extracted from the fruit of *Genipa americana*. In the extraction process, the fruit of gardenia are washed, ground, and dried at 60 °C. Then, the obtained material is boiled with water for 30 min and the extract is recovered. Genipin works as a cross-linker for chitosan, by interacting with its amine groups and producing peptide bonds and tertiary amines. To obtain an optimal hydrogel, genipin (0.10 wt%) is added to the chitosan solution at pH = 5, followed by curing for 12 h at 37 °C (Figure 8).

**Table 8.** Overview of chitosan green hydrogel production methods.

| | Cross-Linkers | Chemicals and Conditions | Open Issues |
|---|---|---|---|
| **Chitosan Green Hydrogel** | Citric acid | RT, 40 min, under stirring [100] | - |
| | GP | GP 0.10 wt%, cure (12 h, 37 °C). [102,103,105,106] | genipin extraction costs? |
| | UV-Vis light | tetramethylammonium hydroxide catalyst 10% *v*/*v*, reaction with 4-azidobenzoic acid (70 °C, 7 h) [115] | alternatives to azidobenzoic acid? |
| | Limonene | 2 wt% acetic acid solution, 1200 rpm for 60 min, 2 mol/L sodium hydroxide solution [113] | evaluation of the overall energy cost |
| | Doubled cross-linkers | reaction between alpha-beta unsaturated acylated chitosan and sulphydrilated chitosan, soak in ethanol [114] | need for preliminary functionalization of chitosan |

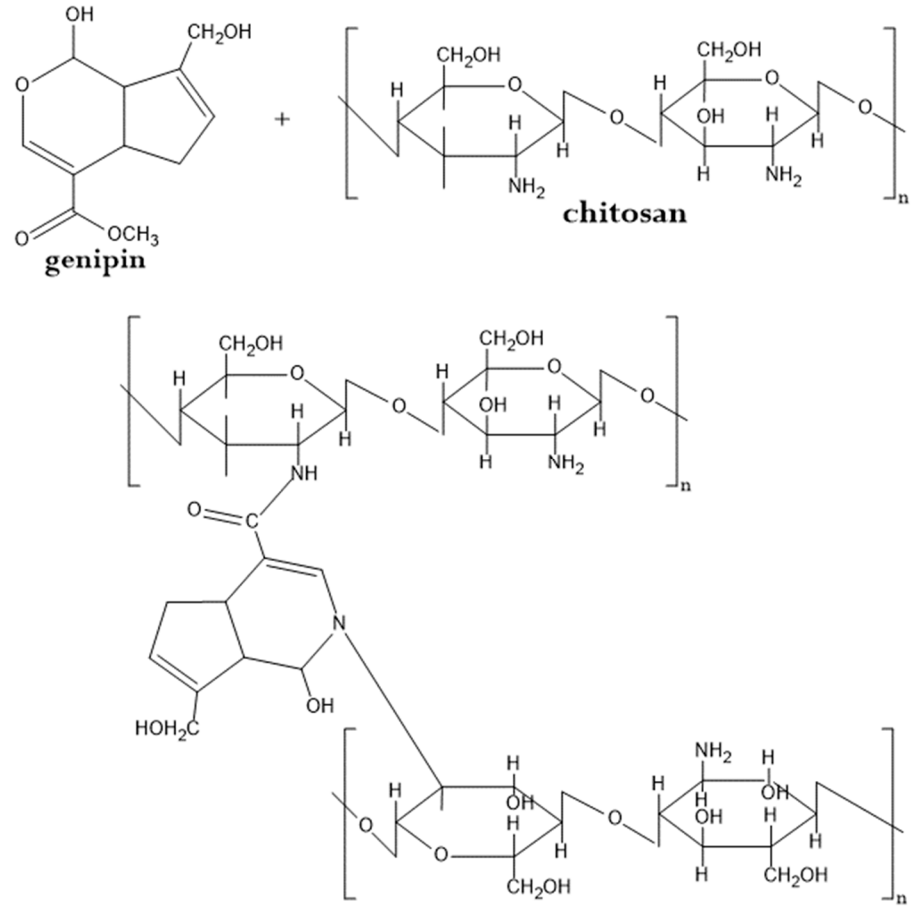


**Figure 8.** Suggested crosslinking reaction mechanism between chitosan and genipin. See text and Ref. [111] for details.

The use of another molecule of natural origin, limonene, instead has been tested for the preparation of chitosan-based microspheres, using limonene as an emulsifier [113].

Another alternative is represented by the formation of a double crosslinking [114]. This process requires the reaction between alpha-beta unsaturated acylated chitosan and sulphydrilated chitosan to obtain a single-cross-linked hydrogel. The double cross-linking is lastly obtained by soaking the hydrogel in an ethanol solution. This route has the advantage to be fast, safe and it doesn't require the use of toxic compounds.

Moreover, it has been proved that the use of chemical cross-linkers can be avoided by exploiting photo-crosslinking to induce the formation of a covalent bond between polymer chains [115]. In this

case, it is necessary to make the starting chitosan solution photoreactive, by introducing inside the chitosan structure an azide group. This can be done by means of alkalization in isopropanol (a reaction between chitosan and propylene oxide in the presence of tetramethylammonium hydroxide as a catalyst). Then, the azide group is obtained by making the derived hydroxypropyl chitosan react with 4-azidobenzoic acid.

The newly described routes allow for minimizing of impacts on the environment thanks to the use of chemicals of natural origin. In addition, they allow the reuse of by-products of chitin and chitosan extraction, minimizing waste originated from the synthesis processes.

## 5. Conclusions and Perspectives

The increase in world population has led to the research of new methods for the sustainable disposal and reuse of food waste. In particular, the awareness that food waste can be a source of molecules with high added value (e.g., proteins, polysaccharides, lipids, etc.) has resulted in new investigations in the field of material science and bio-engineering. Chitin, and its derivative chitosan, are key examples of polysaccharides that can be extracted from food waste, in particular crustaceans and fungi. Renewability, biodegradability, and non-toxicity are the main advantages of their employment in the preparation of functional advanced materials for different applications. However, the processes at the basis of their production are not always paired with the same environmentally-sustainability. In fact, both molecule extraction and hydrogel production show some points of criticism. In the extraction process, the use of strong bases and acids, high temperatures, and a large amount of water for washing steps represent major limitations that still need to be addressed. In the case of hydrogel formation, instead, the major concerns come from the use of toxic and not eco-friendly solvents and cross-linkers. This Perspective summarizes most of the work that can be found in recent literature, aimed at the exploitation of eco-friendly alternative routes. These unconventional strategies are based on the employment of green solvents for chitin extraction and dissolution (DES-based on choline chloride, urea, organic acids), alternative sources of heating (microwave), and/or the use of non-toxic and natural crosslinkers (citric acid, genipin or limonene). In this regard, we would like to highlight that citric acid and DESs/NADES can combine good results during chitin extraction, with the possibility of reusing them in subsequent processing steps: DES can be reused for chitin dissolution before its conversion into chitosan, while citric acid shows the interesting application as cross-linker in hydrogel production. The opportunity of purifying and recycling these extraction chemicals introduces the possibility of developing a whole circular process in which by-products of a single step can be used as reagents in the following one, creating inner loops that are associated with the concepts of "system chemistry" and "circular economy". We want also to underline that most of the chemicals proposed an alternative "green" procedures (choline chloride, urea, organic acids, glycerol, limonene, or genipin) can be extracted from natural sources, and, in some cases, they are by-products of other chemical processes, leading to the development of interconnected sustainable systems. Anyway, these are only a limited number of examples and a huge amount of work has still to be done in this framework. In the future, more attention should be focused on (a) intensive study of DES and NADES and their application in the extraction of chitin (b) low temperature and low energy process for extraction of chitin and chitosan and production of hydrogel (c) evaluation of the cost of production and disposal of chemicals used in the green routes. In addition, to obtain a truly sustainable system, it is necessary to evaluate not only the toxicity of the involved chemicals but also the embodied energy linked to their production, the $CO_2$ footprint of the whole process, and the life cycle assessment of the final product. This information can be scarcely found in literature, but the new analysis could be performed, also with the support of machine learning and artificial intelligence-based algorithms.

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