

# Biopolymer chitosan: Potential sources, extraction methods, and emerging applications

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## ABSTRACT

Food manufacturing generates a considerable amount of leftovers. Garbage disposal could cause environmental and ecological issues. Nevertheless, it is often possible to convert waste into high-value usable goods. Researchers have combed through natural wastes and discovered substances that could be re-utilised to address the issues. One of the materials discovered in marine waste is chitin, which could be transformed into chitosan. Chitosan is a natural biopolymer derived from chitin, which is non-toxic, biodegradable, and biocompatible. Therefore, chitosan has a wide range of possible applications. Moreover, chitosan has been widely acknowledged to be an effective biomaterial in a variety of ways. This review aims to examine more closely the primary sources of chitosan, extraction methods, and applications.

## 1. Introduction

As the global population grows, so does waste production. According to M. Yadav et al. (2019), seafood waste is frequently burnt, buried in landfills, dumped in the sea, or left to disintegrate. The disposal of food waste is a significant issue that industries and society face during food production. A large portion of the by-products generated by processed food remains unutilised, which might contain high-value compounds [1].

Living organisms in the ocean generate approximately  $10^{12}$ – $10^{14}$  tonnes of chitin per year [1,2]. Chitosan, the second most abundant natural resource after cellulose, is the product of the deacetylation of chitin in seafood waste [3]. Chitin exists in a vast variety of biomass, including fungal cell walls, crustacean exoskeletons, insects [4], and fish scale as depicted in Fig. 1 [5]. Chitin exhibits non-toxic, biocompatible, and biodegradable polymeric properties but has inferior solubility at neutral pH [6].

Chitin is a linear amino polysaccharide comprised of poly- $\beta$ (1–4)-N-acetyl-D-glucosamine [7]. The three crystalline allomorphs that vary in microfibrils orientation are recognised as  $\alpha$ -chitin,  $\beta$ -chitin, and  $\gamma$ -chitin. The  $\alpha$ -chitin comprised molecular chains arranged in an antiparallel arrangement. It is also the most abundant and easily accessible. The molecular structure promotes the formation of strong intermolecular

hydrogen bonds, suggesting that it is the most stable. Meanwhile, molecular chains in  $\beta$ -chitin, on the other hand, are bundled in parallel configurations, resulting in weaker intermolecular forces. Ergo  $\beta$ -chitin has a lower stability than  $\alpha$ -chitin. The parallel and antiparallel arrangements of  $\gamma$ -chitin indicate a mixture of the  $\alpha$ - and  $\beta$ -forms [8]. Fig. 2 displays the structures of chitin that comprised N-acetyl-D-glucosamine polysaccharides. Chitosan is produced after the deacetylation process of chitin. The applications of chitosan in drug delivery [9], tissue engineering technology [10], wound healing [11], and other applications are currently being explored. Some studies have also used chitosan to substitute other materials in electrical applications such as sensor, actuator and transducer [12].

## 2. Potential sources

Following past studies, chitin is present in abundance in the shells of crustaceans, such as shrimp, crab, and lobster, and the cell walls of mushrooms, coral, algae, and nematodes. Chitosan, which is deacetylated chitin, could be produced by treating chitin with a high concentration of sodium hydroxide [13]. Table 1 illustrates previous research on chitosan extraction.

Chitosan becomes positively charged in acidic conditions due to  $-\text{NH}_2$  protonation, thus making it soluble in aqueous mediums [7].

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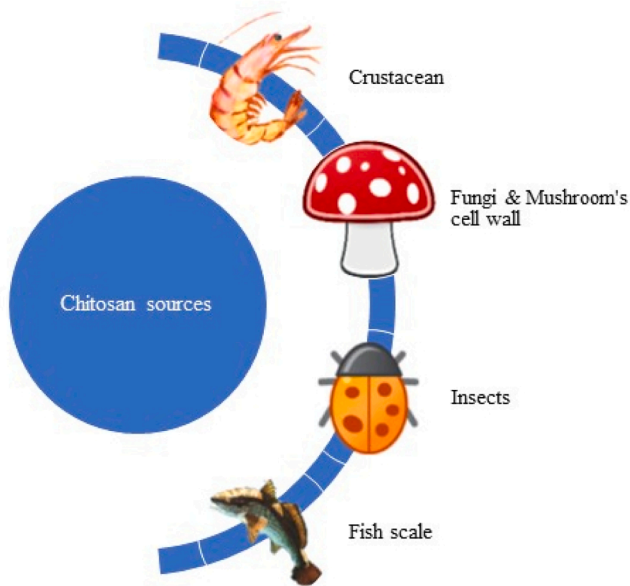


Fig. 1. Common primary sources of chitosan.

Additionally, Jampafuang et al. (2019) stated that the solubility of chitosan in aqueous acidic solutions is attributable to the amino and hydroxyl groups on the chitosan backbone. Nevertheless, to determine if the extracted chitosan has the ideal properties, it is necessary to comprehensively examine the isolated chitosan from a few perspectives, such as deacetylation, viscosity, moisture content, and ash content.

2.1. Crustacean shells

Annually, 18 to 30 million tonnes of fish waste are produced worldwide [30]. Kumari et al. (2015) stated that because of their high biological oxygen demand (BOD), chemical oxygen demand (COD), total suspended solids (TSS), fat-oil-grease (FOG), pathogens, organic

matters, and other nutrients, fishery wastes, such as crustacean shells, are perilous to the environment [30]. Despite the potential dangers, fish waste is abundant in the ecosystem and has the potential to be transformed into valuable resources.

Although chitosan has various applications in biotechnology, agriculture, and medicine, only shrimp, crab, and krill have been recognized as commercial sources for this compound. The reason behind this is that chitosan is primarily derived from crustaceans since their skeletons are readily available as by-products of food processing [31]. Crustacean shells are primarily composed of chitin, protein, and mineral salts. A mineral-protein matrix integrated into the chitin network in the crustacean shells makes it stiff. As a result, demineralisation and deproteination are necessary to isolate the chitin [32]. Deacetylation removes the acetyl in the chitin, thus resulting in a residue known as chitosan. Deacetylation is relatively simple in shrimps and fish compared to the deacetylation of crab shells. However, shrimp shells are the best option because the physicochemical properties of the chitin obtained are close to those of commercially manufactured chitosan [33]. Table 2 displays previous investigations on extraction of chitin and chitosan from crustacean sources.

2.2. Fish scale

Fish scales are regarded as garbage and produced on a considerable scale, 1% of the total weight of a fish, making it one of the leading sources of pollutants in river systems in several nations [34]. The substances constituted in fish scales include chitin, calcium, proximate, alkaloids, steroids, saponins, phenol hydroquinone, molisch, benedict, biuret, and ninhydrin [35]. According to Djais et al. (2021), the amount of chitosan in milkfish could reach 37.4% after dehydration. In addition, antimicrobial compounds found in fish scales with bone-like compositions could be employed as dental materials [35]. As a result, fish scales could be utilised for various purposes, including paper filling [36], biomass in energy generation [37], and heavy metal removal [38].

Previous research demonstrated that chitosan from fish scales could be isolated and used in novel water treatment techniques. Liaw et al. (2020) suggested that fish scale-extracted hydroxyapatite or chitosan composite scaffolds exhibited excellent ability to extract heavy metal

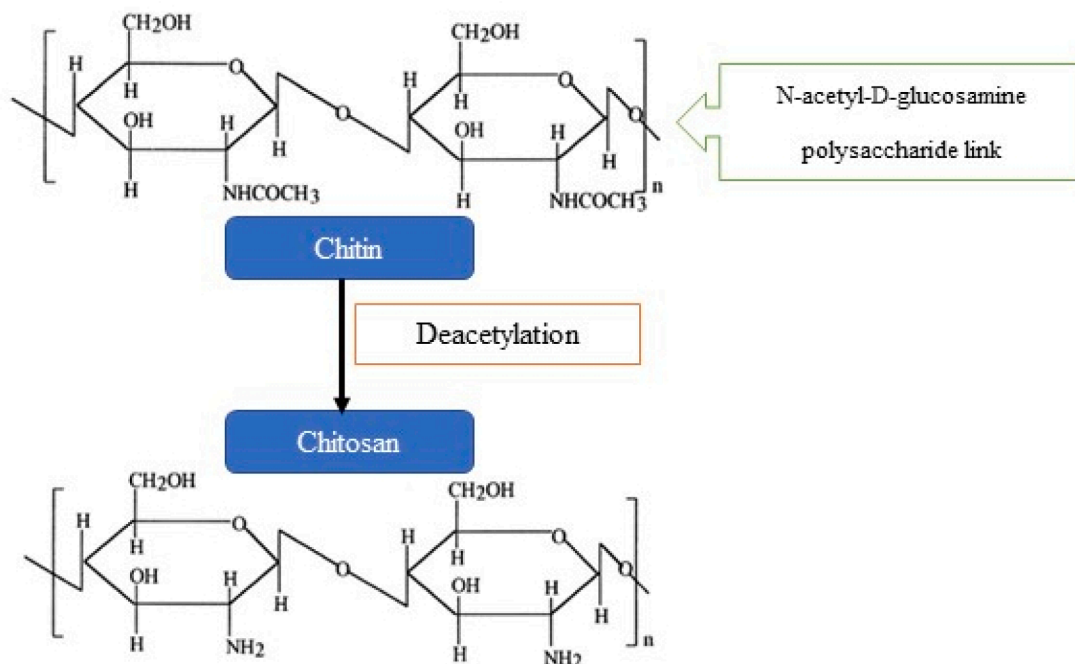


Fig. 2. Schematic diagrams of chitin and chitosan structures.

ions from wastewater. Their tenable channel sizes enable applications in numerous fields under static and flowing conditions [39]. On the other hand, fish scales comprise biologically active substances and a structure resembling bone tissue due to their type I collagen, hydroxyapatite, and unique collagen arrangement. The bio-composite scaffolds displayed cytocompatibility and exhibited promising effects as polymeric scaffold reinforcement agents, including bone tissue regeneration applications [40].

### 2.3. Fungi and mushroom

Fungi are the second-largest community of organisms on the planet, with an estimated population of 5,100,000 individuals and over 70,000 species [41]. Having similar structures to crustaceans, approximately 1 to 15% of the mass of fungal cell walls is chitin, making them the second most common source of chitin after crustaceans [42]. According to Lopez-Moya et al. (2019), chitosan is a defensive modulator in plants. The cell walls of fungal consist of chitin and  $\beta$ -glucan oligomers, which are biosynthesized by chitinases and glucanases [43].

According to Joseph et al. (2021), chitosan isolated from crab and fungi exhibited more effective free radical scavenging potentials than the chitosan obtained from insects and shrimps [56]. In addition, fungi did not require the same severe acid treatment as crustaceans to purify, demineralise, and remove calcium carbonate and other minerals to obtain their chitosan [44].

**Table 1**

Previous research on chitosan isolation from various raw sources.

Source	The degree of acetylation (DA)	The degree of deacetylation (DDA)	Intrinsicviscosity ( $\eta$ )	Molecular Weight	Moisture content	Ash content	Ref.
Horseshoe crab		86%	98.80 cP	187,128.42 gmol <sup>-1</sup>			[14]
Blue crab	8%		3432 mL/g	115 kDa			[15]
Chilean crab	4%			201 $\pm$ 5 kDa			[16]
Sand crab		70.85%			9.78%	0.48%	[17]
<i>Macropipus holsatus</i> crab		82.5%	432 $\pm$ 11 mL/g	194 $\pm$ 60 kDa	9.49 $\pm$ 1.7 %		[18]
<i>Litopenaeus vannamei</i> shrimp		84.76 %		235 kDa		0.08 %	[19]
<i>Solenocera hextii</i> shrimp		85 $\pm$ 0.38 %	15.67 $\pm$ 0.58 cP	52.61 $\pm$ 0.44 kDa	1270 $\pm$ 11 %		[20]
<i>Litopenaeus vannamei</i> shrimp		79%		260 kDa			[21]
<i>Litopenaeus vannamei</i> shrimp		90.7%		140 kDa		9%	[22]
Iraqi Shrimp		52%	19 cp	102.5 KDa	6.2%	0.72%	[23]
Cuttlefish bones	79%			620 $\times$ 10 <sup>3</sup> gmol <sup>-1</sup>			[24]
<i>Illex Argentinus</i> squid pen		85.4%		98.8 3 KDa			[25]
<i>Loligo formosana</i> squid pen		89.72 $\pm$ 0.37	3.24 $\pm$ 0.02 (dL/g)	1.2 $\times$ 10 <sup>5</sup> Da			[26]
Squid pen		96%		8 kDa	10.05 $\pm$ 0.03 %	0.43 $\pm$ 0.01%	[27]
<i>B. magna</i> insect		89.89 $\pm$ 1.34 %	491.88 $\pm$ 3.11 mL/ g	696.95 $\pm$ 4.73 g mol <sup>-1</sup>	34.28 $\pm$ 0.21 %		[28]
<i>B. portentosus</i> house cricket		80.5%			3.33 %	1.00 %	[29]

Poverenov et al. (2018) reported that high-quality mushrooms were not necessarily the only source of chitosan but wastes from the mushroom business might also be utilised. Moreover, after solid-state fermentation, edible mushrooms such as *Agaricus sp.*, *Pleurotus sp.*, and fungi, including *Ganoderma sp.*, were considered sources of chitosan [58,59]. Table 3 lists the findings of previous researchers.

### 2.4. Insects

Chitosan is commonly derived from wastes from the food and fishing sectors, such as shrimp and crab. Nevertheless, due to the constraints in raw material supply, such as seasonal and geographical obstacles, recent research has concentrated on searching for alternative sources. Insects have certain advantages over crustaceans in that they are not seasonal and could readily be reproduced due to their high fertility and reproductive rate. Furthermore, insect-breeding services are springing up worldwide [42]. Consequently, several insect species have been investigated and identified as potential biopolymer sources.

Berezina and Hubert (2019) reported that they divided insects into three main categories, flying (flies and butterflies), jumping (crickets and grasshoppers), and others. Flying insects require a high amount of energy to fly. Hence, their pulp and cuticles comprised a high-fat content, typically exceeding 50%. On the other hand, jumping insects employ their muscles. Therefore, their protein content was significantly higher, approaching 80% [53]. The rest of the insects were usually in

**Table 2**  
Previous research on extraction of chitin and chitosan from crustacean sources.

Sources	Species	Degree of deacetylation (DDA%)	Yield (%)	Finding	Potential applications	Ref
Crab shell	<i>Chionoecetes opilio</i>			As effective antimicrobial agent	Medical industries	[35]
Prawn shell		69.9%		Utilized fungal fermentation to recover chitin from prawn shells	Cost-effective microbial fermentation	[36]
Crab shell		89%		Regenerate chitosan from BMIMCI	Blending medium of polymer.	[37]
Crab shell	<i>S. olivacea</i>	53.4%	44.57%	Good antioxidant properties	Medical industries	[38]
Crab shell		60.69%	41.29%	Optimize chitin recovery by fermentation	Drug delivery	[39]
Blue crab shell	<i>Callinectes sapidus</i>		77.78%	Increasing the crosslinker concentration affected the properties of cryogels	Polymeric scaffold – tissue engineering	[40]
Shrimp shells		70.96%		Optimum efficiency of Pb removal by absorption of chitosan	Alternative way to treat heavy metal	[41]
Shrimp shells	<i>Penaeus monodon</i>		35%	Anticancer activity of chitin and chitosan against human ovarian cancer cell line	Pharmaceutical industries	[42]
Shrimp shells		88%		Removal of Eriochrome black T from aqueous solutions and as alternatives to expensive adsorbents	Dye removal	[43]
Horse mussel shell	<i>Modiolus modiolus</i>	57.43%	10.21%	Chitosan's antimicrobial effectiveness against a diverse range of microorganisms	Biomedical applications	[44]
Lobster shell	<i>Thenus unimaculatus</i>		35%	Antioxidant scavenging effects on the major free radicals	Antioxidant, anti-diabetic and anticoagulant agents in pharmaceutical applications	[45]

between the two categories. The considerations are critical in the extraction and purification of chitin contained in the cuticles of insects.

According to Saenz-Mendoza et al. (2020), the chitin derived from *Brachystola magna* and *Tenebrio molitor* were 10.4% and 11.6%, respectively, making them potential sources of chitin. Besides, *Tenebrio molitor* was able to be artificially bred using simple and low-cost processes. The dry weight (DW) chitin and chitosan yields from *Bombyx mori*, *Ephestia kuehniella*, *Dendrolimus punctatus*, *Argynnis pandora*, and *Clanis bilineata* were 2.59–56%, 3.1–88.40%, 9.5–10.5%, 8–22%, and 31.37–96.2%, respectively [54]. Consequently, the extraction of chitosan from insects gained attention as its sources are easy to cultivate. The numerous potential applications of chitosan isolated from insects are presented in Table 4.

### 3. Extraction methods

#### 3.1. Chemical method

Varun et al. (2017) reported that the traditional chemical approach was frequently employed to isolate chitin. Although chemical extraction is environmentally damaging, inefficient, alters the physical and chemical properties of chitin, and eliminates minerals and proteins, the technique has been the most widely utilised on a commercial scale [78].

Demineralisation, deproteinisation, and deacetylation are the three main steps in the chitosan extraction process as depicted in Fig. 3. The demineralisation stage is conducted in a dilute hydrochloric acid solution. The step removes calcium carbonate and calcium chloride, the key inorganic compounds in crustacean exoskeletons [79,80]. Subsequently, using various organic and inorganic solvents such as sodium hypochlorite, acetone, and hydrogen peroxide, an optional step called decolourisation might be applied to remove any pigments present, primarily Astaxanthin and  $\beta$ -carotene [64].

The deproteinisation step involves the depolymerisation of the biopolymer by breaking the chemical bonds between proteins and chitin using chemicals. Sodium hydroxide at concentrations between 0.125 and 5.0 M is utilised, with various temperatures and treatment durations. Sodium hydroxide results in deproteinisation, biopolymer hydrolysis, molecular weight loss, and partial chitin deacetylation [65].

Deacetylation removes the acetyl groups from chitin and replaces them with reactive amino groups. The percentage of free amino groups within a structure is determined by the degree of deacetylation, which could help differentiate chitin from chitosan. Alkalis are considered the safer chemical alternative for this step because glycosidic bonds are highly vulnerable to acids [54]. Therefore, sodium hydroxide solution within the 45–50% range is generally employed [66].

Lee et al. (2017) claimed that the chitosan extracted from blue crab

**Table 3**  
The findings on fungi/mushroom chitosan from previous studies.

Sources	Species	Degree of deacetylation (DDA%)	Yield	Finding	Potential applications	Ref
Fungi	<i>Auricularia</i> sp.	86.81%	5.81%	Compared to commercial chitosan, the chitosan isolated from <i>Auricularia</i> sp. had better antibacterial activity against both Gram-positive and Gram-negative bacteria.	Medical industries	[45]
Fungi	<i>Tricholoma terreum</i>			Chitosan-fungal extract films were discovered to have substantially stronger anti-quorum sensing and antibacterial activity than gentamicin.	Food packaging technology	[46]
Fungi	<i>Rhizopus oryzae</i>	72.51%	0.288 g/l	Because of the large molecular weight, extracted chitosan had better antioxidant activity than shrimp chitosan.	Medical industries	[47]
Fungi	<i>Amylomyces rouxii</i>	88.7%		The antimycotic activity of the finished textiles with Flu/NACT nanoconjugates was improved.	Health care disciplines	[48]
Fungi	<i>Aspergillus flavus</i>		53.8%	The extracted chitosan increased antibiotic antibacterial activity and exhibited synergistic effects.	Treatment for bacterial infection	[49]
Mushroom's cell wall	<i>Lactarius vellereus</i> alternative chitin source and <i>Phyllophora ribis</i>		73.1%	Both can be used as an	Antimicrobial	[50]
Mushroom's cell wall	<i>Agaricus bisporus</i>		75.3% 46 wt%	The filters may also aid in lowering the environmental effect of typical membrane production procedures.	antioxidant agents Water treatment	[51]
Mushroom's cell wall	<i>Ganoderma lucidum</i>	85%		When compared to similar crustacean products, they had lower viscosity and MW. These features, in combination with the high DD, allow mushroom chitosan to be easily processed and has a high bioactivity.	Antimicrobial agents	[52]

and shrimp shells deproteinised using calcium oxide and then deacetylated did not require the demineralisation stage. The approach required less chemical consumption while still protecting the environment [67]. Compared to crustacean shells, fungal mycelia comprised less inorganic materials, and no demineralisation treatment was needed during processing [68].

### 3.2. Biological method

The application of highly concentrated mineral acids in the bio-extraction of chitin and chitosan has recently sparked interest due to the various challenges that the chemical technique presents, such as energy consumption, risk of harm, and environmental threat [69]. Several biotechnological techniques were developed to address the limitations of chemical chitin purification and are considered effective alternative approaches for recovering high-quality chitin [70]. Enzymatic deproteinisation and fermentations utilising microorganisms are the two most widely used biological methods for chitin extraction [65].

The combination of lactic acid fermentation by *Lactobacillus* with demineralisation and deproteinisation by proteolytic bacteria shows potential for further research. These microbial processes offer environmentally sustainable and beneficial advantages when compared to traditional chemical methods [70]. Younes et al. (2014) suggested

employing proteolytic microorganisms or proteolytic enzymes as another approach. Compared to chemically prepared shellfish, chitins obtained after the deproteinisation of shrimp shell waste with various proteolytic microorganisms exhibited higher molecular weights [71].

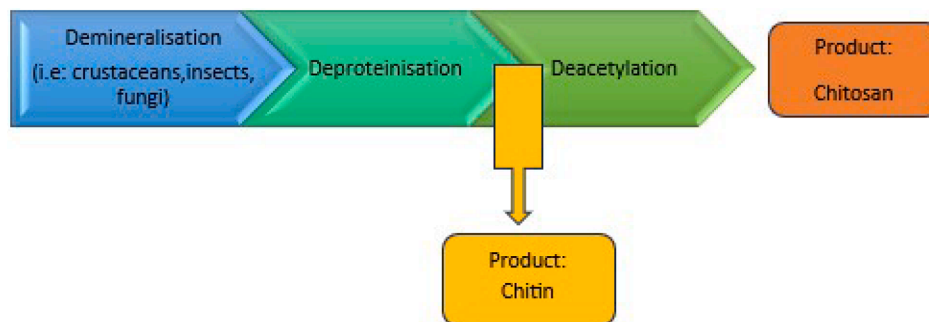
The high cost of purified enzymes is a disadvantage of the biological method. On the other hand, due to coexisting proteases, specific microbial enzyme preparations might be used for deproteinisation, making the method inexpensive and more effective. Nonetheless, although biological extraction is a less expensive and safer option for chitin isolation, it is only available on a laboratory scale [65].

### 3.3. Microwave irradiation

Recently, microwave irradiation has received much interest because the method could speed up reactions by order of magnitude compared to traditional heating. The conventional demineralisation, deproteinisation, decolourisation, and deacetylation technique could take up to two days to fully extract chitosan [72]. The concept underlying microwave heating is the generation of an electromagnetic field that stimulates vibrations on molecular levels of materials. Microwave irradiation for chitin deacetylation was demonstrated to be more efficient than the traditional heating approach. Moreover, a high degree of deacetylation was reached by employing microwave heating for a few minutes [73].

**Table 4**  
The studies on chitosan extraction from insects and their potential applications.

Sources	Method of harvest (source)	Yield of chitin/chitosan	Degree of deacetylation (DD)	Molecular weight	Potential applications	Ref.
<i>Brachystola magna</i>	Collected from local field	10.4%			Transparent film for food packaging	[28]
<i>Tenebrio molitor</i>	Artificial breeding	11.6%			Packaging for UV sensitive food	
<i>Hermetia illucens</i>	Breeding of larvae	47%	43%			[55]
<i>Tabanus bovinus</i>	Collected from field		60.77%		Drug carrier	[56]
<i>Hermetia illucens</i>	Obtained from insect farm	1.56%	91.3%	88.600 Dalton	Potent Antimicrobial and Wound Healing Composites	[57]
<i>Gryllus bimaculatus</i>	Obtained from insect farm	41.75%	84.98%		Nanocapsules	[58]
<i>Zophobas morio</i>	Obtained from insect farm	4.60%	80%		Antibacterial material for food, environmental, fiber industries	[59]
<i>Allomyrina dichotoma</i>	Obtained from insect farm	10.53%	83.37%		Food industry	[60]
<i>Tenebrio molitor</i>	Obtained from insect breeding site	31.9%	53.9%			[60]
<i>Acheta domesticus</i>	Obtained from insect farm	69.0	80%	344 kDa	Hypolipidemi	[61]
<i>Grylloides sigillatus</i>	Obtained from insect farm	62.3	80%	524 kDa	Antimicrobial Agent	
<i>Ephemeroptera</i>	Collected from field	78.43%	84.3%	3.69 kDa	Anti-proliferative material	[62]
<i>Tenebrio molitor</i>	Obtained from insect laboratory	3.65%	92.16%		An oligosaccharide source for pet, animal, and human nutrition.	[63]



**Fig. 3.** General flow of chitosan isolation.

Microwave heating instead of conventional heating would minimise chitosan extraction time from hours to minutes while achieving the same degree of deacetylation. The reactants are stimulated non-uniform and slowly during traditional heating, while microwave heating occurs at the molecular level, resulting in a uniform rapid temperature rise [74]. As a result, the deacetylation time for chitosan isolation through microwave heating decreased from 180 to 60 min. Furthermore, the same degree of deacetylation (DDA) percentage was achieved using the same amount of heat [73]. According to H. EL Knidri et al. (2019), the deacetylation method used sodium hydroxide (NaOH) at a lower concentration, 30%, compared to the traditional process at 40–50%. Therefore, microwave heating is more environmentally friendly, offers fewer chances of damage, and requires fewer chemical expenses [75].

Titik et al. (2018) reported that extraction via microwave irradiation enhanced reaction speed and affected the protein content. The greater

the power of the microwave, the faster the reaction time. Microwave heating is substantially more effective than conventional heating for demineralisation as every molecule in the solution that interacts with the microwave generates heat, ensuring homogenous heating [76]. Mahardika et al. (2019) reported that microwave-irradiated chitosan with a 40-minute reaction time exhibited a greater absorbance than traditionally isolated chitosan that required a 120-minute (2 h) reaction time. Resultantly, the degree of deacetylation of microwave irradiated chitosan was higher [77].

## 4. Emerging applications

### 4.1. Piezoelectric

New uses for chitosan are being explored in various fields, including

the developing field of biodegradable piezoelectric energy harvesters and sensors. To continually power various electrical appliances, the materials used in this application must have inherent piezoelectric properties, or the capacity to produce electrical charges when subjected to mechanical stress [78]. The piezoelectric properties of chitin and chitosan are attributed to the intrinsic molecular polarisation resulting from the non-centrosymmetric crystal structure of  $\alpha$ -chitin and  $\beta$ -chitin polymorphs [79]. Ahmad et al.'s research revealed the potential of chitosan for piezoelectricity, despite focusing solely on indirect piezoelectricity and using chitosan in powder form. However, a comprehensive understanding of chitosan's piezoelectric properties requires further exploratory research [79]. This investigation should involve studying chitosan in various forms beyond powder, to explore its direct piezoelectric properties such as piezoelectric coefficient. Additionally, researchers could explore different processing techniques and conditions to optimize the material's piezoelectric performance. By conducting a more extensive exploration, scientists can fully uncover chitosan's piezoelectric capabilities, which may have applications in various fields like sensors, energy harvesting devices, and biomedical applications.

A potential difference is produced when a compressive or tensile force is applied to piezoelectric materials, referred to as the positive piezoelectric effect. On the other hand, an inverse piezoelectric effect occurs when an electric field is applied to a piezoelectric component, causing mechanical stress. There are a few main parameters to consider during the evaluation of the efficiency of piezoelectric materials. First is the piezoelectric coefficient, which shows how the mechanical and dielectric properties of piezoelectric bodies are related. The electromechanical coupling coefficient ( $k$ ), representing the degree of energy transformation, is the second parameter [80]. Two other critical requirements for a material to be considered as an excellent piezoelectric material are a high value of dielectric permittivity ( $\epsilon$ ) and a low dissipation factor or dielectric loss ( $\tan \delta$ ) [81].

#### 4.2. Biomedical

Pellá et al. (2018) reported that chitosan is a polysaccharide that demonstrated biomaterial growth properties, including biocompatibility, biodegradability, non-toxicity, and low cost. Additionally, chitosan has long been used for wound healing due to its hemostatic properties [82]. Chitosan hastens wound healing through interactions between its amino groups and platelets [83]. Chitosan could also be degraded *in vivo* by several enzymes, the most common lysozyme, a generalised protease found in all mammalian tissues [84].

The cationic nature and electrostatic contact with nucleic acids make chitosan an effective drug carrier and immune adjuvant for cancer vaccines. Generally, chitosan has been widely applied in numerous biomedical applications, including an antibacterial agent in wound dressing, gene delivery [85], tissue engineering, and peripheral nerve [86]. Moreover, chitosan is also utilised as nano-sized drug carriers to target cancerous cells in melanoma, bladder, lungs, breast, colon, pancreatic, and metastatic cancer treatments [87].

#### 4.3. Sensing layer

A sensor is a device that employs a biological element as the sensing element and a transducer to detect a quantifiable signal. According to Muthusankar and Ragupathy (2018), biosensors are expected to play a critical role in clinical and non-clinical applications because of their specificity, mobility, rapid reaction time, durability, and low cost. Biosensor systems utilise isolated enzymes, immunosystems, tissues, organelles, or entire cells to facilitate specific biochemical reactions aided by isolated enzymes, immunosystems, tissues, organelles, or whole cells to detect chemical molecules [104].

Biosensors comprise three main parts, receptor, transducer, and electronic parts. The receptor forms the sensing layer. A receptor might

be a biological or non-biological substance that could capture and interact with target analytes [105]. Selectivity, sensitivity [106], response time, recovery time, detection limit, stability [107], and linearity of response [88] are all critical qualities in the sensing layer. These characteristics provide a foundation for understanding the capabilities of each biorecognition element and how the biorecognition element selection measures the behaviour of the biosensor.

High sensitivity is defined as a substantial detectable change in the signal transmitted by the biosensor due to modest changes in the concentration of bioanalytes [89]. The limit of detection (LOD), the smallest amount of an analyte that might cause a recognisable output signal, reflects the sensitivity of a system [90]. The sensor should also have a broad working range (linear range), which determines the range of analyte concentration that the sensor could detect. Additionally, the linear response range of the system should span the concentration range in which the target analyte would be monitored. Ideally, the response time should be short enough to allow for efficient real-time monitoring of the target analyte. However, the recovery time should be long enough to allow reusability of the biosensor system [88].

Natural polymer materials, such as cellulose and chitosan, are low-cost and environmentally sustainable with a strong gel-forming capacity, thus making them ideal for use in biosensor fabrication. Chitosan, a widely used natural polymer, has physical advantages involving excellent mechanical properties, hydrophilicity, easy operation, and chemical advantages, including biocompatibility and bio-environmental stability [91]. Table 5 tabulates the characteristics of the sensing layer in biosensors investigated in previous research.

#### 4.4. Food packaging

The extensive usage of conventional plastic packaging has contributed to environmental pollution. One of the options to avoid non-renewable petroleum-based plastics packaging is to employ biodegradable materials. Ashrafi et al. (2018) elucidated that active packaging is a cutting-edge concept that could be characterised as a type of packaging in which the product and the environment interact to extend shelf life, improve safety, or enhance sensory characteristics while retaining product quality [101].

Chitosan could be dissolved in dilute acidic solutions and formed into different materials, including edible films. Several types of chitosan-based films could be employed in food packaging materials, including pure chitosan films, chitosan/biopolymer films, chitosan/synthetic polymer films, and chitosan derivative films. Pure chitosan films were reported to delay qualitative and nutraceutical feature shifts, prevent microbial development, retain antioxidant activity, and extend shelf life [102]. Nonetheless, the value of deacetylation of chitosan was demonstrated to affect its acid-catalysed breakdown, where the degradation rate constant increased as the deacetylation value increased [103]. Chitosan forms dimers in acetic acid solution, indicating that the intermolecular interaction is quite strong, and chitosan films produced with acetic acid comprised a more compact structure than those fabricated with other acid solutions [104].

Chitosan is known for its antioxidant and antimicrobial properties. However, chitosan possesses disadvantages, such as low mechanical and thermal stability and high moisture sensitivity, limiting its industrial applicability. Blending chitosan with other biopolymers to combine their benefits while minimising their disadvantages is a technique employed to overcome these difficulties [105]. Furthermore, the properties of chitosan films enable them to be fabricated to acquire excellent mechanical qualities, selective permeability to carbon dioxide and oxygen, and antibacterial capabilities, which could be applied directly in food industries to promote food safety and shelf life [106]. Table 6 displays previous studies on food packaging from chitosan film.

**Table 5**  
Previous studies on sensing layer.

Application	Material	Fabrication method	Linear detection	Detection limit	Sensitivity	Response time	Recovery time	Ref.
Humidity sensor	MWCNTs-CS	Drop-coating			46.7 Hz/% RH	75 s	34 s	[92]
Sarcosine and Creatinine Biosensing	CNT-CS	Drop-casting	$\leq 0.75$ mM	$\sim 6$ $\mu$ M	$\sim 0.5$ $\mu$ A/mM	8 s		[93]
Laccase biosensor	Chitosan/ionic liquid/ phthalocyanine	Immersion into solution	2.4 $\mu$ M to 26 $\mu$ M	8.96-10- 10 M (3- $\sigma$ /m)	0.237 A·M <sup>-1</sup>			[94]
Gas sensing	CS-Pt@SnO <sub>2</sub> NFs	Electrospinning solution				12 s	44 s	[95]
Anti-androgen drug flutamide (FLU) sensor	CS-Au CG	Drop-coating	0.01–1245 $\mu$ M	4.8 nM	0.63 $\mu$ A $\mu$ M <sup>-1</sup> cm <sup>-2</sup>			[115]
Immunosensors	Chitosan/gold nanoparticles	Immersion into solution	0 to 1 $\mu$ g/mL	9x10-4 $\mu$ g/mL	0.27x10-6A/ $\mu$ g mL <sup>-1</sup>			[96]
Caffein sensing	rGO: chitosan: silica sol gel	Dip-coating		1.994 nM		16 s		[97]
Alcohol sensing	quartz crystal microbalance (QCM)	Immersion into solution			4.4 Hz·mg <sup>-1</sup> ·L	26 s		[98]
Acetic anhydride vapor sensing	Quartz crystal microbalance-coated cellulose acetate nanofibers overlaid with chitosan	Immersion into solution	10–1000 ppm	5 ppm	0.234 Hz/ppm	44 s		[99]
Non-Enzymatic Hydrogen Peroxide Sensing	Au/chitosan:CuOx/chitosan/Au	Drop-coating			10,000 $\mu$ A·mM <sup>-1</sup> cm <sup>-2</sup>			[100]

**Table 6**  
Application of chitosan in food packaging.

Film	Tensile strength (MPa)	Elongation (%)	Antioxidant properties DPPHABTS	Ref.
Pure chitosan film	18.14 $\pm$ 0.72	–	7.58%-	[107]
Chitosan/Man go leaf extract (MLE) film	23.06 $\pm$ 0.19	–	87.16-%	[107]
Chitosan-TiO <sub>2</sub> film	46.33 $\pm$ 1.88	25.77 $\pm$ 2.91	–	[108]
Chitosan/Zinc oxide/Neem oil film	60	15.6	–	[109]
Chitosan/Olive pomace film	22.40 $\pm$ 0.22	33.01 $\pm$ 0.66	42.56%-	[110]
Quercetin based Chitosan-gelatin film	17.11 $\pm$ 0.3464	5.100 $\pm$ 0.3162	81.45 %72.2%	[111]
Chitosan/gelatin /silver nanoparticles film	28.87 $\pm$ 0.49	17.99 $\pm$ 0.68	–	[112]

## 5. Conclusion

Biopolymer chitosan has gained popularity because of its attractive qualities, including biodegradability, biocompatibility, and non-toxicity. Notably, biopolymer chitosan can be obtained from two primary sources: recycling fishery waste, including crustacean shells and fish scales, or through cultivation from fungi, mushrooms, and insects. These raw materials are readily available and cost-effective, contributing to the sustainability of chitosan production. To isolate chitosan from its raw sources, different extraction methods are available, ranging from chemical and biological methods to the use of microwaves. These extraction techniques offer flexibility and options for optimizing the yield and properties of chitosan, making it suitable for diverse applications. Several investigations have showcased the versatility of chitosan in various industrial settings, demonstrating its potential in biomedicine, pharmaceuticals, packaging, and energy harvesting. Its biocompatibility and non-toxic nature make it an attractive choice for medical

and pharmaceutical applications. Additionally, its ability to form films and coatings enhances its use in packaging materials, while its potential in energy harvesting opens up new possibilities for sustainable technologies. Despite the promising attributes of crustacean shells and fishery waste as sources for chitosan production, they are currently underutilized. Unlocking the full potential of these resources requires further research and a more thorough investigation of their applications. By exploring these untapped sources and conducting in-depth studies, we can enhance the utilization of chitosan in industrial sectors and contribute to a more sustainable and environmentally friendly future.

## Declaration of Competing Interest

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

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