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REVIEW ARTICLE

β-Chitin and chitosan from waste shells of edible mollusks as a functional ingredient

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Abstract

The marine food-processing industries were producing large quantities of shell wastes as a discard. Currently, this waste material was underutilized and leads to the landfill as a significant environmental issue. The outer shells or exoskeletons of mollusks serve as the best source of chitin. Three different allomorphs of chitin (γ, β, and γ) were extracted from different species of crustaceans, mollusks, and fungi. β-Allomorphs predominantly exist in the shells of mollusks. β-Chitin and its deacetylated product chitosan has been utilized for its special characteristic features, including biocompatibility, environmental friendly, and nontoxic properties. The extraction of β-chitin and chitosan from the mollusk shell waste were evaluated in this work. Hence, this review aims to explore edible mollusk shell waste sources and its suitable extraction techniques, characterizations, and functional properties of mollusk-based β-chitin and chitosan. Further, the genetic pathway of synthesizing mollusk chitin was discussed. The entire life cycle assessment with techno-economic aspects were extrapolated to study the bottlenecks and tangible solution for the industrial upscaling of obtaining β-chitin and chitosan from the edible mollusk shell waste have been reviewed herein.

KEYWORDS
chitin, chitosan, extraction, life cycle assessment, mollusk shell waste, techno-economic assessment

1 | INTRODUCTION

The seafood industry serves as the chief source of food supply for the human population. Seafoods, including fish, crustaceans, and mollusks were attracted by the consumers for their high protein content and other nutritional value. This rising usage led to produce huge volumes of shell wastes. The accumulation of bio-waste in landfills can cause severe deleterious effects on the environment and humans.

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For instance, Cadano et al. (2021) reported that 250,000 MT wastes were produced from aquatic animals. Besides, the conversion of waste resources into valuable biomass is an emerging concept as per United Nations sustainable development goals.

The consumption of mollusks has significantly increased due to their health benefits. Mollusks serve as the best source of polyunsaturated fatty acids next to fish (Abedi & Sahari, 2014; Moniruzzaman et al., 2021). The outer shell of mollusks were inedible which produce huge quantities of shell wastes (Morris et al., 2019; Murphy & Kerton, 2017). In addition, the mollusk shell waste contains calcium carbonate and some minerals (Laskar et al., 2018). These wastes serve as an excellent source of chitin, proteins, and mineral traces (Mathew et al., 2021). Extraction of value-added products after acid and alkali treatments have several demerits (Kumari et al., 2015). In particular, toxic and corrosive chemicals utilized in the biorefining are considered as the major bottlenecks. Furthermore, the chemical residues present on chitosan restrict their usage in food, feed, and pharmaceutical applications. Although it can also be extracted using natural deep eutectic solvents (Bradic et al., 2019), further research is required to find a biodegradable solvent for extraction.

Chitin is a complex polysaccharide generally present in exoskeletons of crustaceans (Mohan et al., 2021), insects (da Silva Lucas et al., 2021), and mushrooms (Benamar et al., 2022; Mesa Ospina et al., 2015). The total yield of chitin and chitosan might vary between the species. The hydrogen bonds present in the chitin lower the solubilizing properties. Chitin is insoluble in water, some organic, and inorganic solvents (Hajji et al., 2014). In addition, three different allomorphs of chitin were reported. For instance, α-chitin exists in the cell wall of fungi and crustaceans (Mohan et al., 2021; Younes & Rinaudo, 2015), β-chitin has been reported from mollusks and insects (Mohan et al., 2020; Weiss, 2012). Furthermore, γ-forms of chitin exist only in cocoons of insects and fungi with three glycosidic chains (Pellis et al., 2022). The multifunctional role of chitin and chitosan depends on its solubilizing properties and molecular weight. These two properties are vital in determining the potential application of chitin, which proves to be a functional ingredient in human application. For instance, (1) it enhances the proliferation of immune lymphocytes, (2) it has inhibitory effects on various types of cancer cell lines, (3) it inhibits the production of reactive oxygen species as an antioxidant, (4) potent surfactant on the food formulation, (5) and it possess better mechanical property on food-packaging.

According to the bibliometric analysis, approximately 100 research papers published over the period of the past two decades that have been indexed in databases, including Elsevier, Springer, Wiley, Taylor and Francis, the Royal Chemical Society, and the American Chemical Society using the keywords "mollusk shell waste," "extraction methods," "chitin," "chitosan," and "biological activity." The majority of research outcomes are from crustacean shell wastes, fungi- and insects-derived chitin and chitosan. The multifunctional properties of chitin and chitosan have been documented in previously published literature (Alimi et al., 2023; Arbia et al., 2013; El Knidri et al., 2018; Hamed et al., 2016; Islam et al., 2023; Joseph et al., 2021; Kaur & Dhillon, 2015; Khajavian et al., 2022; Mohan et al., 2020, 2022, 2023; Saenz-Mendoza et al., 2023; Silva et al., 2017; Özel & Elibol, 2021).

Meanwhile, the structural and functional properties of chitin and its derivatives from various mollusk shells have been reported earlier (Akpan et al., 2018; Aylanc et al., 2020; Cabrera-Barjas et al., 2021; Hazeena et al., 2022; Mohan et al., 2019; Nouj et al., 2022). However, no comprehensive review were focused on the edible mollusk shell waste derived chitin and chitosan. As outlined before, this review aims to discuss the sources of β-chitin and its extraction process. Furthermore, the physicochemical characterization and multifunctional properties were evaluated to emphasis its role as a functional ingredient. Finally, the bottlenecks and viable solutions for producing β-chitin in large-scale were also discussed meticulously.

2 | CHEMICAL STRUCTURE AND SOURCES OF β-CHITIN AND CHITOSAN

Chitin is the second most abundant polysaccharide on earth, next to cellulose. The structure of chitin comprised of repeating β-(1,4)-*N*-acetylglucosamine units, and chitosan was obtained by deacetylation of chitin. Chitosan is acetylated and deacetylated units of D-glucosamine linked with β-(1,4) glycosidic bonds. During deacetylation, acetate ions and amino groups (NH_2) were formed by the hydrolysis of acetamide functional groups (Yadav et al., 2019). Based on the crystalline properties, chitin has been categorized into three different forms, including α, β, and γ-chitin (Rudall & Kenchington, 1973). Furthermore, α-allomorphs exist with antiparallel chains, β-allomorphs contain parallel chains, whereas γ-allomorphs consist of both parallel and antiparallel chains (Rinaudo, 2006). The best sources of α-chitin were exoskeletons of crustaceans and beetles (Kaya et al., 2014b; Pakizeh et al., 2021). Figure 1 illustrates the chemical structures of α, β, and γ-chitin and chitosan. Most importantly, β-chitin exists in squid pens, clams, oysters shell and bones of cuttlefish. The characteristic features, including solubility, flexibility, hardness, and permeability of shells, were determined using the ratio of α-chitin and β-chitin. When compared to α-chitin, β-chitin exhibits higher solubilizing properties (Seenuvasan et al., 2020).

3 | CHITIN SYNTHESIS VIA GENOMIC PATHWAY

The chitin production in mollusk shells can be enhanced through the chitin synthesis pathway, a total of eight enzymes were involved in this process. The chitin synthase was the core enzyme in this pathway, which catalyzes the conversion of *N*-acetyl-D-glucosamine to chitin. This chitin synthase was commonly present in insects, fungi, arthropods, and mollusca. The crustaceans including shrimp and crab was known for higher chitin production (Zhang et al., 2021). The crustaceans have a chitin content of 36.43% and whereas in mollusk with 23% respectively. This difference in chitin synthesis can be identified by exploring the enzyme chitin synthase (CHS). RNAi-mediated silencing of the CHS gene in the insect *Atta sexdens* results in its

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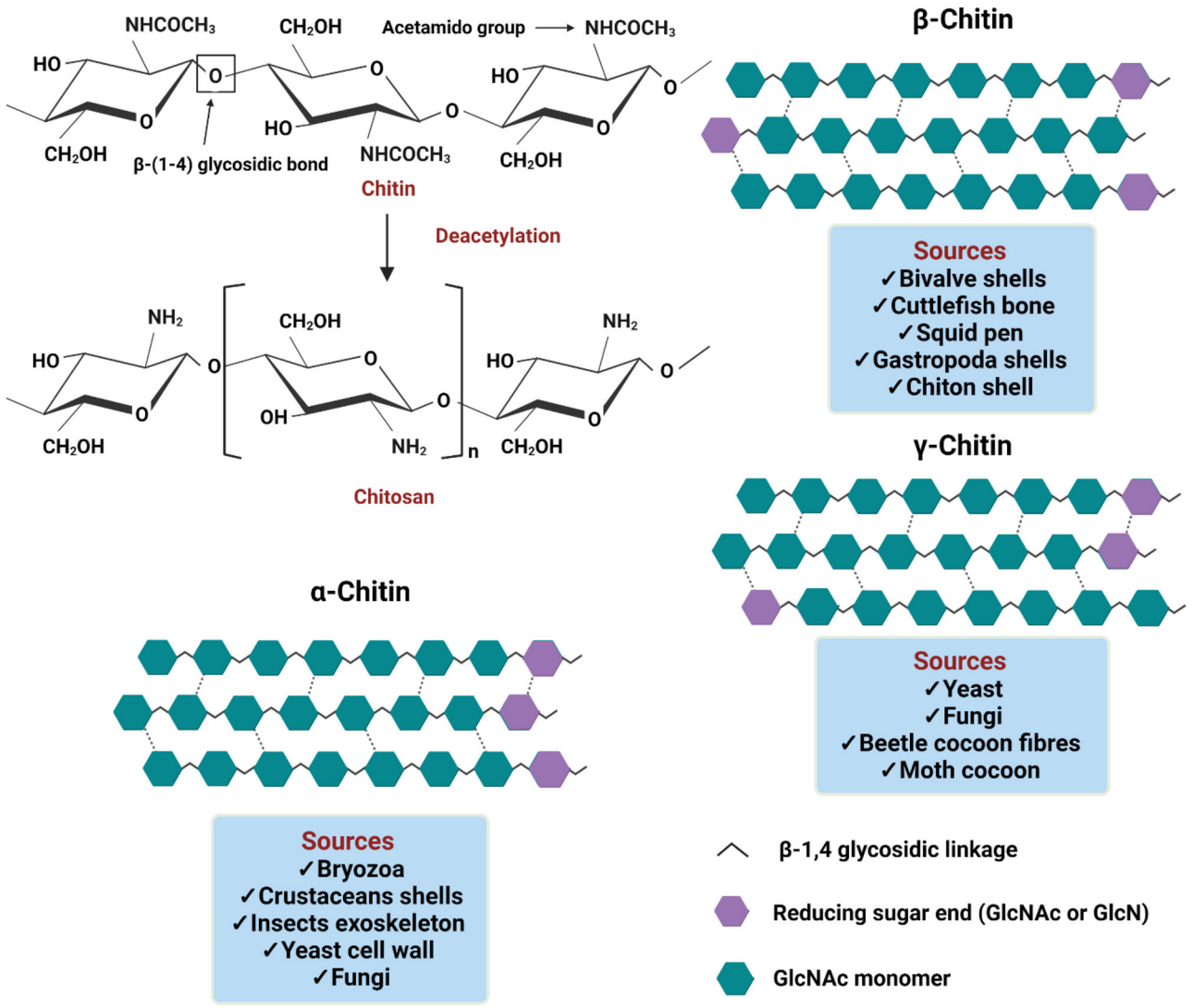

FIGURE 1 Chemical structure of α, β, and γ-chitin.

downregulation, which leads to morphological modification and malfunction in chitin synthesis (Moreira et al., 2020). More exploration into the CHS gene was needed by using the recent advancements in genomics and genetics tools such as Sanger sequencing, whole-genome sequencing (WGS), transcriptome, recombinant DNA technology, and in silico bioinformatics analysis. Phylogenetic analysis revealed that CHS genes of most crustacean species were clustered together and more specific to arthropods closely related to insects (Zhang et al., 2021). This made them distant from mollusk CHS (Zheng et al., 2015). Further phylogenetic analysis revealed that the CHS gene of mollusk *Hyriopsis cumingii*, *Pinctada fucata*, *Atrina rigida*, and *Mytilus galloprovincialis* were similar in identity. The theoretical molecular mass of mollusk was higher (264 kDa) than shrimp CHS and insect CHS protein. This difference was due to the presence of myosin motor head domain in the mollusk CHS protein (Zhang et al., 2021; Zheng et al., 2015). In the mollusk family, the first attempt at sequencing CHS gene was on *A. rigida* and *M. galloprovincialis* with a complete cDNA of the mussel CHS gene found in myosin motor head domain; however, this was not present in crustacean's and insects CHS gene (Weiss et al., 2006). CHS gene from freshwater pearl mussel *H. cumingii* was sequenced using a molecular cloning technique, revealing the presence of myosin head domain (Zheng et al., 2015). Myosin motor head domain function was predicted to be inhibiting the mineralization process and thereby regulating the chitin deposition. WGS of polychaete *Branchipolynoe onnuriensis* identified seven putative CHS genes (Choi et al., 2022), more than 200 CHS isoforms identified in *D. magna* (Zhang et al., 2021), whereas crustaceans possess one or two CHS genes. Nevertheless, the WGS of different mollusks was necessary to find the presence of CHS genes and chitin synthesis-related genes. At the minimal level, the CHS of different mussels can be sequenced using Sanger sequencing and analyzed. Transcriptome analysis of *Lamellidens marginalis* (Indian mussel) identified some of the genes involved in chitin biosynthesis such as CHS, chitin deacetylase isoform, chitinase-3, and chitinase domain–containing protein-1 (Suman et al., 2023). As the CHS genes

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of crustaceans and mollusks were completely different, a comparative analysis of the CHS gene by molecular cloning technique might increase chitin production. Furthermore, the genomic and genetics-based approach of selective breeding of high chitin-producing mussels can be implemented by (1) mussels free of mutation in the CHS gene and the other genes involved in the chitin pathway, (2) expression level of chitin pathway genes in mussels, and (3) also identification of other potential genomic markers. Genomic tools such as WGS, genetic linkage maps, genetic marker panels, and identification of quantitative trait loci were already recommended in the selective breeding of mollusks species for improving the key production traits (Moreira et al., 2020). In this case, key production traits can be high chitin production.

4 | PRETREATMENT AND PROCESSING OF MOLLUSK SHELLS

The seafood industries produce huge quantities of shell wastes, which must be cleaned up before being discarded. However, in most cases it became untreated and leads to landfill as an environmental burden (Lim et al., 2021). The waste shells contain few organic tissue materials after processing the edible part. They can be removed using a variety of techniques, including mechanical or heat treatment, high-pressure washing, enzyme hydrolysis, and acid/alkali washing. The extraction of β-chitin from mollusk shells is a laborious process compared to crustaceans shells that includes the separation of the shell from the gonads/meat. This process requires special tools for declumping, trimming, cooking, and hydrolyzing operations that remain developing at industrial scale. The processing of mollusk shells through cooking (direct steam) opens the mollusk shell and separates the shells and meat (Iribarren et al., 2010a). After the processing, a predominant amount of shell wastes produces $CaCO_3$ and chitin.

5 | EXTRACTION OF β-CHITIN AND CHITOSAN FROM MOLLUSK SHELLS

The extraction of β-chitin from mollusk shell wastes is the most challenging process. Mollusk shells are made up of calcium carbonate (90%), lipid, proteins, and 10% of chitin (Furuhashi et al.,2009; Hou et al., 2016). The most common methods employed for chitin extraction are chemical and green extraction methods. The sources, extraction, characterization, and biological activities of mollusk derived chitin and chitosan are summarized in Table 1.

5.1 | Chemical (conventional) extraction methods

β-Chitin extraction process follows a similar extraction method like α-chitin. There are four essential steps involved in conventional chitin and chitosan extraction (1) deproteinization, which removes protein, (2) demineralization, which removes minerals, (3) decolorization, which removes pigments, and (4) deacetylation (Ianiro et al., 2014). The key advantages of using chemical methods for extraction are the short processing time and low cost.

5.1.1 | Deproteinization

Deproteinization process can be achieved by disrupting the chemical bonds between proteins and chitin (Manni et al., 2010). The mollusk shell powder was subjected to NaOH or KOH treatment for 2 h at 90°C, with constant stirring and it was filtered using a vacuum filter (Abed et al., 2017; Adekanmi et al., 2023). Finally, the deproteinized shells were washed for 30 min to neutralize the pH and dried at 60°C for 24 h. The concentrations of alkali differs between 0.4 and 3 M (Gbenebor et al., 2017; Mohan et al., 2019) and wider ranges of temperatures up to 120°C (Hazeena et al., 2022; Li et al., 2021). The usage of basic alkali solution made chitin to partially deacetylate and it may result in low molecular weight (Mw) and hydrolysis. Alternately, enzymes, including proteases, alcalase, and esperase were utilized to remove the proteins from mollusk shells (Hajji et al., 2015; Vázquez et al., 2017). These enzymes might take longer reaction time, low protein degradation, and expensive to maximize the yield at industrial scale.

5.1.2 | Demineralization

The elimination of mineral components, including $CaCO_3$ and $Ca_3(PO_4)_2$, can be achieved via the demineralization process. The acids, such as H_2SO_4, HCl, HNO_3, CH_3COOH, $HO_2C{-}CO_2H$, and HCOOH, were often utilized in this process. Among them, HCl is predominantly utilized in extraction of β-chitin from mollusk shells (Čadež et al., 2018; Rajathy et al., 2021; Singh et al., 2019; Varma et al., 2021). Followed by, vacuum- filtration and neutralization, the demineralized shells were heated for 24 h at 60°C. During this process, a decomposition reaction occurs which transforms $CaCO_3$ into $Cacl_2$ and CO_2. Generally, this process was carried out at a high temperature for a long duration which adversely affects the physical properties of chitin (Kaur & Dhillon, 2015).

5.1.3 | Decolorization

Removal of pigments attached in the shells could improve the purity of chitin at the final stage. This can be performed by treating with decolorizing agents, including acetone, chloroform, and ethyl alcohol (Dinculescu et al., 2023). After decolorization, resulting pigments can be extracted and utilized for other industrial applications (Rinaudo, 2006). The samples were decolored by subjecting them to an acetone or ethyl alcohol solution for 10 min followed by drying at room temperature for 2 h. The leftover wastes was neutralized and used as a soil enhancer.

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TABLE 1 Extraction methods, characterization, and biological activities of chitin and chitosan from mollusk shells.

Common/scientific name	Extraction methods			Yield (%)		Physical and structural characterizations	Biological activities	References
	Deproteinization	Demineralization	Deacetylation	Chitin	Chitosan			
Bivalve								
Razor clam shell (*Ensis arcuatus*)	10% NaOH at 70°C for 2 h	20% HCl for 16.5 h	50% NaOH at 90°C for 10 h	n.d.	19.36	Ash—8.08%, DDA—48.22%, and Mw—482.38 kDA	n.d.	Zamri et al. (2020)
Razor shell (*Ensis* sp.)	1 M NaOH at 40°C for 5 h	1 M HCl at room temperature for 2 h	n.d.	n.d.	n.d.	FT-IR, SEM, TGA	n.d.	Aylanc et al. (2020)
West African clam (*Egeria radiata*)	4% w/v NaOH at room temperature for 24 h	4% v/v HCl at room temperature for 12 h	65% w/v NaOH for 3 days at room temperature	n.d.	48.6	DDA—67.8%, FT-IR, SEM, XRD	n.d.	Majekodunmi et al. (2017a)
Pinna deltoides	1 M NaOH at 100°C for 20 min	1 M HCl at 70°C for 15 min	45% NaOH at 110°C for 24 h	70.67/100 g	45.01/100 g	DDA—55.17%, FT-IR, XRD, TGA, SEM	n.d.	Varma et al., 2021
Oyster shells (*Mytilus edulis* and *Laevicardium attenuatum*)	4% w/v NaOH at room temperature for 24 h	4% v/v HCl at room temperature for 12 h	65% w/v NaOH for 3 days at room temperature	n.d.	51.8 and 43.8	DDA—69.6% and 37.3%, moisture—3.28% and 3.84%, viscosity—3.24 and 3.86 mPa s XRD, EDAX and micrograph	n.d.	Majekodunmi et al. (2017b)
Oyster shell	3 N NaOH at 90°C for 48 h	1 N HCl at 90°C for 2 h	50% NaOH at 120°C for 1 h	30.01	61.1	DDA—89.14% SEM	n.d.	Handayani et al., 2018
Oyster shell **Mussel shell**	10% NaOH at 30°C for 24 h	10% HCl at 30°C for 72 h	n.d.	69.65 35.03	n.d.	DDA—85.62% and 91%, solubility—77.78% and 85.71%	n.d.	Alabaraoye et al. (2018)
Mussel shell	0.62 M NaOH at 30°C for 16 h	0.68 M HCl at 30°C for 6 h	25 M NaOH for 20 h at 75°C	23.25	15.14	DDAFT-IR	n.d.	Abdulkarim et al. (2013)
Horse **Mussel (*Modiolus modiolus*)**	1 M NaOH at 100°C for 20 min	1 M HCl 70°C at 15 min	45% NaOH at 110°C for 24 h	40.13	10.21	DDA—57.43%, Mw—345.94 kDa FT-IR, XRD, RAMAN	Antibacterial activity	Varma and Vasudevan (2020)
Donacid clam (*Donax scortum*)	1N NaOH at 80°C for 24 h	2 N HCl for 24 h	40% NaOH at 110°C for 6 h	11.96	18.8	DDA—74.35%, Mw—373.80 kDa, UV–Vis, FT-IR, DSC	Antioxidant activity	Shanmugam et al. (2012)
D. scortum	n.d.	n.d.	40% NaOH	n.d.	84.03	Mw—18.65 × 10^4 Da, FT-IR	Anticoagulant and antioxidant activity	Subhapradha et al. (2013a)

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TABLE 1 (Continued)

Common/scientific name	Extraction methods			Yield (%)		Physical and structural characterizations	Biological activities	References
	Deproteinization	Demineralization	Deacetylation	Chitin	Chitosan			
Cockle shell (*Anadara granosa*)	3.5 N NaOH for 1 h	120 mL of HCl for 48 h	120 mL 50% NaOH at 80°C for 3 h	n.d.	n.d.	n.d.	n.d.	Zainal et al. (2014)
Blood cockle shells (*Anadara granosa*)	3% NaOH at 85°C for 30 min	1.25 N HCl at 75°C for 1 h	45% NaOH solution for 1 h	n.d.	n.d.	SEM, FT-IR	Removal of total suspended solids and turbidity of well-water	Siswoyo et al. (2023)
Asian green mussel (*Perna viridis*)	1 N NaOH	1 N HCl	15% NaOH at room temperature for 24 h	41.60	39.5	DDA—79.8%, Mw—16.5 kDa, FT-IR	n.d.	Danarto and Distantina (2016)
Tropical oyster (*Crassostrea iredalei*)	1 M NaOH at 80°C for 2 h	1 M HCl at 75°C for 2 h	50% NaOH at 100°C for 2 h	22.5 17	11.8 9.8	FT-IR-ATR	n.d.	Cadano et al. (2021)
P. viridis	5% NaOH at 70°C for 48 h	1 M HCl at room temperature for 48 h	40% NaOH at 90°C for 6 h	n.d.	18	DDA—55%, FT-IR, XRD, SM, TG/DTA	Antibacterial and anticoagulant activity	Gobinath et al. (2021)
P. viridis	3% NaOH at 70°C for 48 h	5% HCl at room temperature for 48 h	60% NaOH at 84 h	n.d.	n.d.	XRD, EDXRF, FT-IR	n.d.	Sangwaranatee et al. (2018)
P. viridis								
Pen shell (*Pinna bicolor*)	1 M NaOH at 100°C for 20 min	1 M HCl at 75°C for 15 min	45% NaOH at 110°C for 24 h	80.15/100 g	0.021/100 g	DDA—59.76% XRD, FT-IR, RAMAN	Antibacterial activity	Sudatta et al. (2020)
Pen shell (*Atrina pectinata*)	NaOH 70°C for 1 h	HCl treatment (2, 4, and 6 N) at 33°C and 60°C for 3 h	n.d.	0.72	n.d.	n.d.	n.d.	Nugroho et al. (2020)
Scallop shell (*Amusium* sp.)	5% NaOH at 90°C for 140 min	4 M HCl at 90°C for 120 min	50% NaOH at 90°C	n.d.	9.7	DDA—71.8%, FT-IR	n.d.	Rokhati et al. (2017)
Cephalopoda								
Cuttlefish								
Kobi cuttlefish (*Sepia kobiensis*) chitosan	n.d.	n.d.	n.d.	n.d.	n.d.	DDA—85.55%, Mw—322.04 kDa, FT-IR	Antibacterial activity	Shanmugam et al. (2016)
S. kobiensis	NaOH at 60°C for 3 h	4 M HCl at 90°C for 120 min	40% NaOH at 90°C for 120 min	29.87	43.77	DDA—85.55%, Mw—322.04 kDa, FT-IR, NMR, XRD, SEM, DSC	Antioxidant activity	Ramasamy et al. (2014)
S. kobiensis	NaOH at 60°C for 3 h	4 M HCl at 90°C for 120 min	40% NaOH at 90°C for 120 min	n.d.	n.d.	FT-IR, NMR, XRD, SEM, DSC	Anticoagulant and Antibacterial activity	Ramasamy et al. (2022)

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TABLE 1 (Continued)

Common/scientific name	Extraction methods			Yield (%)		Physical and structural characterizations	Biological activities	References
	Deproteinization	Demineralization	Deacetylation	Chitin	Chitosan			
Common cuttlefish (*Sepia officinalis*)	2 M NaOH at 80°C for 2 h	0.55 M HCl at 24°C	n.d.	n.d.	n.d.	XRD, FT-IR, AFM, SEM	n.d.	Čadež et al. (2018)
S. officinalis	4% NaOH 90°C for 20 min	0.55 M HCl at 60°C for 1 h	12.5 M NaOH at 140°C for 4 h	5	n.d.	^{13}C NMR, FT-IR, XRD	n.d.	Hajji et al. (2014)
S. officinalis	A21 crude protease at 90°C for 20 min	0.55 M HCl for 15 min	12.5 M NaOH at 140°C for 4 h	5	1.2	FT-IR	Antimicrobial, antioxidant, and anticancer activity	Hajji et al. (2015)
S. officinalis	1 M NaOH at 70°C for 2 h	36% HCl at 90°C for 30 min	n.d.	n.d.	n.d.	SEM/EDX, FT-IR, XRD, UV–Vis, ^{1}H-NMR	Food industry wastewater treatment	Nouj et al. (2022)
S. officinalis	2 M NaOH at 70°C for 2 h	2 N HCl at 60°C for 3 h	40% NaOH for 6 h at 110°C	n.d.	32	FT-IR, ^{1}H-NMR, SEM	Cadmium removal from wastewater	Kavisri et al., 2023
Hooded cuttlefish (*Sepia prashadi*)	2 M NaOH 70°C for 3 h	1 M HCl at 60°C for 3 h	10 M NaOH at 120°C for 4 h	24	62.6	DDA—55.95%, FT-IR	n.d.	Jothi and Kunthavai (2014)
S. prashadi	1 N NaOH at 80°C for 24 h	2 N HCl for 24 h	40% NaOH for 6 h at 110°C	29	15	Mw—FT-IR, NMR, DSC, SEM	Antioxidant and anticoagulant activities	Seedevi et al. (2017)
Cuttlefish	1 N NaOH at 100°C for 60 min	1 N HCl at room temperature for 30 min	n.d.	2	n.d.	SEM, ^{1}H-NMR, ATR-IR, XRD,	Wound healing activity	Jung et al. (2018)
Cuttlefish	1 N NaOH at 100°C for 60 min	1 N HCl at room temperature for 30 min	33% NaOH at 90°C for 10–240 min	50	70	XRD, TGA, DSC	n.d.	Jung et al. (2019)
Pharaoh cuttlefish (*Sepia pharaonisi*)	NaOH at 80°C for 60 min	1.82% HCl at 3–4 h	40%–50% NaOH at 120°C for 60 min	27.76	39.45	UV–Vis, FT-IR, FT-Raman spectra, MALDI–TOF/MS, TGA, ^{1}H, ^{13}C, 2D COSY and 2D HSQC NMR	Anticoagulant activity	Karthik et al. (2016)
S. pharaonis	NaOH at 60°C for 3 h	4 M HCl at 90°C for 120 min	40% NaOH at 90°C for 120 min	n.d.	21.5	FT-IR, NMR, DSC, SEM	Antioxidant activity Anticoagulant activity	Jayalakshmi et al. (2016)
S. Pharaonis	NaOH at 60°C for 24 h	HCl at 25°C for 24 h	50% NaOH at 100°C for 120 min	20	50	DDA—85.55%, Mw—350.06 kDa, FT-IR, NMR, XRD	n.d.	Shushizadeh et al. (2015)

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TABLE 1 (Continued)

Common/scientific name	Extraction methods			Yield (%)		Physical and structural characterizations	Biological activities	References
	Deproteinization	Demineralization	Deacetylation	Chitin	Chitosan			
S. Pharaonis	10% (w/w) NaOH (2.5 M) for 24 h at 60°C	10% (w/w) HCl (1.04 M) for 24 h at 25°C	40% NaOH aqueous solution	56.47	n.d.	DDA—81.3%, FT-IR, SEM, ^{1}H NMR	Anti-oxidant activity	Hazeena et al. (2022)
***Sepia* sp.**	2 M NaOH for 20 h at 100°C	2 M HCl solution for 4 h at 50°C	n.d.	n.d.	n.d.	SEM, XRD, BET, TGA, FT-IR	n.d.	Kaya et al. (2016)
***Sepia* sp.**	1.0 M NaOH at 70°C for 24 h	0.25–1 M HCl at 90°C for 60 min	45% NaOH at 110°C for 10 h	n.d.	n.d.	FT-IR, XRD, TGA, SEM, NMR	n.d.	Al Sagheer et al. (2009)
Needle cuttlefish (*Sepia aculeata*)	2 M NaOH at 70°C for 4 h	1 M HCl at 60°C for 3 h	25 M NaOH for 20 h at 90 °C	21	49.71	FT-IR	Antioxidant activity	Vino et al. (2012)
Spineless cuttlefish (*Sepiella inermis*)	1 N NaOH at 80°C for 24 h	2 N HCl for 24 h	40% NaOH for 6 h at 110°C	16.67	18.75	DDA—79.64%, Mw—297 kDa, FT-IR, NMR, XRD, SEM	Antioxidant activity	Vairamani et al. (2013)
Shortclub cuttlefish (*Sepia brevimana*)	0.5 N NaOH for 16 h	1% HCl for 36 h at room temperature	50% NaOH for 2–3 h	n.d.	n.d.	DDA—82.92%, FT-IR, NMR, MALDI-TOF/MS	n.d.	Narasimman and Ramachandran (2023)
Squid								
Bigfin reef squid (*Sepioteuthis lessonianai*)	0.5 N NaOH for 16 h	1% HCl for 36 h in room temperature	50% NaOH for 3 h	40	50	FT-IR, NMR, MALDI—TOF	Anti-tuberculosis activity	Ramachandran et al. (2022)
S. lessoniana	1 N NaOH for 14 h	2 N HCl for 24 h	40% NaOH for 8 h	n.d.	n.d.	FT-IR	Antimicrobial activity	Subhapradha et al. (2013b)
S. lessoniana	1 N NaOH at 80°C for 24 h	2 N HCl for 24 h	40% NaOH for 8 h	n.d.	81	FT-IR	Antioxidant activity	Subhapradha et al. (2013c)
S. lessoniana	1 N NaOH at 80°C for 24 h	2 N HCl for 24 h	40% NaOH for 8 h	n.d.	90	DDA—88%, Mw—246 kDa, NMR	Anticoagulant and antioxidant activity	Subhapradha et al. (2013d)
S. lessoniana	1 M NaOH at 80°C for 24 h	2 M HCl for 24 h	40% NaOH for 6 h at 110°C	39.7	n.d.	DDA—88%, Mw—246 kDa, ^{1}H NMR, FT-IR, NMR, DSC, XRD, SEM	n.d.	Subhapradha et al. (2013e)
S. lessoniana	4% NaOH for 1 h	1% HCl at 80°C for 6 h	n.d.	n.d.	n.d.	XRD, FT-IR,	n.d.	Nagahama et al. (2008)

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TABLE 1 (Continued)

Common/scientific name	Extraction methods			Yield (%)		Physical and structural characterizations	Biological activities	References
	Deproteinization	Demineralization	Deacetylation	Chitin	Chitosan			
Argentine shortfin squid (*Illex argentinus*)	Alcalase 2.4L and Esperase 8L for 30 and 80°C	0.2 to 2 M NaOH for 25–75°C	30%–70% NaOH for 24 h at 90°C	n.d.	n.d.	DDA—93%, Mw—143–339 kDa	n.d.	Vázquez et al. (2017)
I. argentinus	1 M NaOH at room temperature for 24 h	n.d.	n.d.	31%	n.d.	DDA—96%, FT-IR, CP-MAS ^{13}C NMR, XRD	n.d.	Cortizo et al. (2008)
I. argentinus	10% NaOH at 100°C for 1 h	6% HCl for 2 h	50% NaOH at 60°C for 4 h	81.9	69.3	DDA-85.4%, Mw- 98.83 kDa, FT-IR, TGA, SEM, NMR	Antioxidant activity	Huang and Tsai (2020)
I. argentinus	NaOH at 120°C for 4 h	0.5 M HCl at 25°C for 30 min	40% NaOH at room temperature for 10 h	n.d.	26.1	Mw—377.1 kDa, DD—92.7, TGA, DSC, FT-IR, CD, SEM, POM	n.d.	Li et al. (2021)
I. argentinus	1 N NaOH at 80°C for 1 h	1 N HCl for 2 h	8 N NaOH for 30 min	n.d.	2.20	XRD, FT-IR	Antibacterial activity	Huang et al. (2018)
European squid (*Loligo vulgaris*)	Alcalase for 3 h at 50°C	1.5 M HCl for 2, 4, 6, and 8 h at 50°C	50% NaOH at 120°C for 4 h	31.2	19.52	^{13}C NMR, FT-IR, XRD	Antimicrobial activity Antioxidant activity	Abdelmalek et al. (2017)
L. vulgaris	1.0 M NaOH for 2, 6, or 24 h	n.d.	n.d.	n.d.	n.d.	XRD, FT-IR, SEM, AFM, TGA	n.d.	Ianiro et al. (2014)
L. vulgaris	0.3 M NaOH at 80–85°C for 1 h	0.55 M HCl for 2 h	40% NaOH for 80°C	40	n.d.	^{1}H NMR, CP/MAS ^{13}C NMR	n.d.	Tolaimate et al. (2000)
L. vulgaris	Deep eutectic solvents extraction at 100 and 120°C for 2 or 3 h	n.d.	n.d.	32.3%	n.d.	DDA—77 and 88%, ATR-FT-IR, TGA, SEM, XRD,	n.d.	McReynolds et al. (2022)
***Loligo* sp.**	n.d.	n.d.	Ultrasound irradiation at 50°C for 30 min	n.d.	75	NMR, SEM, XRD	n.d.	Delezuk et al. (2011)
Bigfin reef squid (*Loligo lessoniana*) and *Loligo formosana*	1.0 M NaOH at 50°C for 5 h	n.d.	50% NaOH at 100°C	35–38	n.d.	n.d.	n.d.	Chandumpai et al. (2004)
L. formosana	Ultrasonicated at amplitude 69% for 41.46 min at the solid/solvent ratio of 1:18	n.d.	50% (w/v) NaOH at 60°C for 12 h	34	65	DDA—78% to 90%, FT-IR, XRD, ^{1}H-NMR,	n.d.	Singh et al. (2019)
***Loligo* sp.**	1 M NaOH at 80°C for 3 h	n.d.	n.d.	32.5	n.d.	FT-IR, XRD	n.d.	Chaussard and Domard (2004)

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TABLE 1 (Continued)

Common/scientific name	Extraction methods			Yield (%)		Physical and structural characterizations	Biological activities	References
	Deproteinization	Demineralization	Deacetylation	Chitin	Chitosan			
Loligo chenisis	NaOH at 80°C for 10 h	n.d.	n.d.	35.8	n.d.	SEM, XRD, NMR, FT-IR	n.d.	Cuong et al. (2016)
Japanese squid (*Loligo Japonica*)	1% NaOH at 90°C for 3 h	n.d.	40% NaOH at 95°C for 6 h	n.d.	30	FT-IR, HPLC, XRD, NMR, AFS	Antiviral activity	He et al. (2016)
***Loligo* sp.**	NaOH at 80–85°C for 1 h	0.55 M HCl at 25°C for 2 h	n.d.	40–42	n.d.	XRD, FT-IR, SEM, TGA, AAS	n.d.	Lavall et al. (2007)
***Loligo* sp.**	3% NaOH for 3 days at room temperature	1.25 N HCl at room temperature for 1 h	50% NaOH at room temperature	n.d.	n.d.	n.d.	n.d.	Santhosh et al. (2010)
Squid pens	10% NaOH at 100°C for 1 h	6% HCl for 2 h	50% NaOH for 2 h at 80°C	n.d.	n.d.	FT-IR, XRD, TGA, SEM	Cu (II) ions adsorption capacity	Lin et al. (2017)
Squid pens	4.0% NaOH at 80°C for 10 h	0.8 M HCl at 30°C for 12 h	4.0% NaOH at 80°C for 4 h	n.d.	n.d.	TEM	Antibacterial activity	Van Hoa et al. (2021)
Squid pens	10% NaOH at 100°C for 1 h	6% HCl for 2 h	50% NaOH for 2 h at 80°C	24	70	Mw—963 Da, FT-IR	Antioxidant activity	Huang et al. (2012)
Squid pens	10% NaOH at 100°C for 1 h	6% HCl for 2 h	50% NaOH for 2 h at 80°C	34.43	n.d.	FT-IR, TEM	Antioxidant activity	Huang et al. (2013)
Squid pens	10% NaOH at 100°C for 1 h	6% HCl for 2 h	50% NaOH for 2 h at 80 °C	n.d.	n.d.	DDA—90%, FT-IR, ^{1}H NMR, XRD	Antioxidant activity	Huang et al. (2014)
Squid pens	10% NaOH at 100°C for 1 h	6% HCl for 2 h	50% NaOH for 2 h at 80°C	n.d.	n.d.	DDA—90%, FT-IR, ^{1}H NMR, ^{13}C NMR, XRD	Antioxidant activity	Huang et al. (2015)
Squid pens	4% NaOH at 10°C for 24 h	2 N HCl at room temperature for 6 h	n.d.	n.d.	n.d.	Mw—612 kDa, FT-IR, CP–MAS ^{13}C NMR, TGA, XRD, DSC	n.d.	Jang et al. (2004)
Squid pens	10% NaOH at 100°C for 1 h	6% HCl for 0.5 h	Ultrasonic (25 kHz) for 60°C at 12 h			DDA—71.32% to 92.91%, FT-IR	Antibacterial	Huang et al. (2011)
Jumbo squid (*Dosidicus gigas*)	n.d.	n.d.	40% NaOH at 90°C for 6 h 50% NaOH at 90°C for 6 h 50% KOH at 90°C for 4 h	n.d.	n.d.	DDA—93%	n.d.	Jung and Zhao (2011)
D. gigas	5% NaOH for 3 days at room temperature	40% HCl or NaOH for 1–4 h	NaOH (40% or 50%), temperatures (60 or 90°C), and reaction times (2, 4, or 6 h)	n.d.	n.d.	Mw—441,737 kDa, DDA—58.66%, XRD, FT-IR	n.d.	Jung and Zhao (2014)
D. gigas	1 M NaOH at 100°C for 3 h	1 M HCl at room temperature for 2 h	n.d.	30.2	n.d.	FT-IR, TGA, NMR, SEM, TEM, XRD	n.d.	Cabrera-Barjas et al. (2021)

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TABLE 1 (Continued)

Common/scientific name	Extraction methods			Yield (%)		Physical and structural characterizations	Biological activities	References
	Deproteinization	Demineralization	Deacetylation	Chitin	Chitosan			
D. gigas	2 M NaOH at 25°C for 12 h	1 M HCl at 25°C for 12 h	n.d.	n.d.	n.d.	XRD, ATR-FT-IR, XRF, TGA, SEM	n.d.	Moreno-Tovar et al. (2021)
D. gigas	1 M NaOH at room temperature for 24 h	n.d.	50% NaOH at 85–100°C for 2 h	38.6	n.d.	SEM	n.d.	Reys et al. (2013)
Loliginid squid (*Doryteuthis sibogae*)	1 N NaOH at 80°C for 24 h	2 N HCl for 24 h	40% NaOH for 6 h at 110°C	33.02	n.d.	FT-IR	n.d.	Barwin Vino et al. (2011)
Long barrel squid (*Doryteuthis singhalensis*)	NaOH at 60°C for 3 h	4 M HCl at 90°C for 120 min	40% NaOH at 90°C for 120 min	37.65	85	DDA—83.76%, Mw—226.6 kDa, FT-IR, NMR, CHN, SEM, DSC	Antioxidant and Anticoagulant properties	Ramasamy et al. (2017)
***Doryteuthis* spp.**	n.d.	n.d.	40% (w/w) aqueous NaOH ultrasound-irradiation for 50 min at 60°C	n.d.	88	^{1}H NMR, XRD	n.d.	Fiamingo et al. (2016)
Japanese flying squid (*Todarodes pacificus*)	1 M NaOH at 50°C for 5 h	1 M HCl for 2 h at room temperature	50% NaOH at 60°C for 2 h	0.0078	n.d.	XRD, EDX, FE-SEM	n.d.	Anusha et al. (2015)
T. pacificus	1 mol/L NaOH for 2 h at 90°C	0.1 mol/L HCl for 16 h at 15°C	n.d.	30	n.d.	XRD, FT-IR, HAADF-STEM, FE-SEM, TEM	n.d.	Suenaga et al. (2016)
T. pacificus	3% NaOH for 30 min at 121°C	1 N HCl for 30 min at room temperature	n.d.	25.5	n.d.	DDA—94.02%, FT-IR,	n.d.	Youn et al. (2013)
Indian Squid (*Uroteuthis duvauceli*)	1 M NaOH at 100°C for 20 min	1 M HCl at 50°C for 15 min	n.d.	n.d.	n.d.	FT-IR, NMR, XRD, SEM, TGA, DSC	n.d.	Balitaan et al. (2020)
Gastropoda								
Periwinkle shell (*Tympanotonus fusatus*) **Snail shell (*Achatina fulica*)**	0.4, 0.8, and 1.2 M NaOH at 100°C for 1 h	1.5, 1.7, and 1.9 M HCl at 32°C	n.d.	n.d.	n.d.	XRD, FT-IR, TGA, SEM, EDS	n.d.	Gbenebor et al. (2017)
Periwinkle shell (*T. fusatus*) **Snail shell (*Lissachatina fulica*)**	0.4, 0.8, and 1.2 M NaOH at 100°C for 1 h	1.5, 1.7, and 1.9 M HCl at 32°C	n.d.	n.d.	n.d.	DDA—50%, FT-IR, XRD, SEM, EDS	n.d.	Akpan et al. (2018)

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TABLE 1 (Continued)

Common/scientific name	Extraction methods			Yield (%)		Physical and structural characterizations	Biological activities	References
	Deproteinization	Demineralization	Deacetylation	Chitin	Chitosan			
Mangrove gastropod (*Nerita crepidularia*) (shell and operculum)	NaOH at 60°C for 3 h	4 M HCl at 90°C for 120 min	40% NaOH at 90°C for 120 min	23.91 31.14	35.43 44.29	FT-IR	n.d.	Palpandi et al. (2009)
Horn snail shell (*Telescopium telescopium*)	3% NaOH at 70°C for 48 h	5% HCl at room temperature for 48 h	60% NaOH for 4 h	42	n.d.	DDA—74.96%, FT-IR, XRD, SEM, TGA, EDX	Antibacterial activity	Rajathy et al. (2021)
Golden apple snail (*Pomacea canaliculata*)	1%–10% NaOH at 65–100°C for 48 h	HCl at room temperature for 2–3 h	40%–50% NaOH for 100°C	n.d.	17.48	SEM, EDS, XRD, FT-IR, NMR,	n.d.	Kaewboonruang et al. (2016)
Kentish snail shell (*Monacha cantiana*)	2%–4% NaOH for 1 h	1% HCl for 24 h	50% NaOH at 100°C for 2 h	n.d.	n.d.	FT-IR	n.d.	Abed et al. (2017)
Snail shells	1 M NaOH at room temperature for 14 min	1.2 M HCl at 80°C for 2 h	n.d.	n.d.	n.d.	FT-IR, SEM	n.d.	Oyekunle and Omoleye (2019a)
Snail shells	1 M NaOH for 2 h	1.2 M HCl for 2 h	n.d.	n.d.	39.69	DDA—54.98%, FT-IR	n.d.	Oyekunle and Omoleye (2019b)
Snail shells	2.0% KOH at 60°C for 24 h	2.5% HCl for 6 h at room temperature	40% NaOH for 2 h at 105°C	n.d.	n.d.	DDA—70%	n.d.	Adekanmi et al. (2023)
Sea snail (*Cerithidea obtuse*)	Natural deep eutectic solvent in the ratio of 1:10 (w/v) at 40°C	n.d.	n.d.	n.d.		DDA—91%, Mw—481 kDa, FT-IR, XRD, SEM	n.d.	Kimi and Hamdi (2023)
Sea snail (*Conus inscriptus*)	3 M NaOH at 80°C for 120 min	1 M HCl at 60°C for 30 min	50% NaOH at 100°C for 2 h	21.65	n.d.	Mw—25 kDa, CrI-82.13%, XRD, FT-IR, EDAX, SEM, TGA	n.d.	Mohan et al. (2019)
Rare Spined Murex (*Murex trapa*)	5% NaOH at 70°C for 48 h	1 M HCl at room temperature for 48 h	40% NaOH at 90°C for 6 h	n.d.	17	FT-IR, XRD, SEM, TG/DTA	anticoagulant and antibacterial activity	Anoop et al. (2022)
Sea snail (*Rapana venosa*) egg capsules	4%, 5%, or 6% NaOH for 65, 85, and 120 min at 80, 85, or 90°C	n.d.	n.d.	n.d.	7	DDA—75%, Fluorescence microscopy	n.d.	Dinculescu et al. (2023)
Giant African Snail (*Achatina fulica*)	n.d.	n.d.	Ultrasound-assisted extraction	n.d.	n.d.	FT-IR	n.d.	Adeboyejo and Oyesanya (2023)

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TABLE 1 (Continued)

Common/scientific name	Extraction methods			Yield (%)		Physical and structural characterizations	Biological activities	References
	Deproteinization	Demineralization	Deacetylation	Chitin	Chitosan			
Freshwater snail (*Belamya javanica*)	4% NaOH for 2 h	1 N HCl for 30 min	60% NaOH at 120°C for 1 h	21.76	n.d.	DDA—97.70%, FT-IR	n.d.	Hardani et al. (2021)
Green ormer (*Haliotis tuberculata*)	1 M NaOH for 8 h	1 M HCl at room temperature for 30 min	n.d.	n.d.	0.064	DDA—88%, FT-IR	n.d.	Zentz et al. (2001)
Polyplacophora								
Persian Gulf chiton shell (*Acanthopleura vaillantii*)	1 M NaOH (20 mL/g) at 70°C	1 M HCl for 3 h	5% NaOH at 110°C for 24 h	n.d.	n.d.	n.d.	Anticancer activity	Rasti et al. (2016)
Chiton shell	1 M NaOH at 70°C for 72 h	1 M HCl for 3 h	5% NaOH at 110°C for 5, 15, and 24 h	4.3	n.d.	DDA—90%, ^{1}H NMR, FT-IR, XRD, EDX, SEM	Antioxidant activity	Rasti et al. (2017)

Abbreviations: ^{1}H NMR, proton nuclear magnetic resonance; 2D COSY NMR, two-dimensional correlation nuclear magnetic resonance spectroscopy; 2D HSQC NMR, heteronuclear single quantum coherence spectroscopy; ATR-FT-IR, attenuated total reflection Fourier transform infrared spectroscopy; CP–MAS ^{13}C NMR, solid-state cross-polarization magic angle spinning carbon-13 nuclear magnetic resonance; CrI, crystallinity index; DDA, degree of acetylation; DSC, differential scanning calorimetry; EDX, energy-dispersive X-ray analysis; EDXRF, energy-dispersive X-ray fluorescence; FESEM, field emission scanning electron microscopy; FT-IR, Fourier-transform infrared spectroscopy; HAADF-STEM, high-angle annular dark-field scanning transmission electron microscopy; MALDI–TOF/MS, matrix-assisted laser desorption/ionization-time of flight mass spectrometry; Mw, molecular weight; n.d., not detected; SEM, scanning electron microscopy; TEM, transmission electron microscopy; TG/DTA, thermogravimetric/differential thermal analyzer; TGA, thermogravimetric analysis; UV–Vis, ultra violet–visible spectroscopy; XRD, X-ray diffraction analysis.

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5.1.4 | Deacetylation

The process of removing acetyl groups that are connected to chitin replaces them with reactive amino groups. The degree of deacetylation (DDA) can affect the percentage of free amino groups, which is considered as an essential factor that differentiates chitin and chitosan. Deacetylation increases the possibilities of producing chitosan in large quantities by converting chitin into chitosan using chemical procedures. This process was performed by conventional chemical deacetylation by alkali-NaOH (Čadež et al., 2018; He et al., 2016; Siswoyo et al., 2023; Zamri et al., 2020). It is suggested that alkali would be a preferable chemical alternative to acid. Furthermore, glycosidic linkages are extremely vulnerable to acid (Hajji et al., 2014).

5.2 | Green extraction methods

The utilization of NaOH at high concentration and temperature during the deproteinization process could deacetylate and depolymerize chitin and restrict their applications. Similarly, this requires a lot of energy and cause adverse effects to the environment (Gortari & Hours, 2013). Furthermore, chemicals utilized during the chitin extraction process spoil or degrade the nature of proteins, and it cannot be used as a food or feed applications (Xu et al., 2008). Therefore, greener extraction approaches are gaining popularity for their environment-friendly nature, safe, and reduced energy consumption. To date, few greener techniques like ultrasound-assisted and deep eutectic solvent extraction methods are used for its versatility in β-chitin production.

5.2.1 | Ultrasound-assisted extraction (UAE) method

The cavitation impact of ultrasound enhances the solubility of protein coupled with chitin, which could be due to depolymerization of macromolecules, dissociation of covalent links in polymer chains, and aggregation dispersion (Suryawanshi et al., 2020). The UAE method is simple, cost-effective, which can be applied to produce chitin in large quantities. It improves mass balance, shortens extraction time, reduces energy input and environmental impact compared to conventional extraction methods (Tiwari, 2015). Most importantly, very few studies evidenced the effectiveness of ultrasound based approaches to remove β-chitin and chitosan from the shells of mollusks (Delezuk et al., 2011; Fiamingo et al., 2016; Huang et al., 2011; Singh et al., 2019). Most importantly, chitosan extracted from squid pens using high-intensity ultrasonic signals reduced the extraction time, temperature, power consumption, and operation frequency of 20 kHz $\pm$ 50 Hz (Huang et al., 2011; Singh et al., 2019). On the other hand, β-chitin particles showed remarkable morphological changes as a result of ultrasound cavitation, that is, small particle size and enhanced *N*-deacetylation efficiency (Delezuk et al., 2011). Furthermore, the volume of β-chitin is influenced by the position of ultrasound probe on the surface this will enhance the conversion of 88% of β-chitin (from <10% of crude chitin) to chitosan (Fiamingo et al., 2016). Thus, further research investigations are required to optimize the UAE to increase the yields of β-chitin. Most importantly, this technique could serve as an alternative for chemical based extraction procedures in the near future.

5.2.2 | Deep eutectic solvent (DES) extraction methods

DES provides a sustainable alternative to the traditional approaches that are used to produce β-chitin. DES has the potential to minimize the multiple steps involved in the conventional chemical processes into single-step-approach. $CaCO_3$ is the main component of mollusk shell. The extraction process requires an acidic solution to act as a co-solvent hydrogen bond donor. Recently, McReynolds et al. (2022) reported that DES breaks strong hydrogen bonds between chitin amino groups and H^+ by using citric acid, malonic acid, and lactic acid. These green solvents are highly biodegradable and less or nontoxic nature compared to conventional alkali extraction (Zhang et al., 2012). DES coupled with potassium carbonate: glycerol enhances the purity of β-chitin from snail shell and squid pen at 100 or 120°C for 2 or 3 h. So far, this combination of solvents has been used only for α-polymorph. But, these results proved that DES is efficient β-polymorph as well (Kimi & Hamdi, 2023; McReynolds et al., 2022). Other physical properties such as acetylation degree and crystallinity improved between 77% and 88%, and 88% and 91%, respectively, and a maximum degradation temperature was around 350°C. DES used in this extraction was recovered and recycled over three times without modifying the final yield of chitin (30%) and C/N ratio (6.45–6.52). Other physical properties were also not modified, including DA and thermal stability (Td_{max} around 350°C). This process is proving that environmentally and economically beneficial compared to the alkali treatment. It is also important to optimize acid, neutral and alkali DES system to increase the final purity of β-chitin.

6 | PHYSICO-CHEMICAL CHARACTERIZATIONS

6.1 | Extraction yield

The extraction yield of β-chitin and chitosan from different mollusk sources is summarized in Table 1. The final β-chitin and chitosan may vary from species to species. The maximum yield was obtained from *Pinna bicolor* powder (100 g of grounded), that is, 80.15% chitin and 0.021% chitosan (Sudatta et al., 2020), whereas other species had less chitin yield, such as 39.7% β-chitin extracted from squid pens *Sepioteuthis lessoniana* (Subhapradha et al., 2013c), followed by cuttlefish bone (*Sepia aculeata*) had 21% chitin and 49.71% chitosan (after deacetylation) (Vino et al., 2012), *Sepia officinalis* yield 5% β-chitin (Hajji et al., 2014), *Sepia pharaonis* cuttle bone yielded 27.6% of β-chitin and 83% of chitosan (from chitin) (Karthik et al., 2016) similar amount recorded in *Sepia prashadi* cuttlebone 29% and 15% β-chitin and chitosan (Seedevi et al., 2017). All these extraction processes used

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acid-alkali (HCl and NaOH) treatment with variable concentration and temperature. Other common edible mollusk species recorded with 30%–49% of chitin and chitosan from Razor clam shell (*Ensis arcuatus*), West African clam *Egeria radiate*, *Pinna deltoids*, *Mytilus edulis*, *Laevicardium attenuatum*, horse mussel (*Modiolus modiolus*), Asian green mussel (*Perna viridis*), Donacid clam (*Donax scortum*), *Scylla serrata* carapace and *P. viridis* shells, Scallop shell (*Amusium* sp), *Scylla tranquebarica* (refer to Table 1). Overall, *P. bicolor* had a high chitin percentage compared to other reported species.

6.2 | Solubility

Chitin has less solubility due to its high cohesive energy between molecules in the solid state (George & Roberts, 1992). This makes chitin more resistant against variety of organic solvents, but chitosan has a high degree of solubility in dilute acidic solutions (<pH 6.0) (Chang et al., 2015; Kumari et al., 2017). The solubilizing properties of chitosan are associated with time, temperature, alkali ratio, Mw, crystalline nature, and acetylation degree (Araanz et al., 2021). The DDA and Mw affects the chitosan solubility in water (Qin et al., 2002). Chitosan has good solubilizing properties due to the presence of an amino group. The amino group contains active sites that influence the attraction of ionic compounds and soluble in inorganic solvents (Varma & Vasudevan, 2020). The dissolvability of chitosan increases by using ionic liquids that dissolves in plain water (Li et al., 2019). Generally, chitosan with high Mw exhibits poor solubility in neutral pH and limits its application potential (Blunt et al., 2018; da Salva et al., 2018). The presence of sulfate plays an important role in chitosan solubility (Ramachandran et al., 2022) but differs between mollusk species. Chitosan from *Telescopium telescopium* was soluble in 1% acetic acid, whereas chitin from *Conus inscriptus* displayed 72.35% solubility in 40% acetic acid solution (Mohan et al., 2019). The solubility of crab, pang scale, silver scale, and prawn chitin was 70.78%, 70.67%, 68%, and 58.33% respectively (Alabaraoye et al., 2018). Mussels' chitin had a highest solubility of 85.71% followed by squid pen and oysters chitin with 78% and 77.78%, respectively (Subhapradah et al., 2013; Huang et al., 2014). Chitin derived from crustacean sources are comparatively less soluble than mollusc chitin. Overall, these outcomes proved more than 70% solubility of mollusk chitin/chitosan is viable for many biodegradable applications.

6.3 | Molecular weight (Mw) distribution of β-chitin and chitosan from mollusk shells

The biological performance of chitin is directly linked to Mw and purity. It has been proven that extraction process and solvents influences the Mw of chitin. Chitosan extracted from *Periplaneta americana*, Orthoptera, *Leptinotarsa decemlineata*, *Mesobuthus gibbosus*, and *Tenebrio molitor* was observed to be 230,300 Da, 5.2–6.8 Da, 2.722–2.676, 3.22, and 308.3 kDa respectively (Kim et al., 2017; Kaya et al., 2014b, 2015, 2016; Song et al., 2018). Chitosan from *E. arcuatus* displayed a high Mw of 482.38 kDa (Zamri et al., 2020), compared to chitosan from *D. scortum*, *M. modiolus*, and *Sepia kobiensis* showed a molecular weight range of 373.80, 345.94, and 322.04 kDa (Ramasamy et al., 2014; Shanmugam et al., 2012; Varma & Vasudevan, 2020). Chitosan extracted from squid pen displayed highest Mw of β-chitin (a → b) was 1800 kDa and β-chitin (b → a) was 1150 kDa (Suenaga et al., 2016), conversely, α-chitin extracted from the shells of *C. inscriptus* displayed Mw of 25 kDa (Mohan et al., 2019). The sulfated chitosan from *S. lessoniana* through gamma irradiation (25, 50, and 100 kGy) displayed different Mw, including 1449, 1051, and 663 Da respectively (Ramachandran et al., 2022). Chitosan was extracted using high hydrostatic pressure (HHP) and untreated squid pen samples had low Mw of 98.83 and 136.11 KDa respectively (Huang & Tsai, 2020). Most interestingly, low Mw chitosan displayed stronger cytotoxic activity against squamous cell carcinoma (Wimardhani et al., 2014). Chitosan's with high Mw makes it insoluble in water and viscous, and this unique property is attractive and helpful in food packagaing application. According to the previous reports, low molecular weight shrimp chitosan exhibits effective bacterial inhibiting effects than the medium and high Mw chitosan (Du et al., 2009). In summary, the Mw of chitin and chitosan influences functional application. Low Mw chitin and chitosan from mollusk shells offer remarkable antibacterial and anticancer characteristics that can be used in the development of new pharmaceuticals.

6.4 | Degree of deacetylation (DDA)

The DDA of chitin and chitosan is the significant characteristic that influences the biological, physicochemical, and mechanical properties of the extracted material, and these attributes are reliant on the method of extraction (Khan et al., 2002). The DDA of chitin was 97.70%, 96%, 94.02%, 90%, 89.14%, 88%, 85.55%, and 74.35% in *Belamya javanica*, *Illex argentinus*, *Todarodes pacificus*, chiton shell, oyster shell *S. lessonianai*, *S. kobiensis*, and *D. scortum*, respectively (Cortizo et al., 2008; Handayani et al., 2018; Hardani et al., 2021; Ramasamy et al., 2014; Rasti et al., 2017; Shanmugam et al., 2012; Subhapradha et al., 2013; Youn et al., 2013). The DDA of chitosan was 88% in *Haliotis tuberculata* (Zentz et al., 2001), 83.76% in *Doryteuthis singhalensis* (Ramasamy et al., 2017), 93% in *Dosidicus gigas* (Jung & Zhao, 2011), 85.4% in *I. argentinus* (Huang & Tsai, 2020), 85.55% in *S. kobiensis* (Shanmugam et al., 2016), 71.8% in Scallop shell (Rokhati et al., 2017), and 89.14% in oyster shell (Alabaraoye et al., 2018). DDA can be determined by Fourier transform infrared spectroscopy (FT-IR) (Kaya et al., 2014a), conductometric (Khayrova et al., 2019), acid-base (Zhang et al., 2011), and potentiometric titration methods (Ma et al., 2015). Chitosan derived from fish, shrimp, and crab shells have DDA percentages of 75%, 78% and 70%, respectively (Kumari et al., 2017). The research findings that were discussed earlier have revealed that an increasing DDA of chitin and chitosan that can be employed as scaffolds for skin/tissue engineering and implantations in the field of biomedicine (Akpan et al., 2018).

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7 | STRUCTURAL CHARACTERIZATIONS

The structural characterization of β-chitin and chitosan from mollusk shells requires instruments including X-ray diffractometry (XRD), energy-dispersive X-ray spectroscopy (EDX), FT-IR, scanning electron microscopy (SEM), thermogravimetric analysis (TGA), and nuclear magnetic resonance spectroscopy (NMR). Identifying the structural components is necessary to optimize its extraction process as well as its suitable applications.

7.1 | Crystalline properties

XRD is a non-destructive method for characterizing crystalline materials that provide information on structural arrangements, the orientation of the crystals, grain size, and crystallinity. In this method, the X-rays filtered to produce monochromatic radiation on the material. The peak intensities represent specific angles from each set of lattice planes in the sample (Bunaciu et al., 2015). A total of 13 strong peaks, including two strong peaks between 23° and 50° and three strongest peaks at 29.3°, 19.63°, and 20°, were observed in pen shells (Sudatta et al., 2020), and a strong peak at 20.04° for horse mussel (Varma & Vasudevan, 2020) represents a denser crystalline structure of chitosan. In another study, Majekodunmi et al. (2017a) evidenced seven strong peaks and two highest peaks corresponding to silicon dioxide and calcium carbonate. A recent study by Varma et al. (2021) analyzed the chitosan from *P. deltoids*. The XRD analysis results displayed nine distinct peaks. The chitosan extracted from crab and squilla displayed two typical crystalline peaks at $2\theta = 10.3$ and 19.2; $2\theta = 10.2$ and 19.5, both of which were slightly displaced to a higher diffraction angle and revealed semi-crystalline chitosan (Anand et al., 2014). In conclusion, chitin and chitosan extracted from the mollusk shell wastes revealed more crystalline than amorphous nature of β-chitin and chitosan.

7.2 | Energy-dispersive X-ray spectroscopy (EDX)

The presence of C, H, and N in chitin and chitosan were determined using energy-dispersive X-ray spectroscopy (Scimeca et al., 2018). The mollusk shells derived chitin and chitosan comprises of 10.75%–48.40% carbon, 0.64%—7.02% hydrogen, and 2.90%–12.32% nitrogen (Cadano et al., 2021; Karthik et al., 2016; Ramasamy et al., 2014; Seedevi et al., 2017). The highest carbon content of 48.40% in *Crassostrea iredalei* (Cadano et al., 2021), 7.02% of hydrogen in *P. viridis* (Cadano et al., 2021), and 5.70% of nitrogen in *Sepioteuthis pharaonis* (Karthik et al., 2016) were documented. Hence, it can be concluded that the chitosan isolated from mollusk shells contains high C% followed by N and H. In addition, the carbon, hydrogen, and nitrogen analytical results for the chitin from crabs were found to be 6.03%, 42.9%, and 5.65%, respectively; from crayfish it was noticed as 6.09%, 42.88%, and 6.02%, respectively; and from shrimp it was confirmed as 6.17%, 43.2%, and 6.42% (Kaya et al., 2015). The N content of chitin is an essential indicator to determine the purity index, and it has been discovered that the pure (acetylated) chitin had 6.89% N; however, higher N indicates protein residue in chitin. However, <6.89% suggests inorganic elements are still present (Liu et al., 2012; Sajomsang & Gonil, 2010). Especially, N-rich source indicates the purity nature of chitin and chitosan.

7.3 | Fourier transform infrared spectroscopy (FT-IR)

FT-IR is a modern spectroscopic technique used for both qualitative and quantitative analyses of chitin and chitosan to differentiate or distinguish α-form and β-form by evidencing amide I band. In the α-form, the amide I band splits into two bands at the ranges of 1650 and 1620 cm^{-1} (Wang et al., 2013), whereas in the β-form, there is only one amide I band at the 1656 cm^{-1} region. Shanmugam et al. (2016) demonstrated that chitosan extracted from *S. kobiensis* confirmed the presence of bands corresponding to the functional groups such as NH_2 bonded with H, and OH (3435.37 cm^{-1}), aliphatic CH (2921.94 and 2852.95 cm^{-1}), C=O (1640.62 cm^{-1}), C–O–C (1020.99 cm^{-1}), and P=O (1386.30 cm^{-1}). However, chitosan from *S. officinalis* confirmed the presence of OH (3340 and 3350 cm^{-1}), C–O–C and C–O (1200-950 cm^{-1}), amide (1658, 1558, and 1627 cm^{-1}), and CH_X (1376 cm^{-1}) groups (Cadez et al., 2018). Chitosan isolated from *P. bicolor* displayed major vibrations ranging between 700 and 3000 cm^{-1} (Sudatta et al., 2020). Furthermore, the functional group corresponding to NH (711, 871, and 872 cm^{-1}) stretching vibrations, C–O–C (1016 cm^{-1}), amide I and II (1741 and 1395 cm^{-1}), aliphatic CH (2855 cm^{-1}), asymmetric CH_2 (2926 cm^{-1}), and OH (3448 cm^{-1}). Varma and Vasudevan (2020) documented five peaks between the frequency range of 4000 and 400 cm^{-1} which corresponding to NH, C–O (564 cm^{-1}), NH (711 cm^{-1}), C–O–C (1174 cm^{-1}), CH2 (2685 cm^{-1}), and OH (3594 cm^{-1}) in the chitosan of *M. modiolus*. Furthermore, Hajji et al. (2015) highlighted four peaks at the frequency ranges of 3490, 2800–3100, and 1558 cm^{-1} that correspond to OH-stretching vibrations that overlap the NH, CH, and NH of chitosan isolated from *S. officinalis*. The FT-IR spectrum of squilla, crab, krill, lobster, and shrimp chitin and chitosan exhibits I band at the positions of 1643, 1634, 1625, 1628 and 1667 cm^{-1}, respectively (Anand et al., 2014; Mohan et al., 2021; Sayari et al., 2016; Srinivasan et al., 2018; Wang et al., 2013). Overall, the functional group determination results suggested that the chitin and chitosan extracted from mollusk shells are in β-form.

7.4 | Scanning electron microscopy (SEM)

SEM is a useful technique for determining the surface morphology of chitin and chitosan. The chitosan isolated from the mollusk *M. edulis* and *L. attenuatum* showed uneven size and shape at low magnification and brick-like structures at high magnification (Majekodunmi et al., 2017b). Varma et al. (2021) identified a porous free smooth texture of chitosan isolated from *Plebidonax deltoides*, whereas *S. kobiensis* chitosan showed flakey, highly porous, and fibril structures

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(Ramasamy et al., 2014). A rough surface on chitosan was observed at a higher magnification of *M. edulis* (Majekodunmi et al., 2017b); however, uneven particle size and shape were observed under low magnification. The chitosan from *S. prashadi* showed porous bridge shaped crystalline particles (Seedevi et al., 2017). Vairamani et al. (2013) observed and reported highly porous structure of chitosan isolated from cuttlebone. However, the chitosan isolated from *P. deltoides* showed a porous-free smooth texture under SEM micrographs. Chitin from conus shell had microfibrillar crystalline structure with pores (Mohan et al., 2019). Moreover, chitin extracted from krill, shrimp, and lobster shells was also found to contain the similarly densely packed fibers (Al Sagheer et al., 2009; Srinivasan et al., 2018; Wang et al., 2013). Surface morphology is one of the essential factors that determines the efficient usage of chitin and chitosan. Chitin and chitosan in their nanofiber and nanopore forms may have uses in textiles, food, and medicine (Aranaz et al., 2009; Synowiecki & Al-Khateeb, 2003).

7.5 | Thermogravimetric analysis

The thermal stability based on the amount of mass that is lost at two different stages. The loss that occurs in the first step can be attributed to the water molecules during evaporation in the chitin and chitosan molecules, and the loss that occurs in the second step can be attributed to the degradation of chitin and chitosan (Ofem, 2015). The first loss of the chitin and chitosan from different mollusk shells ranged from 5% to 9.61%, whereas the second mass loss ranged from 51.9% to 63.5% (Aylanc et al., 2020; Cabrera-Barjas et al., 2021; Huang & Tsai, 2020; Ianiro et al., 2014; Kaya et al., 2016; Mohan et al., 2019; Varma et al. 2021). The maximum temperatures (DTG max) for the degradation of chitin and chitosan from several mollusk shells ranged from 380 to 444°C (Mohan et al., 2019; Rajathy et al., 2021). In the TGA analysis of chitosan obtained from crab and squilla, the process of mass loss occurred in three stages: The first stage was observed at temperatures below 100°C; the second stage had occurred at temperatures between 213 and 269°C; and the third stage had occurred at temperatures between 350 and 384°C (Anand et al., 2014). In addition, De Andrade et al. (2012) have reported that the maximum decomposition temperature range for crab chitosan is between 400 and 500°C. This thermal stability of chitosan was influenced by Mw, and crystallinity (Kumari et al., 2017; Kaya et al., 2016). Overall, it could be evidenced that chitin and chitosan from mollusk shells showed better thermal stability at >350 °C.

7.6 | Nuclear magnetic resonance spectroscopy

NMR spectroscopy is used to identify and characterize chemical compounds. The nature of neighboring units determines the change in the resonance signal. The presence of hydrogen bonds in the molecules shifts the resonance signal to lower fields (Zia et al., 2019). Ramasamy et al. (2014) investigated the chitosan from *S. kobiensis* showed four peaks at 1.95–1.97, 3.05–3.09, 3.65–3.68, and 3.81–3.85 ppm, which represent three protons of *N*-acetyl glucosamine, H-2 proton of glucosamine, and non-anomeric proton, respectively. Similar units were identified in *S. prashadi* chitosan at 3.503–3.583, 3.002–3.044, 2.983, and 1.918–1.923 ppm that indicate the presence of glucosidic amide proton, sulfated amide proton, H_2 of GlcN, N-alkylated GlcN, and unfold amide of α-carbon proton, respectively (Seedevi et al., 2017). The ^{13}C CP/MAS NMR spectra of the insect cicada slough chitin spectrum have eight well-defined peaks of C1–C6, CH3, and C=O carbons. These peaks can be identified by a chemical shift that ranges from 20 to 190 ppm (Sajomsang & Gonil, 2010). The peaks identified in the NMR studies revealed that they can be used to characterize, identify, and confirm the presence of chemical molecules in the chitin and chitosan extracted from various mollusk species.

8 | FUNCTIONAL PROPERTIES

Edible mollusk shells chitin and chitosan exhibited a wide range of biological functions that include antibacterial, antifungal, antiviral, antituberculosis, anticoagulant, antioxidant, anticancer, and anti-obesity properties (Adhikari & Yadav, 2018; He et al., 2016; Ramasamy et al., 2022; Van Hoa et al., 2021). The valorization of functional ingredient from mollusk shell waste is shown in Figure 2.

8.1 | Antioxidant and anticoagulant activities

The antioxidant property of any natural compound is determined by its potency against free radical scavenging properties. Chitosan from *Sepia aculeata* cuttle bones exhibits potent antioxidants against DPPH and hydroxyl radicals at 88.6% and 72%, respectively (Vino et al., 2012). However, other species of cuttlefish *S. prashadi* exhibited 64.5% and 83.5% against hydroxyl and superoxide radical scavenging activity. This difference could be due to alkali concentration, time, and temperature applied in extraction process that influences the antioxidant percentage. Less DDPH scavenging action of 38.92% was observed from shells of *D. scortum* (Shanmugam et al., 2012). These chitin displayed both anticoagulant and antioxidant activity. They exhibited partial thromboplastin time (PTT) and PT assay value of 6.90 and 1.2 IU/mg, respectively. Most importantly, chitosan exhibited anticoagulant properties in human plasma with PTT of 1.73 and activated a PTT of 6.45 IU (Subhapradha et al. 2013d). However, increasing the pressure of 300 MPa/min and a release time of <3 s through HHP increases antioxidant activity of 98 %and 99% against DDPH and the chelating ability, comparatively HHP process displayed better antioxidant activity (Huang & Tsai, 2020) to the conventional acid/alkali method. These antioxidant properties is essential for food application to preserve food and also act as a functional ingredient while consuming. However, the percentage of mollusk chitin addition in food is lacking. It is therefore recommended to identify the concentration dependent mode of action in animal models.

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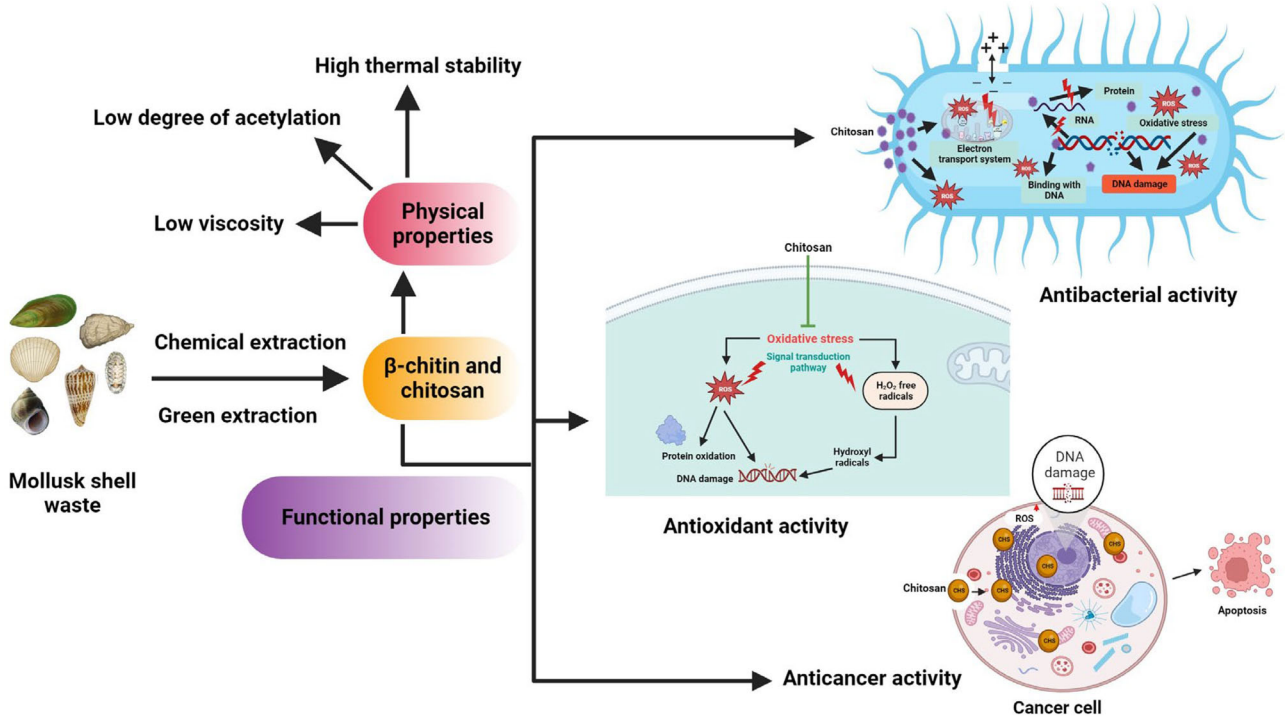

FIGURE 2 Valorization of functional ingredient from mollusk shell waste.

8.2 | Antimicrobial activities

Chitin and its derivatives have been well recognized for their antimicrobial potential (Benhabiles et al., 2012; Rakkhumkaew & Pengsuk, 2018). For instance, Abdelmalek et al. (2017) evaluated the antibacterial efficacy of β-chitosan against a wide range of pathogens, including *Salmonella enterica*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Bacillus cereus* at 49 mg/mL concentration on the selected pathogens. They evidenced that chitosan has the ability to inhibit the growth of these pathogens at 0.5–2.3 mm zone of inhibition. Moreover, the antibacterial effectiveness of chitosan and phosphorylated chitosan from *S. kobiensis* has been evaluated (Shanmugam et al. 2016) against *Proteus vlulgaris* and *Staphylococcus aureus* showed 17 and 16 mm inhibition at 100% concentration. However, 50% chitin concentration had less inhibition of 14 mm against the same bacterial species. The gamma-irradiated sulfated chitosan 100 Gy (GIR-SCH) displayed anti-tuberculosis effects against *Mycobacterium smegmatis* (Ramachandran et al., 2022). Most interestingly, it displayed three folds higher action than the commercial chitosan but less action against commercial drug rifampin. Chitosan from *S. kobiensis* displayed potent action against *Vibrio cholerae* with 7 mm zone of inhibition (Ramasamy et al., 2022). At a low concentration of 200 μg/mL chitin, *P. bicolor* shells had displayed better antibacterial activity against G +ve and G −ve pathogens (Sudatta et al., 2020). For instance, the highest zone (17 $\pm$ 0.5 mm) of inhibition was observed in cuttlefish bone-derived chitosan against *E. coli*, which comparatively higher than crustaceans chitosan (14 $\pm$ 0.3 mm) (crab) and shrimp-derived chitosan (10 $\pm$ 0.8 mm) (Hajji et al., 2015). Similarly, He et al. (2016) evaluated in vitro effects of β-chitosan against Newcastle disease virus that had a potency to enhance immune function. The mechanism involved in antimicrobial properties are cell wall lysis, release of intracellular components, including protein and genetic materials. Meanwhile, chitosan interacts with the outer membrane and forms vesicles like structure and leads to cell wall disruption (Helander et al., 2001).

8.3 | Anticancer activity

The anticancer action of β-chitin was not much evaluated on various cancer cell lines. As the main focus was given to α-chitin that was found to be well established on various tumor cells and also commercialized in pharmaceutics. There are few research outcomes proven the efficacy of β-chitin/chitosan action on tumor cells such as RT112, and HepG2 (Hajji et al., 2015; Rasti et al., 2016). Cuttlefish chitosan's low AD (<20%) holds promising industrial use. It exhibited superior activity against human bladder cancer cells, with an EC_{50} of 62 μM after 48 h and 50 μM after 72 h of RT112 cell incubation. In contrast, crab chitosan (AD: 12%; M_W: 6120 g/mol) had EC_{50} values of 100 μM after 48 h and 50 μM after 72 h. Therefore, this confirms that low Mw, water-soluble chitosan forming low viscous solution likely inhibits tumor growth (sarcoma in mice) by promoting T-cell proliferation and enhancing cytotoxic activity against tumors through induced

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lymphocyte cytokines, leading to enhanced natural killer cell activity, contributing to their antitumor effects.

8.4 | Wound-healing activity

Karthik et al. (2016) demonstrated that low Mw-sulfated chitosan from *S. pharaonis* showed cytostatic activity, followed by anticoagulant and antiviral activities. Similarly, β-chitin from cuttlefish bone exhibited wound-healing properties against skin damage that reduces wound radius on the rat models (Jung et al., 2018). To obtain a better functionality of chitin, electrospinning or nanopartilces will improve chitin action on the target site. However, the mode of action, molecular mechanisms, and effectiveness of chitosan may provide better insight to identify the unknown or novel mechanisms in future.

9 | FOOD INDUSTRIAL APPLICATION

The food industry generates substantial effluents during processing, which need to be treated/adsorped before eliminating to the environment. Chitin and chitosan have gained attention for their adsorption properties and low cost. Furthermore, presence of amino and hydroxyl groups significantly absorbs the toxic effluents. Their unique chemical structure (β-chitin), stability, chelation behavior, high reactivity, and selectivity toward contaminants make them excellent for adsorption (Bhatnagar & Sillanpää, 2009; Boamah et al., 2015). Recent studies have explored mollusk-derived β-chitin and chitosan as adsorbents for wastewater contaminants (Kavisri et al., 2023; Nouj et al., 2022; Siswoyo et al., 2023). For instance, chitosan from cephalopod waste proved effective in removing cadmium (Cd) from aqueous solutions, with optimal conditions at pH 7, 42.5°C, 220 min, and 1 g/L sorbent dosage (Kavisri et al., 2023), highlighting the role of surface pores in adsorption. Nouj et al. (2022) demonstrated that β-chitin from cuttlefish bone significantly reduced turbidity, BOD, and COD in food processing wastewater. Similarly, Siswoyo et al. (2023) found that chitosan from blood cockle shells reduced total suspended solids and turbidity in well-water. These findings suggest that mollusk-derived β-chitin and chitosan serve as natural coagulants, promising biopolymers and potential alternatives for replacing the chemical coagulants. However, there is a need for further research on their ecological impact in wastewater treatment from various industrial sectors.

10 | LIFE CYCLE ASSESSMENT (LCA) OF MOLLUSK SHELL FOR β-CHITIN PRODUCTION

Life cycle assessment (LCA) is a technique to measure the total carbon footprint from land-use change, carbon uptake, biogenic carbon emission, and so on (ISO14040/14044) during the production process from cradle to grave. The amount of energy involved, the use of operational materials in the production process, and its waste disposal will determine the environmental effect through LCA. Environmental footprint of producing chitin from crustacean's shells (Muñoz et al., 2018) and mussel $CaCO_3$ production (Iribarren et al., 2010a, 2010b) were studied earlier in LCA. However, limited information is available on the carbon-footprint of chitin from mollusk shell waste. LCA inventory analysis starts from hatchery, seedling, cultivation, formation of shells, harvesting, and extraction processes involved in the entire value-chain.

The cultured mollusks such as mussels, clams, and oysters (de Alvarenga et al., 2012; Filgueira et al., 2015; Vélez-Henao et al., 2021) were potent carbon sequestration that uptake eutrophication agents like nitrogen and phosphorus, which positively affects the biodiversity of marine ecosystem. The shell formation is influenced by various abiotic factors such as pH, temperature, and salinity of the growth media. The fluctuation in these factors affects C, N, and P fixation in the clam-shell, which releases CO_2, and climate change (CC) impact category during the formation of the shells. Nevertheless, it was balanced during respiration by C fixation in the shell, and a biogenetic precipitation of $CaCO_3$ (Filgueira et al., 2015). Upto 57% CC and 59% acidification were observed at the shell-forming stage. Followed by the post-harvesting steps such as farming, washing, cleaning, and grading accounts 38% CC and 31.8% acidification of the total environmental impacts. At the industrial processing stage, the strong acid is used for the extraction process that consumes energy (fuel and electricity) up to 5% acidification and 35% ozone layer depletion (Vélez-Henao et al., 2021). Therefore, it can be extrapolated that the purification of fresh mussels became key contributor to environmental impact. However, 100 t of mussel shells produce 65 t of $CaCO_3$ with 44 t of debris (Iribarren et al., 2010b), and the remaining can produce 10%–20% of chitin (Table 1) depending on the origin. Followed by the next step of producing chitin by extracting them using HCl and NaOH for hydrolysis, for instance, 10 kg of feedstock material should be treated with 9 kg HCl and 8 kg NaOH in 300 L water, which could yield 1 kg of chitin. This process releases 0.9 kg CO_2 per kg chitin based on their carbon content and stoichiometry. The entire process, including power, type of power grid, other materials involved in the process, will be evaluated. The majority of emission comes from wastewater as NaOH, HCl consumption and disposal lead to CC and acidification and ammonia emission. These emissions also depend on other impurities like sand, sediments, and organic debris, associated with the pretreatment process, which will influence the wastewater disposal treatment. The chitin recovery process contributes to acidification, photochemical smog, and global warming potential (Zuorro et al., 2020). The LCA for chitin and chitosan production from mollusk shells is shown in Figure 3.

11 | TECHNO-ECONOMIC ASSESSMENT (TEA) FOR CHITIN PRODUCTION

TEA was carried out to test product and process benefit pathways. Based on this profitability, process optimization, production costs, and long-term payback will be estimated. The conversion of shell waste into chitosan is a a multi-step process: raw material pretreatment involving washing and grinding, followed by depigmentation with ethanol, demineralization with HCl, and deproteinization with NaOH to produce

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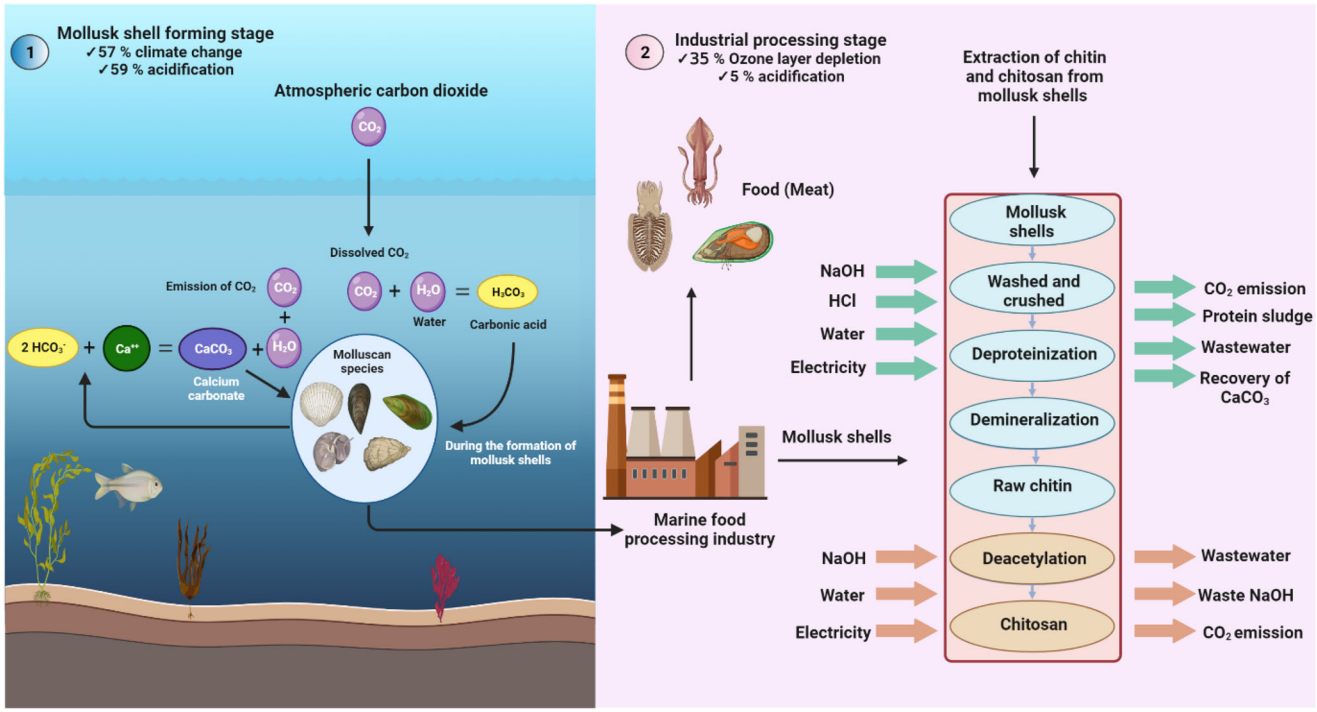

FIGURE 3 Life cycle assessment of chitin and chitosan production from mollusk shells.

chitin. The final step involves deacetylation using NaOH to obtain chitosan (Okoro et al., 2023). The novelty lies in scaling up this chitosan production process from mollusks shell waste, previously developed in the lab, and applying a TES sensitivity assessment to evaluate the impact of economic changes on the process conditions. This economic evaluation involves equipment costs, primarily heat exchangers, washing tanks, a crusher, reactors, and dryer, which are the highest contributors to direct fixed capital investment (DFCI). The raw material cost encompasses cleaning the mollusk exoskeleton (removal of debris), transportation to the negiboring plant, reagents for depigmentation, demineralization, deproteinization, deacetylation, and neutralization, along with catalyzers for necessary reactions. It is advisable that chitosan cost remains within the range of 50,000–120,000 USD/ton in the North Colombian context (Cogollo-Herrera et al., 2018). DFCI impacts operational expenses and makes large-scale production economically challenging. Implementing an integrated biorefinery approach using water pinch analysis can reduce water and recycled NaOH and HCl solution consumption in chitosan production by 80% (Zuorro et al., 2020). Although this method may decrease the production capacity by 28%, it can yield a 65.88% return on investment with a complete investment recovery in 6 years when processing 4113.09 tons/year of fresh shrimp. A similar approach could be applied to mollusk shell biorefineries to optimize costs.

12 | BOTTLENECKS AND POSSIBLE SOLUTION

At the biorefining stage, identifying an efficient solvent combination to replace HCl and NaOH was in the rudimentary stage, though few lab-scale studies showed better performance on green chemicals; it is a high-end technology to upscale. Besides, β-chitin is a highly crystalline structure that is relatively resistant to enzymatic degradation, thus limiting the extraction process through EAE. The tightly packed β-chitin chains require specialized extraction methods to break down the structure and release the chitin molecules. This factor also influences solubility, as they are insoluble in most common solvents, which limits the reaction time with green chemicals and processability. Similarly, β-chitin was found less stable than α-chitin, and thermal decomposition occurs between 25 and 250°C, with an exothermic peak of 230°C compared to its counterparts 330 and 310°C (α- and γ-chitin) (Jang et al., 2004). This allows β-chitin as a low activation energy relatively at a low temperature and insensitive. Another constraint is obtaining byproducts or side-streams generated during β-chitin extraction (less than 20%–25%), as the residual waste (more than 75%–80%) comprises of proteins, and minerals, mixed with debris, and sand, making the separation process complicated. However, the electrostatic attraction created by using selective adsorbents like Fe_3O_4/polyaniline composite (Li et al., 2022), and ion exchange using expanded bed adsorption (Shahid et al., 2021) had proven to recover a high percentage of protein from the processing wastewater. Transitioning to larger scale production while maintaining quality and cost-effectiveness can be complex. Nevertheless, the availability of mollusk shells was limited to specific regions, making it difficult for mass production of chitin and chitosan. In the national and regional context, mollusk shells were used for compost production and the rest accumulated in a landfill. Food and Agriculture Organization (FAO) data shows that 600,000 t of mussels have been used in the European market, with 500,000 t from domestic origin and 100,000 t of international origin. The

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European region's per capita consumption of mussels was between 200 g and 4 kg (FAO, 2023). However, there was a 20% decline in mussel production in the EU region, although it represents more than one third of aquaculture production. With integrated multi-trophic aquaculture techniques involving mollusks in farming might increase the production of raw material (shells). The brightside is that farmed mollusks have a low negative impact and carbon footprint on the environment compared to wild harvest species like squid pens for this reason. Therefore, environmentally sustainable methods for β-chitin extraction must be developed to ensure its long-term viability.

AUTHOR CONTRIBUTIONS

Durairaj Karthick Rajan: Conceptualization; writing—original draft; writing—review and editing. **Kannan Mohan**: Conceptualization; writing—original draft; writing—review and editing. **Jayakumar Rajarajeswaran**: Writing—original draft. **Dharmaraj Divya**: Writing—original draft. **Ragavendhar Kumar**: Writing—original draft. **Sabariswaran Kandasamy**: Writing-original draft. **Shubing Zhang**: Supervision. **Abirami Ramu Ganesan**: Supervision; visualization; writing and reviewing.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflicts of interest.

ETHICS STATEMENT

We have not done any experiments using animal or human in this review article.

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REFERENCES

Abdelmalek, B. E., Sila, A., Haddar, A., Bougatef, A., & Ayadi, M. A. (2017). β-Chitin and chitosan from squid gladius: Biological activities of chitosan and its application as clarifying agent for apple juice. *International Journal of Biological Macromolecules*, *104*, 953–962.

Abdulkarim, A., Isa, M. T., Abdulsalam, S., Muhammad, A. J., & Ameh, A. O. (2013). Extraction and characterisation of chitin and chitosan from mussel shell. *Civil and Environmental Research*, *3*(2), 108–114.

Abed, E. H., Al Ameen, N. İ., & Jazaa, L. A. (2017). Extraction of chitosan from Kentish snail exoskeleton shell's, *Monacha cantiana* (Montagu, 1803) for the pharmaceutical application. *Journal of International Environmental Application and Science*, *12*(2), 125–130.

Abedi, E., & Sahari, M. A. (2014). Long-chain polyunsaturated fatty acid sources and evaluation of their nutritional and functional properties. *Food Science and Nutrition*, *2*(5), 443–463.

Adeboyejo, F. O., & Oyesanya, O. B. (2023). Ultrasound and autoclave-deacetylated *Achatina fulica* shell chitosan: Characterisation and effect on tomato and cucumber fruit qualities during storage. *Food Chemistry*, *415*, 135750.

Adekanmi, A. A., Adekanmi, U. T., Adekanmi, A. S., Ahmad, L. K., & Emmanuel, O. O. (2023). Production and characterisation of chitosan from chitin of snail shells by sequential modification process. *African Journal of Biotechnology*, *22*(2), 39–53. https://doi.org/10.5897/AJB2020.17135

Adhikari, H. S., & Yadav, P. N. (2018). Anticancer activity of chitosan, chitosan derivatives, and their mechanism of action. *International Journal of Biomaterials*, 2952085.

Akpan, E. I., Gbenebor, O. P., & Adeosun, S. O. (2018). Synthesis and characterisation of chitin from periwinkle (*Tympanotonus fusatus* (L.)) and snail (*Lissachatina fulica* (Bowdich)) shells. *International Journal of Biological Macromolecules*, *106*, 1080–1088.

Al Sagheer, F. A., Al-Sughayer, M. A., Muslim, S., & Elsabee, M. Z. (2009). Extraction and characterization of chitin and chitosan from marine sources in Arabian Gulf. *Carbohydrate Polymers*, *77*(2), 410–419.

Alabaraoye, E., Achilonu, M., & Hester, R. (2018). Biopolymer (Chitin) from various marine seashell wastes: Isolation and characterization. *Journal of Polymers and the Environment*, *26*, 2207–2218.

Alimi, B. A., Pathania, S., Wilson, J., Duffy, B., & Frias, J. M. C. (2023). Extraction, quantification, characterization, and application in food packaging of chitin and chitosan from mushroom: A review. *International Journal of Biological Macromolecules*, *237*, 124195.

Anand, M., Kalaivani, R., Maruthupandy, M., Kumaraguru, A., & Suresh, S. (2014). Extraction and characterization of chitosan from marine crab and squilla collected from the Gulf of Mannar Region, South India. *Journal of Chitin and Chitosan Science*, *2*(4), 280–287.

Anoop, A., Gobinath, T., & Ravichandran, S. (2022). Physicochemical properties and structural characterization of chitosan synthesized from rare spined murex, *Murex trapa* (Roding, 1798) shell waste. *Research Journal of Pharmacy and Technology*, *15*(12), 5729–5735. doi :10.52711/0974-360X.2022.00966

Anusha, J. R., Fleming, A. T., Kim, H. J., Kim, B. C., Yu, K. H., & Raj, C. J. (2015). Effective immobilization of glucose oxidase on chitosan submicron particles from gladius of *Todarodes pacificus* for glucose sensing. *Bioelectrochemistry*, *104*, 44–50.

Aranaz, I., Alcántara, A. R., Civera, M. C., Arias, C., Elorza, B., Heras Caballero, A., & Acosta, N. (2021). Chitosan: An overview of its properties and applications. *Polymers*, *13*(19), 3256.

Aranaz, I., Mengíbar, M., Harris, R., Panos, I., Miralles, B., Acosta, N., & Heras, A. (2009). Functional characterization of chitin and chitosan. *Current Chemical Biology*, *3*(2), 203–230.

Arbia, W., Arbia, L., Adour, L., & Amrane, A. (2013). Chitin extraction from crustacean shells using biological methods—A review. *Food Technology and Biotechnology*, *51*(1), 12–25.

Aylanc, V., Ertosun, S., Akyuz, L., Bilican, B. K., Gokdag, S., Bilican, I., Cakmak, Y. S., Yilmaz, B. A., & Kaya, M. (2020). Natural β-chitin-protein complex film obtained from waste razor shells for transdermal capsaicin carrier. *International Journal of Biological Macromolecules*, *155*, 508–515.

Balitaan, J. N. I., Yeh, J. M., & Santiago, K. S. (2020). Marine waste to a functional biomaterial: Green facile synthesis of modified-β-chitin from *Uroteuthis duvauceli* pens (gladius). *International Journal of Biological Macromolecules*, *154*, 1565–1575.

Barwin Vino, A., Ramasamy, P., Vairamani, S., & Shanmugan, A. (2011). Physicochemical characterization of biopolymers chitin and chitosan extracted from squid *Doryteuthis sibogae* adam, 1954 pen. *International Journal of Pharmaceutical Research and Development*, *2*(12), 181–190.

Benamar, M. M., Ammar-Khodja, N., & Adour, L. (2022). Biopolymers isolation (chitin and chitosan) from mushroom biomass of

26438429, 2024, 1, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/fft2.326, Wiley Online Library on [28/01/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Pleurotus ostreatus (Jacq: Fries) Kummer. *Algerian Journal of Environmental Science and Technology*, *8*(3), 2611–2617.

Benhabiles, M. S., Salah, R., Lounici, H., Drouiche, N., Goosen, M. F. A., & Mameri, N. (2012). Antibacterial activity of chitin, chitosan and its oligomers prepared from shrimp shell waste. *Food Hydrocolloids*, *29*(1), 48–56.

Bhatnagar, A., & Sillanpää, M. (2009). Applications of chitin-and chitosan-derivatives for the detoxification of water and wastewater—A short review. *Advances in Colloid and Interface Science*, *152*(1–2), 26–38.

Blunt, J. W., Carroll, A. R., Copp, B. R., Davis, R. A., Keyzers, R. A., & Prinsep, M. R. (2018). Marine natural products. *Natural Products Reports*, *35*, 8–53. https://doi.org/10.1039/C7NP00052A

Boamah, P. O., Huang, Y., Hua, M., Zhang, Q., Liu, Y., Onumah, J., Wang, W., & Song, Y. (2015). Removal of cadmium from aqueous solution using low molecular weight chitosan derivative. *Carbohydrate Polymers*, *122*, 255–264.

Bradić, B., Novak, U., & Likozar, B. (2019). Crustacean shell bio-refining to chitin by natural deep eutectic solvents. *Green Processing and Synthesis*, *9*(1), 13–25.

Bunaciu, A. A., UdriŞTioiu, E. G., & Aboul-Enein, H. Y. (2015). X-ray diffraction: Instrumentation and applications. *Critical Reviews in Analytical Chemistry*, *45*(4), 289–299.

Cabrera-Barjas, G., González, C., Nesic, A., Marrugo, K. P., Gómez, O., Delattre, C., Valdes, O., Yin, H., Bravo, G., & Cea, J. (2021). Utilization of marine waste to obtain β-chitin nanofibers and films from giant Humboldt squid *Dosidicus gigas*. *Marine Drugs*, *19*(4), 184.

Cadano, J. R., Jose, M., Lubi, A. G., Maling, J. N., Moraga, J. S., Shi, Q. Y., Vegafria, H. M., & VinceCruz-Abeledo, C. C. (2021). A comparative study on the raw chitin and chitosan yields of common bio-waste from Philippine seafood. *Environmental Science and Pollution Research*, *28*(10), 11954–11961.

Čadež, V., Šegota, S., Sondi, I., Lyons, D. M., Saha, P., Saha, N., & Sikirić, M. D. (2018). Calcium phosphate and calcium carbonate mineralization of bioinspired hydrogels based on β-chitin isolated from biomineral of the common cuttlefish (*Sepia officinalis*, L.). *Journal of Polymer Research*, *25*(10), 1–12.

Chandumpai, A., Singhpibulporn, N., Faroongsarng, D., & Sornprasit, P. (2004). Preparation and physico-chemical characterization of chitin and chitosan from the pens of the squid species, *Loligo lessoniana* and *Loligo formosana*. *Carbohydrate Polymers*, *58*(4), 467–474.

Chang, S. H., Lin, H. T. V., Wu, G. J., & Tsai, G J. (2015). pH effects on solubility, zeta potential, and correlation between antibacterial activity and molecular weight of chitosan. *Carbohydrate Polymers*, *134*, 74–81.

Chaussard, G., & Domard, A. (2004). New aspects of the extraction of chitin from squid pens. *Biomacromolecules*, *5*(2), 559–564.

Choi, H., Kim, S. L., Jeong, M. K., Yu, O. H., & Eyun, S. (2022). Identification and phylogenetic analysis of chitin synthase genes from the deep-sea polychaete *Branchipolynoe onnuriensis* genome. *Journal of Marine Science and Engineering*, *10*(5), 598.

Cogollo-Herrera, K., Bonfante-Alvarez, H., Avila-Montiel, G. D., Herrera-Barros, A., & Gonzalez- Delgado, A. D. (2018). Techno-economic sensitivity analysis of large scale chitosan production process from shrimp shell wastes. *Chemical Engineering Transactions*, *70*, 2179–2184.

Cortizo, M. S., Berghoff, C. F., & Alessandrini, J. L. (2008). Characterization of chitin from *Illex argentinus* squid pen. *Carbohydrate Polymers*, *74*(1), 10–15.

Cuong, H. N., Minh, N. C., Van Hoa, N., & Trung, T. S. (2016). Preparation and characterization of high purity β-chitin from squid pens (*Loligo chenisis*). *International Journal of Biological Macromolecules*, *93*, 442–447.

da Silva Lucas, A. J., Oreste, E. Q., Costa, H. L. G., López, H. M., Saad, C. D. M., & Prentice, C. (2021). Extraction, physicochemical characterization, and morphological properties of chitin and chitosan from cuticles of edible insects. *Food Chemistry*, *343*, 128550.

da Silva, S. B., Krolicka, M., van den Broek, L. A., Frissen, A. E., & Boeriu, C. G. (2018). Water-soluble chitosan derivatives and pH-responsive hydrogels by selective C-6 oxidation mediated by TEMPO-laccase redox system. *Carbohydrate Polymers*, *186*, 299–309.

Danarto, Y. C., & Distantina, S. (2016). Optimizing deacetylation process for chitosan production from green mussel (*Perna viridis*) shell. In AIP conference proceedings (1710, 1, 030028). AIP Conference Proceedings, *1710*, 030028.

de Alvarenga, R. A. F., Galindro, B. M., de Fátima Helpa, C., & Soares, S. R. (2012). The recycling of oyster shells: An environmental analysis using life cycle assessment. *Journal of Environmental Management*, *106*, 102–109.

de Andrade, S. M. B., Ladchumananandasivam, R., da Rocha, B. G., Belarmino, D. D., & Galvão, A. O. (2012). The use of exoskeletons of shrimp and crab for the extraction of chitosan and production of nanomembrane. *Materials Science and Applications*, *3*, 21160 https://doi.org/10.4236/msa.2012.37070

Delezuk, J. A. D. M., Cardoso, M. B., Domard, A., & Campana-Filho, S. P. (2011). Ultrasound-assisted deacetylation of beta-chitin: Influence of processing parameters. *Polymer International*, *60*(6), 903–909.

Dinculescu, D., Gîjiu, C. L., Apetroaei, M. R., Isopescu, R., Rău, I., & Schröder, V. (2023). Optimization of chitosan extraction process from *Rapana venosa* egg capsules waste using experimental design. *Materials*, *16*(2), 525.

Du, Y., Zhao, Y., Dai, S., & Yang, B. (2009). Preparation of water-soluble chitosan from shrimp shell and its antibacterial activity. *Innovative Food Science & Emerging Technologies*, *10*(1), 103–107.

El Knidri, H., Belaabed, R., Addaou, A., Laajeb, A., & Lahsini, A. (2018). Extraction, chemical modification and characterization of chitin and chitosan. *International Journal of Biological Macromolecules*, *120*, 1181–1189.

FAO. (2023). The European market for mussels, https://www.fao.org/in-action/globefish/fishery-information/resource-detail/en/c/338588/

Fiamingo, A., de Moura Delezuk, J. A., Trombotto, S., David, L., & Campana-Filho, S. P. (2016). Extensively deacetylated high molecular weight chitosan from the multistep ultrasound-assisted deacetylation of beta-chitin. *Ultrasonics Sonochemistry*, *32*, 79–85.

Filgueira, R., Byron, C. J, Comeau, L. A., Costa-Pierce, B., Cranford, P. J., Ferreira, J. G., Grant, J., Guyondet, T., Jansen, H. M., Landry, T., McKindsey, C. W., Petersen, J. K., Reid, G. K., Robinson, S. M. C., Smaal, A., Sonier, R., Strand, Ø., & Strohmeier, T. (2015). An integrated ecosystem approach for assessing the potential role of cultivated bivalve shells as part of the carbon trading system. *Marine Ecology Progress Series*, *518*, 281–287.

Furuhashi, T., Schwarzinger, C., Miksik, I., Smrz, M., & Beran, A. (2009). Molluscan shell evolution with review of shell calcification hypothesis. *Comparative biochemistry and physiology Part B: Biochemistry and molecular biology*, *154*(3), 351–371.

Gbenebor, O. P., Akpan, E. I., & Adeosun, S. O. (2017). Thermal, structural and acetylation behavior of snail and periwinkle shells chitin. *Progress in Biomaterials*, *6*(3), 97–111.

George, A., & Roberts, F. (1992). The use of exoskeletons of shrimp (*Litopenaeus vanammei*) and crab (*Ucides cordatus*) for the extraction of chitosan and production of nanomembrane. In *Chitin chemistry* (pp. 249–267). The Mac Millan Press Ltd.

Gobinath, T., Thamizhselvan, S., Ramakrishnan, A., & Ravichandran, S. (2021). Preparation and characterization of chitosan from *Perna viridis* (Linnaeus, 1758) shell waste as raw material. *Research Journal of Pharmacy and Technology*, *14*(5), 2757–2762.

Gortari, M. C., & Hours, R. A. (2013). Biotechnological processes for chitin recovery out of crustacean waste: A mini-review. *Electronic Journal of Biotechnology*, *16*(3), 14–14.

Hajji, S., Younes, I., Ghorbel-Bellaaj, O., Hajji, R., Rinaudo, M., Nasri, M., & Jellouli, K. (2014). Structural differences between chitin and chitosan extracted from three different marine sources. *International Journal of Biological Macromolecules*, *65*, 298–306.

Hajji, S., Younes, I., Rinaudo, M., Jellouli, K., & Nasri, M. (2015). Characterization and in vitro evaluation of cytotoxicity, antimicrobial and antioxidant

26438429, 2024, 1, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/ffd2.326, Wiley Online Library on [28/01/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

activities of chitosans extracted from three different marine sources. *Applied Biochemistry and Biotechnology*, *177*(1), 18–35.

Hamed, I., Özogul, F., & Regenstein, J. M. (2016). Industrial applications of crustacean by-products (chitin, chitosan, and chitooligosaccharides): A review. *Trends in Food Science & Technology*, *48*, 40–50.

Handayani, L., Syahputra, F., & Astuti, Y. (2018). Utilization and characterization of oyster shell as chitosan and nanochitosan. *Jurnal Kimia Sains Dan Aplikasi*, *21*(4), 224–231.

Hardani, P. T., Sugijanto, N. E. N., & Kartosentono, S. (2021). Heavy metals bioremediation by shells dust and chitosan derived from *Belamya javanica* snail, an eco-friendly biosorbent. *Research Journal of Pharmacy and Technology*, *14*(3), 1555–1560. https://doi.org/10.5958/0974-360X.2021.00274.2

Hazeena, S. H., Hou, C. Y., Zeng, J. H., Li, B. H., Lin, T. C., Liu, C. S., Chang, C. I., Hsieh, S. L., & Shih, M. K. (2022). Extraction optimization and structural characteristics of chitosan from Cuttlefish (*S. pharaonis* sp.) bone. *Materials*, *15*(22), 7969.

He, X., Xing, R., Li, K., Qin, Y., Zou, P., Liu, S., Yu, H., & Li, P. (2016). Beta-chitosan extracted from *Loligo japonica* for a potential use to inhibit Newcastle disease. *International Journal of Biological Macromolecules*, *82*, 614–620.

Helander, I. M., Nurmiaho-Lassila, E. L., Ahvenainen, R., Rhoades, J., & Roller, S. (2001). Chitosan disrupts the barrier properties of the outer membrane of Gram-negative bacteria. *International Journal of Food Microbiology*, *71*(2–3), 235–244.

Hou, Y., Shavandi, A., Carne, A., Bekhit, A. A., Ng, T. B., Cheung, R. C. F., & Bekhit, A. E. D. A. (2016). Marine shells: Potential opportunities for extraction of functional and health-promoting materials. *Critical Reviews in Environmental Science and Technology*, *46*(11–12), 1047–1116.

Huang, C. Y., Kuo, C. H., Wu, C. H., Ku, M. W., & Chen, P. W. (2018). Extraction of crude chitosans from squid (*Illex argentinus*) pen by a compressional puffing-pretreatment process and evaluation of their antibacterial activity. *Food Chemistry*, *254*, 217–223.

Huang, J., Chen, W. W., Hu, S., Gong, J. Y., Lai, H. W., Liu, P., Mei, L. H., & Mao, J. W. (2013). Biochemical activities of 6-carboxy β-chitin derived from squid pens. *Carbohydrate Polymers*, *91*(1), 191–197.

Huang, J., Cheng, Z. H., Xie, H. H., Gong, J. Y., Lou, J., Ge, Q., Wang, Y. J., Wu, Y. F., Liu, S. W., Sun, P. L., & Mao, J. W. (2014). Effect of quaternization degree on physiochemical and biological activities of chitosan from squid pens. *International Journal of Biological Macromolecules*, *70*, 545–550.

Huang, J., Mao, J. W., Hu, S., Zhao, D. K., Mei, L. H., Liu, S. W., Wu, Y. F., Fang, S., & Shao, Q. (2011). Ultrasonic-assisted preparation, characterization and antibacterial activity of β-chitosan from squid pens. In *Advanced materials research* (Vol. 236, pp. 282–287). Trans Tech Publications Ltd.

Huang, J., Xie, H., Hu, S., Xie, T., Gong, J., Jiang, C., Ge, Q., Wu, Y., Liu, S., Cui, Y., & Mao, J. (2015). Preparation, characterization, and biochemical activities of *N*-(2-carboxyethyl) chitosan from squid pens. *Journal of Agricultural and Food Chemistry*, *63*(9), 2464–2471.

Huang, J., Zhao, D., Hu, S., Mao, J., & Mei, L. (2012). Biochemical activities of low molecular weight chitosans derived from squid pens. *Carbohydrate Polymers*, *87*(3), 2231–2236.

Huang, Y. L., & Tsai, Y. H. (2020). Extraction of chitosan from squid pen waste by high hydrostatic pressure: Effects on physicochemical properties and antioxidant activities of chitosan. *International Journal of Biological Macromolecules*, *160*, 677–687.

Ianiro, A., Di Giosia, M., Fermani, S., Samorì, C., Barbalinardo, M., Valle, F., Pellegrini, G., Biscarini, F., Zerbetto, F., Calvaresi, M., & Falini, G. (2014). Customizing properties of β-chitin in squid pen (gladius) by chemical treatments. *Marine Drugs*, *12*(12), 5979–5992.

Iribarren, D., Moreira, M. T., & Feijoo, G. (2010a). Life cycle assessment of fresh and canned mussel processing and consumption in Galicia (NW Spain). *Resources, Conservation and Recycling*, *55*(2), 106–117.

Iribarren, D., Moreira, M. T., & Feijoo, G. (2010b). Implementing by-product management into the life cycle assessment of the mussel sector. *Resources, Conservation and Recycling*, *54*(12), 1219–1230.

Islam, N., Hoque, M., & Taharat, S. F. (2023). Recent advances in extraction of chitin and chitosan. *World Journal of Microbiology and Biotechnology*, *39*(1), 28.

Jang, M. K., Kong, B. G., Jeong, Y. I., Lee, C. H., & Nah, J. W. (2004). Physicochemical characterization of α-chitin, β-chitin, and γ-chitin separated from natural resources. *Journal of Polymer Science Part A: Polymer Chemistry*, *42*(14), 3423–3432.

Jayalakshmi, K., Subhapradha, N., Vairamani, S., & Shanmugam, A. (2016). Anticoagulant and antioxidant property of chitosan sulfate from the internal bone of cuttlefish. *International Journal of Frontiers in Science and Technology*, *4*(4), 32–38.

Joseph, S. M., Krishnamoorthy, S., Paranthaman, R., Moses, J. A., & Anandharamakrishnan, C. (2021). A review on source-specific chemistry, functionality, and applications of chitin and chitosan. *Carbohydrate Polymer Technologies and Applications*, *2*, 100036.

Jothi, N., & Kunthavai, N. R. (2014). Isolation and identification of chitin and chitosan from cuttle bone of *Sepia prashadi* Winckworth. *International Journal of Current Science*, *11*, 18–25.

Jung, H. S., Kim, M. H., & Park, W. H. (2019). Preparation and structural investigation of novel β-chitin nanocrystals from cuttlefish bone. *ACS Biomaterials Science & Engineering*, *5*(4), 1744–1752.

Jung, H. S., Kim, M. H., Shin, J. Y., Park, S. R., Jung, J. Y., & Park, W. H. (2018). Electrospinning and wound healing activity of β-chitin extracted from cuttlefish bone. *Carbohydrate Polymers*, *193*, 205–211.

Jung, J., & Zhao, Y. (2011). Characteristics of deacetylation and depolymerization of β-chitin from jumbo squid (*Dosidicus gigas*) pens. *Carbohydrate Research*, *346*(13), 1876–1884.

Jung, J., & Zhao, Y. (2014). Alkali-or acid-induced changes in structure, moisture absorption ability and deacetylating reaction of β-chitin extracted from jumbo squid (*Dosidicus gigas*) pens. *Food Chemistry*, *152*, 355–362.

Kaewboonruang, S. A. Y. A. N., Phatrabuddha, N., Sawangwong, P., & Pitaksanurat, S. (2016). *Comparative studies on the extraction of chitin–chitosan from golden apple snail shells at the control field* (Doctoral dissertation, Burapha University).

Karthik, R., Manigandan, V., Saravanan, R., Rajesh, R. P., & Chandrika, B. (2016). Structural characterization and in vitro biomedical activities of sulfated chitosan from *Sepia pharaonis*. *International Journal of Biological Macromolecules*, *84*, 319–328.

Kaur, S., & Dhillon, G. S. (2015). Recent trends in biological extraction of chitin from marine shell wastes: A review. *Critical Reviews in Biotechnology*, *35*(1), 44–61.

Kavisri, M., Abraham, M., Namasivayam, S. K. R., Aravindkumar, J., Balaji, D., Sathishkumar, R., Sigamani, S., Srinivasan, R., & Moovendhan, M. (2023). Adsorption isotherm, kinetics and response surface methodology optimization of cadmium (Cd) removal from aqueous solution by chitosan biopolymers from cephalopod waste. *Journal of Environmental Management*, *335*, 117484.

Kaya, M., Baran, T., Erdoğan, S., Menteş, A., Özüsağlam, M. A., & Çakmak, Y. S. (2014a). Physicochemical comparison of chitin and chitosan obtained from larvae and adult Colorado potato beetle (*Leptinotarsa decemlineata*). *Materials Science and Engineering: C*, *45*, 72–81.

Kaya, M., Baran, T., & Karaarslan, M. (2015). A new method for fast chitin extraction from shells of crab, crayfsh and shrimp. *Natural Product Research*, *29*(15), 1477–1480.

Kaya, M., Baublys, V., Can, E., Satkauskien, E. I., Bitim, B., & Tubelyte, V. (2014b). Comparison of physicochemical properties of chitins isolated from an insect (*Melolontha melolontha*) and a crustacean species (*Oniscus asellus*). *Zoomorphology*, *133*(3), 285–293.

Kaya, M., Sargin, I., Aylanc, V., Tomruk, M. N., Gevrek, S., Karatoprak, I., Colak, N., Sak, Y. G., & Bulut, E. (2016). Comparison of bovine serum albumin adsorption capacities of α-chitin isolated from an insect and β-chitin from cuttlebone. *Journal of Industrial and Engineering Chemistry*, *38*, 146–156.

Khajavian, M., Vatanpour, V., Castro-Muñoz, R., & Boczkaj, G. (2022). Chitin and derivative chitosan-based structures-preparation strategies aided

26438429, 2024, 1, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/fft2.326, Wiley Online Library on [28/01/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

by deep eutectic solvents: A review. *Carbohydrate Polymers*, *275*, 118 702.

Khan, T. A., Peh, K. K., & Ch'ng, H. S. (2002). Reporting degree of deacetylation values of chitosan: The influence of analytical methods. *Journal of Pharmacy & Pharmaceutical Sciences*, *5*(3), 205–212.

Khayrova, A., Lopatin, S., & Varlamov, V. (2019). Black soldier fly *Hermetia illucens* as a novel source of chitin and chitosan. *International Journal of Sciences*, *8*, 81–86.

Kim, M. W., Song, Y. S., Seo, D. J., Han, Y. S., Jo, Y. H., Noh, M. Y., Yang, Y. C., Park, Y.-K., Kim, S.-A., Choi, C., Jung, W.-J., & Jung, W. (2017). Extraction of chitin and chitosan from the exoskeleton of the cockroach (*Periplaneta americana* L.). *Journal of Chitin and Chitosan*, *22*(2), 76–81.

Kimi, M., & Hamdi, M. (2023). Direct extraction of chitosan from snail shells by natural deep eutectic solvent. *Current Chemistry Letters*, *12*(2), 275–280.

Kumari, S., Annamareddy, S. H. K., Abanti, S., & Rath, P. K. (2017). Physicochemical properties and characterization of chitosan synthesized from fish scales, crab and shrimp shells. *International Journal of Biological Macromolecules*, *104*, 1697–1705.

Kumari, S., Rath, P., Kumar, A. S. H., & Tiwari, T. N. (2015). Extraction and characterization of chitin and chitosan from fishery waste by chemical method. *Environmental Technology & Innovation*, *3*, 77–85.

Laskar, I. B., Rajkumari, K., Gupta, R., Chatterjee, S., Paul, B., & Rokhum, S. L. (2018). Waste snail shell derived heterogeneous catalyst for biodiesel production by the transesterification of soybean oil. *RSC Advances*, *8*(36), 20131–20142.

Lavall, R. L., Assis, O. B., & Campana-Filho, S. P. (2007). β-Chitin from the pens of *Loligo* sp.: Extraction and characterization. *Bioresource Technology*, *98*(13), 2465–2472.

Li, B., Wang, J., Moustafa, M. E., & Yang, H. (2019). Ecofriendly method to dissolve chitosan in plain water. *ACS Biomaterials Science & Engineering*, *5*(12), 6355–6360.

Li, B., Wu, X., Bao, B., Guo, R., & Wu, W. (2021). Evaluation of α-chitosan from crab shell and β-chitosan from squid gladius based on biochemistry performance. *Applied Sciences*, *11*(7), 3183.

Li, H., Huang, W., Qiu, B., Thabet, H. K., Alhashmialameer, D., Huang, M., & Guo, Z. (2022). Effective removal of proteins and polysaccharides from biotreated wastewater by polyaniline composites. *Advanced Composites and Hybrid Materials*, *5*(3), 1888–1898.

Lim, J., Cho, H., & Kim, J. (2021). Optimization of wet flue gas desulfurization system using recycled waste oyster shell as high-grade limestone substitutes. *Journal of Cleaner Production*, *318*, 128492.

Lin, Y. C., Wang, H. P., Gohar, F., Ullah, M. H., Zhang, X., Xie, D. F., Fang, H., Huang, J., & Yang, J. X. (2017). Preparation and copper ions adsorption properties of thiosemicarbazide chitosan from squid pens. *International Journal of Biological Macromolecules*, *95*, 476–483.

Liu, S., Sun, J., Yu, L., Zhang, C., Bi, J., Zhu, F., Qu, M., Jiang, C., & Yang, Q. (2012). Extraction and characterization of chitin from the beetle *Holotrichia parallela* Motschulsky. *Molecules*, *17*(4), 4604–4611.

Ma, J., Xin, C., & Tan, C. (2015). Preparation, physicochemical and pharmaceutical characterization of chitosan from *Catharsius molossus* residue. *International Journal of Biological Macromolecules*, *80*, 547–556.

Majekodunmi, S. O., Olorunsola, E. O., Ofiwe, U. C., Udobre, A. S., & Akpan, E. (2017a). Material properties of chitosan from shells of Egeria radiata: Drug delivery considerations. *Journal of Coastal Life Medicine*, *5*(7), 321–324.

Majekodunmi, S. O., Olorunsola, E. O., & Uzoaganobi, C. C. (2017b). Comparative physicochemical characterization of chitosan from shells of two bivalved mollusks from two different continents. *American Journal of Polymer Science*, *7*(1), 15–22.

Manni, L., Ghorbel-Bellaaj, O., Jellouli, K., Younes, I., & Nasri, M. (2010). Extraction and characterization of chitin, chitosan, and protein hydrolysates prepared from shrimp waste by treatment with crude protease from *Bacillus cereus* SV1. *Applied Biochemistry and Biotechnology*, *162*, 345–357.

Mathew, G. M., Huang, C. C., Sindhu, R., Binod, P., Sirohi, R., Awsathi, M. K., Pillai, S., & Pandey, A. (2021). Enzymatic approaches in the bioprocessing of shellfish wastes. *3 Biotech*, *11*, 1–13.

McReynolds, C., Adrien, A., de Fraissinette, N. B., Olza, S., & Fernandes, S. C. (2022). Deep eutectic solvents for the extraction of β-chitin from *Loligo vulgaris* squid pens: A sustainable way to valorize fishery by-products. *Biomass Conversion and Biorefinery*, 1–13. https://doi.org/10.1007/s13399-022-03569-9

Mesa Ospina, N., Ospina Alvarez, S. P., Escobar Sierra, D. M., Rojas Vahos, D. F., Zapata Ocampo, P. A., & Ossa Orozco, C. P. (2015). Isolation of chitosan from *Ganoderma lucidum* mushroom for biomedical applications. *Journal of Materials Science: Materials in Medicine*, *26*, 1–9.

Mohan, K., Ganesan, A. R., Ezhilarasi, P. N., Kondamareddy, K. K., Rajan, D. K., Sathishkumar, P., Rajarajeswaran, J., & Conterno, L. (2022). Green and eco-friendly approaches for the extraction of chitin and chitosan: A review. *Carbohydrate Polymers*, *287*, 119349.

Mohan, K., Ganesan, A. R., Muralisankar, T., Jayakumar, R., Sathishkumar, P., Uthayakumar, V., Chandirasekar, R., & Revathi, N. (2020). Recent insights into the extraction, characterization, and bioactivities of chitin and chitosan from insects. *Trends in Food Science & Technology*, *105*, 17–42.

Mohan, K., Muralisankar, T., Jayakumar, R., & Rajeevgandhi, C. (2021). A study on structural comparisons of α-chitin extracted from marine crustacean shell waste. *Carbohydrate Polymer Technologies and Applications*, *2*, 100037.

Mohan, K., Rajan, D. K., Rajarajeswaran, J., Divya, D., & Ganesan, A. R. (2023). Recent trends on chitosan based hybrid materials for wastewater treatment: A review. *Current Opinion in Environmental Science & Health*, 100473.

Mohan, K., Ravichandran, S., Muralisankar, T., Uthayakumar, V., Chandirasekar, R., Rajeevgandhi, C., Karthick Rajan, D., & Seedevi, P. (2019). Extraction and characterization of chitin from sea snail *Conus inscriptus* (Reeve, 1843). *International Journal of Biological Macromolecules*, *126*, 555–560.

Moniruzzaman, M., Sku, S., Chowdhury, P., Tanu, M. B., Yeasmine, S., Hossen, M. N., Min, T., Bai, S. C., & Mahmud, Y. (2021). Nutritional evaluation of some economically important marine and freshwater mollusc species of Bangladesh. *Heliyon*, *7*(5), e07088.

Moreira, A. C., Carneiro, R. L., Fracola, M. F., Micocci, K. C., Bueno, O. C., & Souza, D. H. (2020). Analysis of the gene expression and RNAi-mediated knockdown of chitin synthase from leaf-cutting ant *Atta sexdens*. *Journal of the Brazilian Chemical Society*, *31*, 1979–1990.

Moreno-Tovar, R., Bucio, L., Thions, C., & Tehuacanero-Cuapa, S. (2021). Thermal degradation and lifetime of β-chitin from *Dosidicus gigas* squid pen: Effect of impact at 9.7 GPa and a comparative study with α-chitin. *Carbohydrate Polymers*, *251*, 116987.

Morris, J. P., Backeljau, T., & Chapelle, G. (2019). Shells from aquaculture: A valuable biomaterial, not a nuisance waste product. *Reviews in Aquaculture*, *11*(1), 42–57.

Muñoz, I., Rodríguez, C., Gillet, D., & Moerschbacher, B. M. (2018). Life cycle assessment of chitosan production in India and Europe. *The International Journal of Life Cycle Assessment*, *23*, 1151–1160.

Murphy, J. N., & Kerton, F. M. (2017). Characterization and utilization of waste streams from mollusc aquaculture and fishing industries. *Fuels, Chemicals and Materials from the Oceans and Aquatic Sources*, 189–227. https://doi.org/10.1002/9781119117193.ch8

Nagahama, H., Higuchi, T., Jayakumar, R., Furuike, T., & Tamura, H. (2008). XRD studies of β-chitin from squid pen with calcium solvent. *International Journal of Biological Macromolecules*, *42*(4), 309–313.

Narasimman, V., & Ramachandran, S. (2023). In silico analysis of low molecular weight sulfated chitosan from *Sepia brevimana* as potential inhibitors of white spot syndrome envelope proteins. *Biomass Conversion and Biorefinery*, 1–12. https://doi.org/10.1007/s13399-023-03844-3

Nouj, N., Hafid, N., El Alem, N., Buciscanu, I. I., Maier, S. S., Samoila, P., Soreanu, G., Cretescu, I., & Stan, C. D. (2022). Valorization of β-chitin

26438429, 2024, 1, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/ffd2.326, Wiley Online Library on [28/01/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

extraction byproduct from cuttlefish bone and its application in food wastewater treatment. *Materials*, *15*(8), 2803.

Nugroho, I. L., Masithah, E. D., & Pursetyo, K. T. (2020). The effect of hydrochloric acid concentration and temperature demineralization on characteristics of chitin from penshell (*Atrina pectinata*). *IOP Conference Series: Earth and Environmental Science*, *441*(1), 012152.

Ofem, M. (2015). *Properties of chitin whisker reinforced poly (acrylic acid) composites*.The University of Manchester.

Okoro, O. V., Nie, L., Gunduz, O., Ulag, S., Hamidi, M., & Shavandi, A. (2023). Technoeconomic Assessment of Biopolymer Production from Crustacean Waste with the UK as a Case Study. *Sustainability*, *15*(3), 2280.

Oyekunle, D. T., & Omoleye, J. A. (2019a). New process for synthesizing chitosan from snail shells. *Journal of Physics: Conference Series*, *1299*(1), 012089.

Oyekunle, D. T., & Omoleye, J. A. (2019b). Extraction, characterization and kinetics of demineralization of chitin produced from snail shells of different particle sizes using 1.2 M HCl. *International Journal of Mechanical Engineering and Technology*, *10*(1), 2011–2020.

Özel, N., & Elibol, M. (2021). A review on the potential uses of deep eutectic solvents in chitin and chitosan related processes. *Carbohydrate Polymers*, *262*, 117942.

Pakizeh, M., Moradi, A., & Ghassemi, T. (2021). Chemical extraction and modification of chitin and chitosan from shrimp shells. *European Polymer Journal*, *159*, 110709.

Palpandi, C., Shanmugam, V., & Shanmugam, A. (2009). Extraction of chitin and chitosan from shell and operculum of mangrove gastropod *Nerita* (*Dostia*) *crepidularia* Lamarck. *International Journal of Medicine and Medical Sciences*, *1*(5), 198–205.

Pellis, A., Guebitz, G. M., & Nyanhongo, G. S. (2022). Chitosan: Sources, processing and modification techniques. *Gels*, *8*(7), 393.

Qin, C., Du, Y., Xiao, L., Li, Z., & Gao, X. (2002). Enzymic preparation of water-soluble chitosan and their antitumor activity. *International Journal of Biological Macromolecules*, *31*(1–3), 111–117.

Rajathy, T. J., Srinivasan, M., & Mohanraj, T. (2021). Physicochemical and functional characterization of chitosan from horn snail gastropod *Telescopium telescopium*. *Journal of Applied Pharmaceutical Science*, *11*(2), 052–058.

Rakkhumkaew, N., & Pengsuk, C. (2018). Chitosan and chitooligosaccharides from shrimp shell waste: characterization, antimicrobial and shelf life extension in bread. *Food Science and Biotechnology*, *27*(4), 1201–1208.

Ramachandran, S., Narasimman, V., & Rajesh, P. (2022). Low molecular weight sulfated chitosan isolation, characterization and anti-tuberculosis activity derived from *Sepioteuthis lessoniana*. *International Journal of Biological Macromolecules*, *206*, 29–39.

Ramasamy, P., Sekar, S., Paramasivam, S., Suri, P., Chinnaiyan, U., Singh, R., Tanguturi Raghavaiah, B. P., & Seshadri, V. D. (2022). Sulfation of chitosan from *Sepia kobiensis* as potential anticoagulant and antibacterial molecule. *Natural Product Research*, *36*(12), 3216–3222.

Ramasamy, P., Subhapradha, N., Shanmugam, V., & Shanmugam, A. (2014). Extraction, characterization and antioxidant property of chitosan from cuttlebone *Sepia kobiensis* (Hoyle 1885). *International Journal of Biological Macromolecules*, *64*, 202–212.

Ramasamy, P., Subhapradha, N., Thinesh, T., Selvin, J., Selvan, K. M., Shanmugam, V., & Shanmugam, A. (2017). Characterization of bioactive chitosan and sulfated chitosan from *Doryteuthis singhalensis* (Ortmann, 1891). *International Journal of Biological Macromolecules*, *99*, 682–691.

Rasti, H., Parivar, K., Baharara, J., Iranshahi, M., & Namvar, F. (2017). Chitin from the mollusc chiton: Extraction, characterization and chitosan preparation. *Iranian Journal of Pharmaceutical Research*, *16*(1), 366.

Rasti, H., Parivar, K., Baharara, J., Iranshahi, M., & Namvar, F. (2016). Chitosan extracted from the Persian Gulf chiton shells: Induction of apoptosis in liver cancer cell line. *Iranian Journal of Fisheries Sciences*, *15*(4), 1362–1378.

Reys, L. L., Silva, S. S., Oliveira, J. M., Caridade, S. G., Mano, J. F., Silva, T. H., & Reis, R. L. (2013). Revealing the potential of squid chitosan-based structures for biomedical applications. *Biomedical Materials*, *8*(4), 045002.

Rinaudo, M. (2006). Chitin and chitosan: Properties and applications. *Progress in Polymer Science*, *31*(7), 603–632.

Rokhati, N., Istirokhatun, T., Apriyanti, D. T., & Susanto, H. (2017). Production of chitosan from *Amusium* sp Scallop Shell Waste. *IOP Conference Series: Earth and Environmental Science*, *55*(1), 012071.

Rudall, K. M., & Kenchington, W. J. B. R. (1973). The chitin system. *Biological Reviews*, *48*(4), 597–633.

Saenz-Mendoza, A. I., Zamudio-Flores, P. B., García-Anaya, M. C., Velasco, C. R., Acosta-Muñiz, C. H., Espino-Díaz, M., Tirado-Gallegos, J. M., Hernández-González, M., Vela-Gutiérrez, G., Salgado-Delgado, R., Rendón-Villalobos, J. R., & Ortega-Ortega, A. (2023). Insects as a potential source of chitin and chitosan: Physicochemical, morphological and structural characterization—A review. *Emirates Journal of Food and Agriculture*, *35*(5), 388–407.

Sajomsang, W., & Gonil, P. (2010). Preparation and characterization of α-chitin from cicada sloughs. *Materials Science and Engineering: C*, *30*(3), 357–363.

Sangwaranatee, N. W., Teanchai, K., Kongsriprapan, S., & Siriprom, W. (2018). Characterization and analyzation of chitosan powder from *Perna viridis* shell. *Materials Today: Proceedings*, *5*(6), 13922–13925.

Santhosh, S., Sini, T. K., & Mathew, P. T. (2010). Variation in properties of chitosan prepared at different alkali concentrations from squid pen and shrimp shell. *International Journal of Polymeric Materials*, *59*(4), 286–291.

Sayari, N., Sila, A., Abdelmalek, B. E., Abdallah, R. B., Ellouz-Chaabouni, S., Bougatef, A., & Balti, R. (2016). Chitin and chitosan from the Norway lobster by-products: Antimicrobial and anti-proliferative activities. *International Journal of Biological Macromolecules*, *87*, 163–171.

Scimeca, M., Bischetti, S., Lamsira, H. K., Bonfiglio, R., & Bonanno, E. (2018). Energy dispersive X-ray (EDX) microanalysis: A powerful tool in biomedical research and diagnosis. *European Journal of Histochemistry: EJH*, *62*(1), 1–9.

Seedevi, P., Moovendhan, M., Vairamani, S., & Shanmugam, A. (2017). Evaluation of antioxidant activities and chemical analysis of sulfated chitosan from *Sepia prashadi*. *International Journal of Biological Macromolecules*, *99*, 519–529.

Seenuvasan, M., Sarojini, G., & Dineshkumar, M. (2020). Recovery of chitosan from natural biotic waste. In *Current developments in biotechnology and bioengineering* (pp. 115–133). Elsevier.

Shahid, K., Srivastava, V., & Sillanpää, M. (2021). Protein recovery as a resource from waste specifically via membrane technology—From waste to wonder. *Environmental Science and Pollution Research*, *28*, 10262–10282.

Shanmugam, A., Kathiresan, K., & Nayak, L. (2016). Preparation, characterization and antibacterial activity of chitosan and phosphorylated chitosan from cuttlebone of *Sepia kobiensis* (Hoyle, 1885). *Biotechnology Reports*, *9*, 25–30.

Shanmugam, A., Subhapradha, N., Suman, S., Ramasamy, P., Saravanan, R., Shanmugam, V., & Srinivasan, A. (2012). Characterization of biopolymer "chitosan" from the shell of donacid clam *Donax scortum* (Linnaeus, 1758) and its antioxidant activity. *International Journal of Pharmacy and Pharmaceutical Sciences*, *4*, 460–465.

Shushizadeh, M. R., Pour, E. M., Zare, A., & Lashkari, Z. (2015). Persian gulf β-chitin extraction from *Sepia pharaonis* sp. cuttlebone and preparation of its derivatives. *Bioactive Carbohydrates and Dietary Fibre*, *6*(2), 133–142.

Silva, S. S., Mano, J. F., & Reis, R. L. (2017). Ionic liquids in the processing and chemical modification of chitin and chitosan for biomedical applications. *Green Chemistry*, *19*(5), 1208–1220.

Singh, A., Benjakul, S., & Prodpran, T. (2019). Ultrasound-assisted extraction of chitosan from squid pen: Molecular characterization and fat binding capacity. *Journal of Food Science*, *84*(2), 224–234.

26438429, 2024, 1, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/ffd2.326, Wiley Online Library on [28/01/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Siswoyo, E., Zahra, R. N., Mai, N. H. A., Nurmiyanto, A., Umemura, K., & Boving, T. (2023). Chitosan of blood cockle shell (*Anadara granosa*) as a natural coagulant for removal of total suspended solids (TSS) and turbidity of well-water. *The Egyptian Journal of Aquatic Research*, *49*(3), 283–289.

Song, Y. S., Kim, M. W., Moon, C., Seo, D. J., Han, Y. S., Jo, Y. H., Noh, M. Y., Park, Y.-K., Kim, S.-A., Kim, Y. W., & Jung, W. J. (2018). Extraction of chitin and chitosan from larval exuvium and whole body of edible mealworm, *Tenebrio molitor*. *Entomological Research*, *48*(3), 227–233.

Srinivasan, H., Kanayairam, V., & Ravichandran, R. (2018). Chitin and chitosan preparation from shrimp shells *Penaeus monodon* and its human ovarian cancer cell line, PA-1. *International Journal of Biological Macromolecules*, *107*, 662–667.

Subhapradha, N., Ramasamy, P., Shanmugam, V., Madeswaran, P., Srinivasan, A., & Shanmugam, A. (2013a). Physicochemical characterisation of β-chitosan from *Sepioteuthis lessoniana* gladius. *Food Chemistry*, *141*(2), 907–913.

Subhapradha, N., Ramasamy, P., Srinivasan, A., Madeswaran, P., Shanmugam, V., & Shanmugam, A. (2013b). Sulfation of β-chitosan and evaluation of biological activity from gladius of *Sepioteuthis lessoniana*. *International Journal of Biological Macromolecules*, *62*, 336–340.

Subhapradha, N., Ramasamy, P., Srinivasan, A., Shanmugam, A., & Shanmugam, V. (2013c). Preparation of chitosan derivatives from gladius of squid *Sepioteuthis lessoniana* (Lesson, 1830) and antimicrobial potential against human pathogens. *Journal of Biological Sciences*, *13*(4), 257.

Subhapradha, N., Ramasamy, P., Sudharsan, S., Seedevi, P., Moovendhan, M., Srinivasan, A., Shanmugam, V., Shanmugam, A., & Shanmugam, A. (2013d). Preparation of phosphorylated chitosan from gladius of the squid *Sepioteuthis lessoniana* (Lesson, 1830) and its in vitro antioxidant activity. *Bioactive Carbohydrates and Dietary Fibre*, *1*(2), 148–155.

Subhapradha, N., Suman, S., Ramasamy, P., Saravanan, R., Shanmugam, V., Srinivasan, A., & Shanmugam, A. (2013e). Anticoagulant and antioxidant activity of sulfated chitosan from the shell of donacid clam *Donax scortum* (Linnaeus, 1758). *International Journal of Nutrition, Pharmacology, Neurological Diseases*, *3*(1), 39–45.

Sudatta, B. P., Sugumar, V., Varma, R., & Nigariga, P. (2020). Extraction, characterization and antimicrobial activity of chitosan from pen shell, *Pinna bicolor*. *International Journal of Biological Macromolecules*, *163*, 423–430.

Suenaga, S., Nikaido, N., Totani, K., Kawasaki, K., Ito, Y., Yamashita, K., & Osada, M. (2016). Effect of purification method of β-chitin from squid pen on the properties of β-chitin nanofibers. *International Journal of Biological Macromolecules*, *91*, 987–993.

Suman, S., Kumar, P., Saurabh, S., Katneni, V. K., Prabhudas, S. K., Gireesh-Babu, P., Das, R., Chaudhari, A., & Chaudhari, A. (2023). Transcriptome analysis of Indian freshwater pearl mussel *Lamellidens marginalis* to unravel the genes associated with biomineralization. https://doi.org/10.21203/rs.3.rs-2806499/v1

Suryawanshi, N., Ayothiraman, S., & Eswari, J. S. (2020). Ultrasonication mode for the expedition of extraction process of chitin from the maritime shrimp shell waste. *Indian journal of Biochemistry & Biophysics*, *57*, 431–438.

Synowiecki, J., & Al-Khateeb, N. A. (2003). Production, properties, and some new applications of chitin and its derivatives. *Critical Reviews in Food Science and Nutrition*, *43*, 145–171.

Tiwari, B. K. (2015). Ultrasound: A clean, green extraction technology. *TrAC Trends in Analytical Chemistry*, *71*, 100–109.

Tolaimate, A., Desbrieres, J., Rhazi, M., Alagui, A., Vincendon, M., & Vottero, P. (2000). On the influence of deacetylation process on the physicochemical characteristics of chitosan from squid chitin. *Polymer*, *41*(7), 2463–2469.

Vairamani, S., Subhapradha, N., Ramasamy, P., Raveendran, S., Srinivasan, A., & Shanmugam, A. (2013). Physicochemical characteristics and antioxidant efficacy of chitosan from the internal shell of spineless cuttlefish *Sepiella inermis*. *Preparative Biochemistry and Biotechnology*, *43*(7), 696–716.

Van Hoa, N., Vuong, N. T. H., Minh, N. C., Cuong, H. N., & Trung, T. S. (2021). Squid pen chitosan nanoparticles: Small size and high antibacterial activity. *Polymer Bulletin*, *78*, 7313–7324.

Varma, R., & Vasudevan, S. (2020). Extraction, characterization, and antimicrobial activity of chitosan from horse mussel *Modiolus modiolus*. *ACS Omega*, *5*(32), 20224–20230.

Varma, R., Vasudevan, S., Chelladurai, S., & Narayanasamy, A. (2021). Synthesis and physicochemical characteristics of chitosan extracted from *Pinna deltoides*. *Letters in Applied NanoBioScience*, *11*, 4061–4070.

Vázquez, J. A., Noriega, D., Ramos, P., Valcarcel, J., Novoa-Carballal, R., Pastrana, L., Reis, R. L., & Pérez-Martín, R. I. (2017). Optimization of high purity chitin and chitosan production from *Illex argentinus* pens by a combination of enzymatic and chemical processes. *Carbohydrate Polymers*, *174*, 262–272.

Vélez-Henao, J. A., Weinland, F., & Reintjes, N. (2021). Life cycle assessment of aquaculture bivalve shellfish production—A critical review of methodological trends. *The International Journal of Life Cycle Assessment*, *26*, 1–16.

Vino, A. B., Ramasamy, P., Shanmugam, V., & Shanmugam, A. (2012). Extraction, characterization and in vitro antioxidative potential of chitosan and sulfated chitosan from cuttlebone of *Sepia aculeata* Orbigny, 1848. *Asian Pacific Journal of Tropical Biomedicine*, *2*(1), S334–S341.

Wang, Y., Chang, Y., Yu, L., Zhang, C., Xu, X., Xue, Y., Li, Z., & Xue, C. (2013). Crystalline structure and thermal property characterization of chitin from *Antarctic krill* (Euphausia superba). *Carbohydrate Polymers*, *92*(1), 90–97.

Weiss, I. M., Schönitzer, V., Eichner, N., & Sumper, M. (2006). The chitin synthase involved in marine bivalve mollusk shell formation contains a myosin domain. *FEBS Letters*, *580*(7), 1846–1852.

Weiss, I. (2012). Species-specific shells: Chitin synthases and cell mechanics in molluscs. *Zeitschrift für Kristallographie—Crystalline Materials*, *227*(11), 723–738.

Wimardhani, Y. S., Suniarti, D. F., Freisleben, H. J., Wanandi, S. I., Siregar, N. C., & Ikeda, M. A. (2014). Chitosan exerts anticancer activity through induction of apoptosis and cell cycle arrest in oral cancer cells. *Journal of Oral Science*, *56*(2), 119–126.

Xu, Y., Gallert, C., & Winter, J. (2008). Chitin purification from shrimp wastes by microbial deproteination and decalcification. *Applied Microbiology and Biotechnology*, *79*, 687–697.

Yadav, M., Goswami, P., Paritosh, K., Kumar, M., Pareek, N., & Vivekanand, V. (2019). Seafood waste: A source for preparation of commercially employable chitin/chitosan materials. *Bioresources and Bioprocessing*, *6*(1), 1–20.

Youn, D. K., No, H. K., & Prinyawiwatkul, W. (2013). Preparation and characteristics of squid pen β-chitin prepared under optimal deproteinisation and demineralisation condition. *International Journal of Food Science & Technology*, *48*(3), 571–577.

Younes, I., & Rinaudo, M. (2015). Chitin and chitosan preparation from marine sources. Structure, properties and applications. *Marine Drugs*, *13*(3), 1133–1174.

Zainal, S., Hanum, R., & Rahmah, M. (2014). Optimization of chitoasn extract from cockle shell using response surface methodology (RSM). *Asian Journal of Agriculture and Food Sciences*, *2*(4), 314.

Zamri, A. I., Latiff, N. F., Abdullah, Q. H., & Ahmad, F. (2020). Extraction and optimization of chitosan from razor clam (*Ensis arcuatus*) shells by using response surface methodology (RSM). *Food Research*, *4*, 674–678.

Zentz, F., Bédouet, L., Almeida, M. J., Milet, C., Lopez, E., & Giraud, M. (2001). Characterization and quantification of chitosan extracted from nacre of the abalone *Haliotis tuberculata* and the oyster *Pinctada maxima*. *Marine Biotechnology*, *3*, 36–44.

Zhang, A. J., Qin, Q. L., Zhang, H., Wang, H. T., Li, X., Miao, L., & Wu, Y. J. (2011). Preparation and characterisation of food grade chitosan from housefly larvae. *Czech Journal of Food Sciences*, *29*(6), 616–623.

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Zhang, Q., Vigier, K. D. O., Royer, S., & Jérôme, F. (2012). Deep eutectic solvents: Syntheses, properties and applications. *Chemical Society Reviews*, *41*(21), 7108–7146.

Zhang, X., Yuan, J., Li, F., & Xiang, J. (2021). Chitin synthesis and degradation in crustaceans: A genomic view and application. *Marine Drugs*, *19*(3), 153.

Zheng, H. F., Bai, Z. Y., Lin, J. Y., Wang, G. L., & Li, J. L. (2015). Characterization and functional analysis of a chitin synthase gene (HcCS1) identified from the freshwater pearlmussel *Hyriopsis cumingii*. *Genetics and Molecular Research*, *14*, 19264–19274.

Zia, K., Siddiqui, T., Ali, S., Farooq, I., Zafar, M. S., & Khurshid, Z. (2019). Nuclear magnetic resonance spectroscopy for medical and dental applications: A comprehensive review. *European Journal of Dentistry*, *13*(01), 124–128.

Zuorro, A., Moreno-Sader, K. A., & González-Delgado, Á. D. (2020). Economic evaluation and techno-economic sensitivity analysis of a mass integrated shrimp biorefinery in North Colombia. *Polymers*, *12*(10), 2397.

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