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Pharmacology activities and extraction of α-chitin prepared from crustaceans: A review

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ABSTRACT

Most crustacean shells are not utilized and are discarded to become waste. α-Chitin, a polysaccharide compound, is commonly found in crustaceans shells. This polysaccharide is widely used in agriculture, pharmacy, and industry. Our review aims to add insights into various methods for extracting chitin from crustaceans. Besides, the pharmacological activities of α-chitin are also discussed in this article. The method of finding data was sourced from PubMed with predetermined criteria. From the article search, it was obtained that there are several ways to extract α-chitin from crustaceans, namely the chemical method, the microbiological method, and the combination of the chemical and enzymatic methods. The pharmacology activities of α-chitin from crustaceans, in general, revealed its potential to be developed as anticancer and anti-inflammatory and to accelerate wound healing.

INTRODUCTION

Chitin, a homopolysaccharide structure arranged over N-acetyl-D-glucosamine molecules connected by the β (1→4) glycosidic bonds, is the second largest compound after cellulose. This colorless, crystalline, or amorphous powder is insoluble in water, organic solvents, dilute acids, and bases (Mathur and Narang, 1990).

Chitin occurs in three different polymorphisms isomers (α, β, and, γ) where N-acetyl glycosyl is a crystallographic unit that is common in all forms (Agboh and Qin, 1997). The intermolecular bonds in chitin are arranged like sheets. The bond that presents in one sheet possesses the same orientation "sense"; for example, in β-chitin, the sheets along the c-axis point in the same direction and the arrangement between the sheets is also parallel. In α-chitin, the sheets along the c-axis face the opposite direction (antiparallel) to that of the β-chitin. In γ-chitin, every third sheet has an opposite direction compared to those of the previous two sheets (Aranaz *et al*., 2009; Roy *et al*., 2017).

Compared to β-chitin, the α-chitin form is more widely available in nature. Cuttlefish bone is an example of a source of β-chitin (Jung *et al*., 2018), but the β-chitin form will change into the α-chitin form when it undergoes an excessive deacetylation process using alkaline and acidic solvents (Akpan, 2018). In nature, α-chitin occupies the most amount compared to other polymorphic forms (Maruthiah and Palavesam, 2017). α-Chitin can be found in the sponge (Tarusin *et al*., 2017), crab (Ifuku *et al*., 2009), shrimp (Goodrich and Winter, 2007), (Aranaz *et al*., 2009), insect cuticles (Wu *et al*., 2020), fungi (Hassainia *et al*., 2018), and sea snail (Mohan *et al*., 2019). Of these various sources, most of the industries prefer using crustaceans subphylum in the manufacture of α-chitin. Crustaceans are available in an abundant amount, as recorded globally in 2017 that almost 15.2 million tons of crustaceans had been produced (FAO, 2020). Parts of the shells, claws, heads, and other wasted parts of the crustaceans can reach 70% of the total weight of these sea creatures. Little can be used

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as animal feed and fertilizer, while the majority of the remainder is discarded (Ordóñez-Del Pazo *et al*., 2014). This huge amount of waste could affect the global sea pollution; thus, a further process should be undertaken knowing that chitin contained in crustacean waste has a higher amount (approximately 20%–30%) than other species (Vani *et al*., 2013).

There are two important steps in chitin extraction, for example, deproteination and demineralization. Sometimes an additional step needs to be carried out, that is, decolorization. Currently, the chitin extraction process is growing. Chitin extraction was first carried out using alkali and acids; however, lately, many researchers prefer to employ chemical, enzymatic, microbiological (Bajaj *et al*., 2015), and natural deep eutectic solvents (NADES) and several other methods.

The existence of chitin is now most preferable because it is biocompatible, biodegradable, easily absorbed in tissues, and nontoxic to both humans and the environment. Its functions are very broad which include in pharmacy, biomedical food, textile, packaging, agriculture, and others (Aranaz *et al*., 2009; Jollès and Muzzarelli, 1999; Roy *et al*., 2017). To the best of our knowledge, no existing article has reviewed the pharmacological activities of α-chitin sourced from the subphylum crustaceans. Therefore, this article aims to focus on the up-to-date extraction methods and the pharmacological activities of α-chitin from crustaceans.

METHODS

Articles were obtained from the PubMed database by inputting strategies, population (P) (crustacean); intervention (I) (preparation; extraction); control (C) (α-chitin; chitin); outcome (O) (chitin yield, demineralization, deproteination, and pharmacology effect); MeSH: ("Chitin/analysis" [MeSH] or "Chitin/biosynthesis" [MeSH] or "Chitin/pharmacology" [MeSH] and "Crustaceans" [MeSH]) NOT "Chitosan" [MeSH]. Chitin derivatives and chitin other than the crude form, for example, chitin nanofibers, chitin nanocrystal, nanochitin, and other forms of chitin, which have undergone further processes besides demineralization, deproteination, and decolorization, were excluded from the search. The search was carried out on all articles published with the above keywords until April 2020.

Extraction process

Every sea species that contains chitin is always associated with organic and inorganic substances, which affect its amount. Processes and conditions during extraction also affect the amount of chitin produced (Sorokulova *et al*., 2009). Deproteination and demineralization steps are considered as critical, due to its role in removing the protein, minerals, lipids, and pigments (Hamdi *et al*., 2017). Some extraction processes prioritize the demineralization process. However, it is not uncommon that deproteination takes precedence in eliminating some minerals by breaking up the calcium–protein–chitin complex in skeletal tissues during fermentation (Zhang *et al*., 2012). Before extraction, the shell should be boiled to make it easier to clean the shell from the remaining meat. The rest of the meat must be removed immediately to avoid the occurrence of the odor (Xu *et al*., 2008). The boiling process is also intended to reduce protease activity with the aim of chitin purification (Flores-albino *et al*., 2012). Figure 1 shows the process of all extractions.

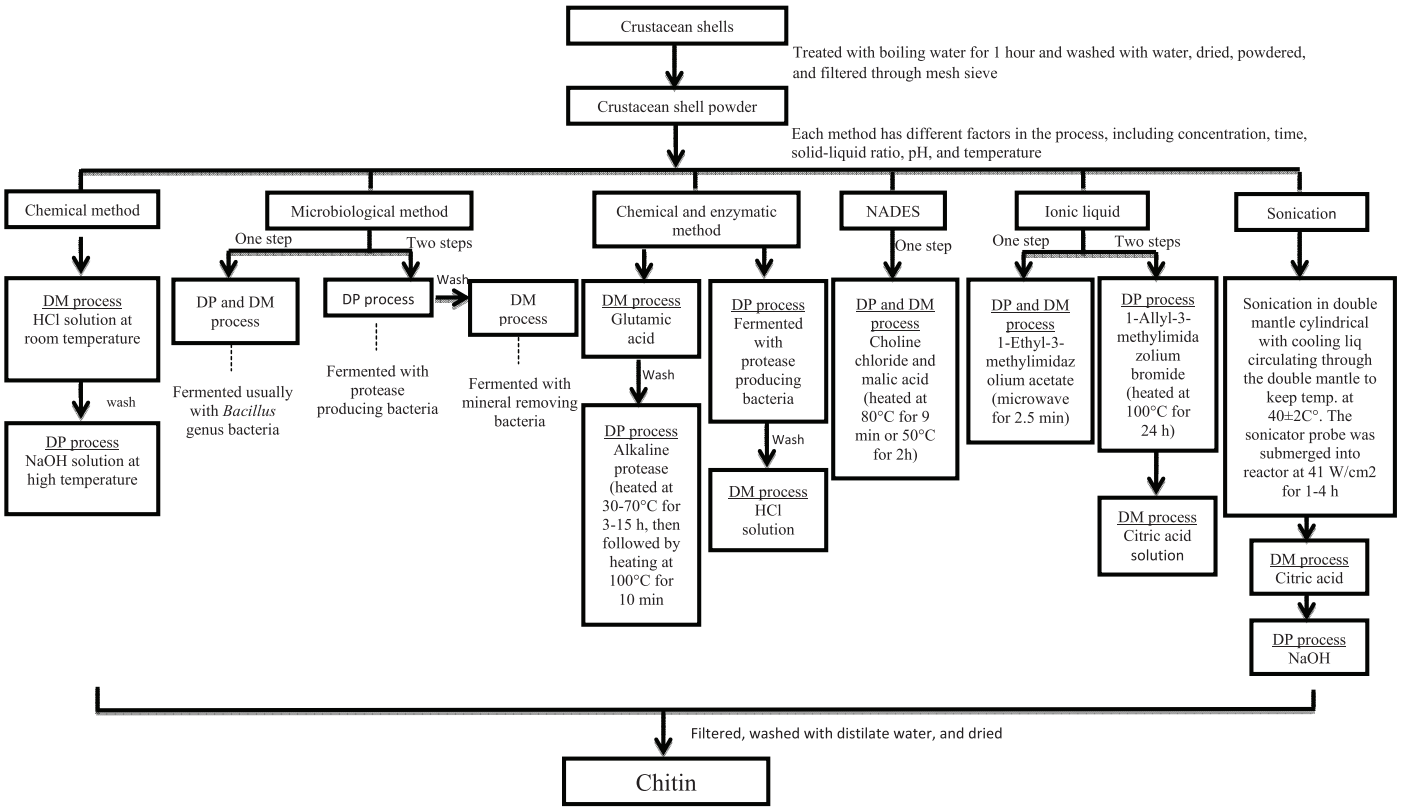

Figure 1. Chitin extractions with various methods. *DM = demineralization; DP = deproteination.

Chemical extraction method

Chemical extraction generally employs alkaline and acidic solvents at high temperatures during the deproteination process (Younes *et al*., 2014). In general, the conditions during the extraction process will greatly affect the molecular weight and the degree of chitin acetylation. The disproportionate time, pH, and temperature during demineralization will produce chitin with a lower molecular weight. Chitin is a sensitive acid compound and can be degraded through several pathways. Hydrolytic depolymerization, heat degradation, and deacetylation are some of the pathways that will cause the physiological properties of the final product to be inconsistent (Ghorbel-Bellaaj *et al*., 2013; Sorokulova *et al*., 2009; Younes *et al*., 2012). Extraction using alkalis and acids produces waste that contains a lot of chloride, sodium, and calcium ions that are difficult to degrade, which will be a problem for the environment (Ding *et al*., 2020; Ghorbel-Bellaaj *et al*., 2013).

The deproteination process produces hydrolyzed protein waste, a hydrolysis solution of protein that is rich in amino acids, peptides, and chitooligosaccharides (Rinaudo, 2006). Hydrolyzed protein from chemical extraction deproteination cannot be used because it contains dangerous alkali solvents (Younes *et al*., 2014). Another disadvantage is that it consumes energy and produces large amounts of corrosive alkaline acids and can damage the environment (Ghorbel-bellaaj *et al*., 2013). However, chemical extraction methods produce a consistent amount of chitin and short time extraction (Kaya *et al*., 2014).

Microbiological extraction method

Given the various limitations of chemical extraction, the researchers keep looking for a safer, environment-friendly, green extraction that could produce more chitin in higher quality. One of the environment-friendly biotechnology methods is the fermentation using lactic acid bacteria (LAB). During fermentation, the bacteria will convert carbon sources, such as glucose or molasses, into lactic acid and pH will decrease due to the production of lactic acid. Crustacean shells have calcium carbonate and will react with lactic acid to form calcium lactate (Mao *et al*., 2013). The acid produced will be responsible for the demineralization process.

The presence of glucose or other carbon sources becomes a critical point that will determine the level of efficiency of the demineralization process (Ghorbel-Bellaaj *et al*., 2012). Although high glucose concentrations will accelerate the fermentation cycle, fermentation will be inhibited and pH will increase when the sugar concentration exceeds 15% (Zhang *et al*., 2012). Glucose at a concentration of 5% can induce protease production even up to four times more than the medium that does not use glucose (Ghorbel-Bellaaj *et al*., 2012).

The use of protease enzymes in the chitin extraction process will facilitate the removal of protein and calcium carbonate. Bacteria were tested first before use to see the ability to produce proteases. The test was continued to see the activity of proteolysis, for example, by looking at the proteolytic zone of casein agar (Harkin *et al*., 2015). The use of protease or lactic acid produced by bacteria to extract chitin is simple and inexpensive (Ghorbel-bellaaj *et al*., 2013).

The deproteination and demineralization can be either done simultaneously or carried out separately into two steps (Dun *et al*., 2019). Two-step fermentation has the advantage of acquiring chitin with a high grade of purity. LAB and protease-producing microbials have different optimal growth conditions so that it is preferably carried out in two steps. However, the two-step fermentation process requires a longer time and higher costs (Xu *et al*., 2008). The fermentation process that includes deproteinizing or demineralizing can be carried out in one step. However, the efficiency of deproteination is difficult to achieve; therefore, optimization of the factors that influence the process of demineralization and deproteination is needed (Oh *et al*., 2007).

Many factors will influence the demineralization and deproteination processes, such as carbon source and glucose concentration, inoculum amount, pH, and fermentation time (Arbia *et al*., 2013) so that several methods of approach for optimization studies in extracting chitin can be used, such as the Box–Behnken design or the Plackett–Burman design. These methods are also useful for understanding interactions between various physicochemical parameters using a minimum number of

Table 1. Various reports on α-chitin extraction using the chemical method.

No.	Crustacean	Demineralization			Deproteination			Note	Chitin yield (%)	Reference
		HCl (w/v)	Time	T(°C)	NaOH (w/v)	Time	T(°C)			
1.	Chilean crab	2 N HCl (1:10)	1 hours	Room	1 M NaOH (1:20)	3 hours	100	–	10,4	(Bernabé *et al*., 2020)
2.	Lobster	HCl 6%	2.5 hours	Room	NaOH 10%	3 hours	90	Decolorize: H2O2 10% at 80°C	16.53 ± 2.3	(Zhu *et al*., 2017)
3.	Barnacle (*Chelonibiapatula*)	1M HCl	10 minutes	Room	2 M NaOH	20 minutes	–	–	3.11	(Kaya *et al*., 2014)
4.	Shrimp(*Metapenaeus monoceros*)	1.25 M HCl (1:10)	6 hours	Room	1.25 M NaOH	4 hours	–	–	20±2	(Manni *et al*., 2010)
5.	Shrimp (*Parapenaeopsis stylifera*)	0.25 M HCl	15 minutes (1:40)	Room	1 M NaOH (1:30)	24 hours	70	DP: 98% DM: 90%	–	(Percot *et al*., 2003)
6.	Krill (*Euphausia superba*)	1.7 M HCl	6 hours	Room	2.5 M NaOH	1 hours	75	Decolorization: 1% potassium permanganate	27.80 ± 1.48	(Wang *et al*., 2013)

*DM = demineralization;

DP = deproteination.

Table 2. α-Chitin extraction using microbial fermentation with demineralization and deproteination yield.

No.	Crustacean	Microbial	Fermentation process	% DM	% DP	Reference
1.	Shrimp	*Paracoccus saliphilus*	Shell powder mixed with halophilic production medium (1:3). After sterilized, 5% *Paracoccus saliphilus* was inoculated into the media at 50°C for 3 hours	–	85.64	(Maruthiah and Palavesam, 2017)
	Crab			–	69.33	
	Lobster			–	40.18	
2.	Shrimp	*Bacillus subtilis*	Using Box–Behnken design, the optimum value will be obtained with conditions: sucrose (5%), shrimp shell (12.5%), inoculum size (10%), and fermentation time (7 days)	82.1	96.0	(Gamal *et al*., 2016)
3.	Shrimp *(Penaeus merguiensis)*	*Pseudomonas aeruginosa, Serratia marcescens*, and *Bacillus pumilus*	Three proteases producing bacteria mixed with 5% shrimp shell and 10% glucose fermented at 60°C for 6 days	78.46	74.76	(Sedaghat *et al*., 2016)
4.	Crab (*Carcinus mediterraneus)*	*B. subtilis*	Crab shell was fermented into medium with 5% glucose, at 37°C for 5 days using protease-producing bacteria	76.7	81.6	(Hajji *et al*., 2015)
		Bacillus mojavensis		73.2	80.4	
		B. pumilus		83.07	95	
		Bacillus amyloliquefaciens		79.8	85.3	
		Bacillus licheniformis		89	92	
		Bacillus cereus		81.8	83.1	
5.	Shrimp (*Metapenaeus monoceros*)	*B. pumilus*	Using Plackett–Burman design, the optimum value will be obtained with conditions: shrimp shell (70 g/l) and 5% glucose sol fermented, pH of 5.0, at 35°C for 6 days	88	94	(Ghorbel-bellaaj *et al*., 2013)
6.	Shrimp (*Metapeneaus monoceros*)	*B. pumilus*	Fermentation is done by adding shrimp shell in 5% glucose medium. After sterilization, the medium was fermented with each microbial for 5 days at 37°C	75.3	91.2	(Ghorbel-Bellaaj *et al*., 2012)
		B. mojavensis		78.7	88	
		B. licheniformis		55.55	90.8	
		B. cereus		77.3	88.6	
		B. amyloliquefaciens		66.05	90.8	
		B. subtilis		79.9	91.25	
7.	Shrimp (*Penaeus vannamei*)	*Lactobacilllus acidophilus*	Shrimp shell, microbial, and glucose were fermented at 37°C for 96 hours	99.5	97.4	(Duan *et al*., 2012)
8.	Shrimp (*Metapeneaus monoceros*)	*P. aeruginosa*	Using the Plackett–Burman design, shrimp shell and 5% glucose (1:1) were fermented with microbial for 5 days	96	89	(Ghorbel-Bellaaj *et al*., 2011a)
9.	Shrimp	*B. cereus*	Shrimp shell 3% was fermented with inoculum microbial 10% for 14 days at 37°C	95	92	(Sorokulova *et al*., 2009)
		Exiguobacteriumacetylicum		97.1	92.8	
10.	Red crab (*Chionoecetes japonicus*)	*Lactobacillus paracasei* subsp. *Tolerans* and *S. marcescens*	Crab shell with 10% glucose and microbial was added and fermented at 30°C for 7 days	97.2	52.6	(Jung *et al*., 2006)

*DM = demineralization;
DP = deproteination.

experiments. The Plackett–Burman design aims to select important factors from a large number of variables. From many important factors, there will then be tested statistics where this will be useful in designing experiments, building models, and evaluating the effects of different factors to find the optimal conditions for getting chitin (de Coninck *et al*., 2000; Ghorbel-Bellaaj *et al*., 2011).

In general, an enzymatic process using bacteria is carried out by the fermentation process. High protease activity shows the ability to hydrolyze protein more and more (Sedaghat *et al*., 2016). Most of the microbes used are free of chitinolytic activity which prevents the reduction of chitin quality during deproteination (Bajaj *et al*., 2015). Deproteination cannot reach 100%; this is because the enzyme does not get access to penetrate some of the protected proteins in the innermost layer, and ultimately proteolysis will not occur (Wang *et al*., 2006). The result of obtaining chitin by this method is chitin which has molecular weight and crystalline which is higher than chemically prepared chitin (Pacheco *et al*., 2011). The other disadvantage of microbial extraction is that it takes a long time. In general, the advantages of chitin extraction using the microbial extraction method are as follows:

1. It is homogeneous and prevents deacetylation caused by strong alkali acid, resulting in high-quality products (Ramírez-Coutiño *et al*., 2006; Zhang *et al*., 2012).
2. It is an ecofriendly and green method (Sedaghat *et al*., 2016).
3. It helps in obtaining protein hydrolysate (amino acid and polypeptide) as a byproduct of the deproteination process (Hajji *et al*., 2015).

Chemical and enzymatic extraction method

Extraction using the chemical and enzymatic method is a method that has two steps in extraction. The combination of chemicals and enzymes is done to get a shorter extraction time.

Table 3. α-Chitin extraction using microbial fermentation with chitin yield.

No.	Crustacean	Microbial	Fermentation process	Chitin yield (%)	Reference
1.	Shrimp (*Metapenaeus monoceros*)	*Aeribacillus pallidus*(VP3), *Lysinibacillus fusiformis*(C250R), and *Anoxybacillus kamchatken sis* (M1V)	Using the Taguchi and Box–Behnken designs, final culture volume of 15 ml with pH 9 containing 20 g/l shrimp powder and 10 g/l sucrose were inoculated with VP3, C250R, and M1V strains at 0.05, 0.1, and 0.2, respectively. The culture was incubated at 45°C for 24 hours under agitation of 200 rpm	16.7	(Jabeur *et al*., 2020)
2.	Tiger shrimp head	*Brevibacillus parabrevis*	Microbial was grown in 100 ml of liquid medium containing 3% (*w/v*) shrimp waste, 0.05% $MgSO_4$•$7H_2O$, and 0.1% K_2HPO_4. Incubation conditions were kept at 37°C and 150 rpm and the culture was incubated for 4 days	14.35 ± 1.40	(Thang *et al*., 2019)
	Tiger shrimp shell			23.23 ± 3.75	
	White leg shrimp head			9.27 ± 0.20	
	White leg shrimp shell			16.87 ± 3.03	
3.	Brown crab (*Cancer pagurus*)	*Exiguobacterium* spp.	Brown crab shell with 10% glucose sol (1:20) was sterilized. A certain amount of bacterial culture is added and then incubated for 5 days at 30°C. After incubation, the pellet was washed with deionized water and sterilize with 70% (*v/v*) ethanol. Referment the previous fermentation results with 10% glucose and bacterial inoculum at 30°C for 7 days	13.8 ± 0.85	(Harkin *et al*., 2015)
		B. licheniformis		14.5 ±.099	
		B. subtilis + *Lactobacillus* spp.		14.7 ± 1.56	
		B. cereus + *Pseudomonas* spp.		15.4 ± 1.56	
		B. cereus + *Arthrobacter luteolus*		14.1 ± 1.41	
		Pseudomonas spp.		16.3 ± 0.42	
		Pseudomonas migulae		15.5 ± 0.85	
		Enterococcus sp.		14.6 ± 1.13	
4.	Red crab (*Chionoecetes japonicus*)	*L. paracasei*	Crab leg shells with 10% glucose sol and inoculated microbial was incubated for 5 days at 30°C. Pellet was filtered and washed with distilled water. Pellet was refermented with 10% glucose soldan microbial at 30°C in a shaking incubator (180 rpm) for 7 days	38.67 ± 1.35	(Jung *et al*., 2007)
		S. marcescens		36.67 ± 1.33	
5.	Shrimp (*Penaeus vannamei*)	Deproteination: *S. marcescens*	Deproteination: using the Taguchi experimental design, it was found that the optimal process is obtained with conditions: 2% shrimp shell, 2 hours sonication, and fermentation time with microbial of 4 daysDemineralization: the optimal process is obtained with conditions: 2% shrimp shell, 15% glucose, and fermentation time with microbial of 2 days	18,9	(Zhang *et al*., 2012)
		Demineralization: *Lactobacillus plantarum*			
6.	Crab (*Callinectes bellicosus*)	*Lactobacillus sp.*	Crab shell fermented with microbial in media containing sugar cane molasses at 35°C for 120 hours	34.4	(Flores-Albino *et al*., 2012)
7.	Shrimp (*Penaeus monodon*)	Deproteination: *Bacterium HP1* (culture GM)	Deproteination: shrimp shell was fermented in medium containing *P. monodon* for 68 hours and in medium containing *C. crangon* for 50 hours, each at 37°C	37	(Xu *et al*., 2008)
	Shrimp (*Crangon crangon*)	Demineralization: *Lactobacillus casei MRS1*	Demineralization:deproteination results with glucose fermented with microbial inoculums at 37 °C for 47 hours for *P. monodon* and 46 hours for *C. crangen*	30	
8.	Crawfish (*Procambarus clarkii*)	*L. paracasei*	Crawfish shell was fermented with 10% dextrose at 30°C for 3 days	20.6	(Cremades *et al*., 2001)
9.	Shrimp	*A. niger0576*	Shrimp shell was fermented with fungi inoculum at 30°C for 4 days. Fungal mycelia decanted from shrimp shell. Entrapped fungal mycelia can release with hot water. Then, soak the shrimp shell in 5% lithium chloride–N, N-dimethylacetamide solvent (1:150) for 48 hours	22 ± 2	(Teng *et al*., 2001)
		A. niger0307		27 ± 3	
		A. niger0474		17 ± 3	

In general, the principle of this method is to replace a microbial in one of the extraction steps with a chemical compound (Table 4).

Natural deep eutectic solvent

NADES is obtained from an adequate mixture of hydrogen bond acceptor and donor which will enables their bonding through the interaction of hydrogen bonds forming eutectic with a low melting point (Abbott *et al*., 2004). The advantage of NADES is that it is a nontoxic and biodegradable solvent, where being environmentally friendly which will be an advantage compared to alkaline, acidic, and ionic liquid (IL) solvents (Huang *et al*., 2018). Besides, NADES can be used in extraction media and as a solvent in several biopolymers, including starch, cellulose, and lignin (Francisco *et al*., 2012). A mixture of choline halide (chloride/bromide)urea, choline chloride–thiourea, chlorocholine chlorideurea, and betaine hydrochlorideurea is a type of NADES suitable for dissolving α-chitin. Dissolution from biopolymers can be carried out using heating under the microwave, conventional heating, or heating by ultrasonication (Sharma *et al*., 2013). This is appropriate with the data extraction process presented in Table 5.

Table 4. α-Chitin extraction using chemical and enzymatic combination.

No.	Crustacean	Demineralization			Deproteination			Note	Chitin yield (%)	Reference
		Solvent (*w/v*)	Time	T (°C)	Proteinase	Time	T (°C)			
1.	Crab (*Scylla serrate*)	5% glutamic acid 1:10	12 hours	75	Alkaline protease	6 hours	55	Decolorization process in 5% (*w/v*) potassium permanganate solution, 30 minutes, and continued to soak in 3% oxalic acid sol	11.88	(Ding *et al*., 2020)
2.	Crayfish (*Procambarus clarkii*)	10% innoculation of *Bacillus coagulans* with 5% (*w/v*) glucose added	48 hours	50	Proteinase K of 1,000 U/g	Demineralization and deproteination processes are carried out simultaneously		DP: 93 % DM: 91 %	94	(Dun *et al*., 2019)
3.	Blue crab (*P. segnis*) Shrimp (*P. kerathurus*)	Proteinase from *Portunus segnis viscera*	3 hours	50	0.55 M HCl	30 minutes	4		19.06 ± 1.65 22.23 ± 0.94	(Hamdi *et al*., 2017)
4.	Shrimp (*Metapenaeus monoceros*)	Proteinase from *B. mojavensis*	3 hours	50	1.5 M HCl 1:10	6 hours	25	DP: 77% ± 3%	–	(Younes *et al*., 2014)
		Proteinase from *Bacillus capriscus*	3 hours	45				DP: 78% ± 2%		
5.	Shrimp (*Metapeneaus monoceros*)	A2 crude enzyme produced by *P. aeruginosa*	3 hours	40	5% HCl (1:10)	6 hours	25	DP: 85%	–	(Ghorbel-Bellaaj *et al*., 2011)
6.	Shrimp (*Metapenaeu monoceros*)	SV1 crude enzyme by *B. cereus*	3 hours	40	1.5 M HCl (1:10)	6 hours	25	DP: 88%	16.55±1.5	(Manni *et al*., 2010)

*DM = demineralization;
DP = deproteination.

Table 5. α-Chitin extraction using NADES.

No.	Crustacean	Extraction	Chitin yield (%)	Reference
1.	Shrimp shell	Choline chloride 1 M and malic acids 1 M (1:1) were heated at 80°C. Shrimp shell and mixture (1:20) were heated under microwave for 9 minutes	Chitin: - DM: 99% DP: 93.8%	(Huang *et al*., 2018)
2.	Lobster Shell	Choline chloride and malic acid (1:2) were heated at 50°C for 2 h. The proportion of lobster shells and mixture is (7:1). Decolorization was continued with 10% (w/v) H_2O_2 at 80°C	Chitin: 20.63 ± 3.30%	(Zhu *et al*., 2017)

*DM = demineralization;
DP = deproteination.

Table 6. α-Chitin extraction using IL.

No.	Crustacean	Extraction	Chitin yield (%)	reference
1.	Black tiger Shrimp	Shrimp shell was suspended with [C2mim][OAc] (1:49). The mixture was placed into a microwave for 2.5 minutes. The results were centrifuged and washed in DI water. The final result is in filament form	2.5	(Berton *et al*., 2018)
2.	Red queen crab	Crab shell was suspended with 1-allyl-3-methylimidazolium bromide at 100°C for 24 hours. The deproteination product was soaked with 1.5% HCl for 3 hours at room temperature	7.5	(Setoguchi *et al*., 2012)

Ionic liquid

The IL is a salt with a low boiling point that will form a liquid at temperatures below the water boiling point, which is useful as a solvent for cellulose or other polysaccharides (Zakrzewska *et al*., 2010). The advantages of the IL method are that it is more economic, efficient, and ecofriendly (Zhu *et al*., 2017). However, this method also has disadvantages, such as high cost and toxicity (Sharma *et al*., 2013), besides handling IL by untrained people is also dangerous (Bajaj *et al*., 2015). Dissolution using IL solvents will damage the hydrogen bonds in the "reassemble" chains into a new arrangement, thus forming amorphous chitin (Shamshina and Rogers, 2020).

Sonication

It is known that the use of high-intensity ultrasound to extract several polysaccharides requires a short time and little

solvent so that it will save production costs (Wang and Wang, 2004). However, the addition of sonication to the chitin extraction process is not very useful in the demineralization step; even chitin can be damaged due to some of the material being dissolved and rinsed with reagents due to depolymerization (Kjartansson *et al*., 2006a). Besides, chitin yields are low due to extensive perforation of the shell (Kjartansson *et al*., 2006b).

The addition of sonication in extraction will be very useful if there is an incomplete deproteination process. Furthermore, the addition of sonication will also trigger changes in the crystalline chitin form so that it will be easier if it will be reacted chemically (Kjartansson *et al*., 2006a). Finally, the use of high-intensity ultrasound will be very useful in accelerating the extraction with a low degree of crystalline, if needed (Kjartansson *et al*., 2006b).

Pilot-scale chitin production

Chitin production on a pilot scale has been carried out in several experiments (Table 8). Chitin produced at the pilot scale is not very different from chitin production at the laboratory scale. Extraction by bacterial fermentation method is suitable for pilot-scale chitin production; this can be seen in all pilot-scale tests using the microbial method.

Pharmacology activities

Pharmacological studies of crude α-chitin are very rare. Table 9 shows some of the best references we can find. In general, pharmacological activities of α-chitin showed its potential to be anticancer and anti-inflammatory and to accelerate wound healing (Anandan *et al*., 2004; Bae *et al*., 2013; Teng *et al*., 2001). Chitin was tested pharmacologically to Hep2 (human larynx carcinoma cell line), RD (human embryo rhabdomyosarcoma cell line), and THP-1 (human monocytic leukemia cell line). Although the cytotoxic effect is not too large, its anticancer potential can be enhanced by changes in the low molecular weight of chitin (Bouhenna *et al*., 2015; Salah *et al*., 2013).

Table 7. α-Chitin extraction using sonication.

No	Crustacean	Demineralization	Deproteination	Chitin yield (%)	Reference
1.	Shrimp (*Pandalus borealis*)	Shrimp shell was suspended with 0.25 M HCl (1:40) at 40°C for 4 hours; sonication was added at 41 W/cm² with temperature 40°C ± 2°C for 1 and 3 hours additional time	The demineralized product was suspended in 0.25 M NaOH (1:40) at 40°C with sonication for 4 hours. Additional deproteination is carried out with soaking to 1 M NaOH for 2 hours	11.4	(Kjartansson *et al*., 2006a)
2.	Freshwater Prawn (*Macrobrachium rosenbergii)*	Prawn shell was demineralized in 0.25 M HCl (1:40) for 4 hours at 40°C and then sonicated at 41 W/cm² for 4 hours	The demineralization product was soaking with 0.25 M NaOH (1:15) at 40°C and sonicated for 4 hours. Additional deproteination is carried out with soaking to 1 M NaOH at 90°C for 2 hours	5.03	(Kjartansson *et al*., 2006b)

Table 8. Pilot scale of α-chitin production.

No.	Crustacean	Scale	Deproteination			Demineralization			Chitin yields (%)	Reference
			Condition	Time	T(°C)	Condition	Time	T(°C)		
1.	Shrimp (*Crangon crangon*)	0.25 l	Shrimp shell: glucose, yeast extract, calcium carbonate (GYC) medium containing inoculum (0.025 kg:0.025 l) with 0.225 l tap water. The inoculum was obtained from shrimp shell. Every 1,500 ml GYC medium containing 5 g/l glucose (G), 20 g/l yeast extract (Y), and 30 g casein (C)	40 hours	37	The deproteination results were fermented with 0.025 l lactobacilli lactic acid with 0.225 l of tap water. Lactobacilli were grown in De Man, Rogosa and Sharpe agar medium containing different hexoses and pentoses	40 hours	37	27	(Bajaj *et al*., 2015)
		10 l	Shrimp shell: GYC medium containing inoculum (1 kg:1 l) with 9 l tap water	40 hours	37	The deproteination results were fermented with 1 l of lactobacilli lactic acid with 9 l of tap water	40 hours	37	32	
		300 l	Shrimp shell: GYC medium containing inoculum (39 kg: 29 l) with 261 l tap water	40 hours	37	The deproteinated product was fermented with 29 l lactobacilli lactic acid with 271 l tap water	40 hours	37	23	
2.	Shrimp	20 l	1.2 kg shrimp shell fermented and 1.2 l *B. cereus* 8-1 strain culture overnight with added 12 l tap water	14 days	37		DP: 78.6 ± 2.6 DM: 73.0 ± 1.5			(Sorokulova *et al*., 2009)
3.	Shrimp (*Penaeus vannamei*)	930 l	50 kg shrimp shell, 7.5 kg glucose, and 51 l of water were mixed. Then the mixture is fermented with *L. acidophilus*	96 hours	40		DP: 90.3			(Duan *et al*., 2012)

Table 9. Pharmacology activities of α-chitin.

No.	Crustacean	Chitin obtain	Pharmacological activities	Mechanism	Reference
1.	Shrimp (*Parapenaeus longirostris*)	Demineralization: the shrimp shell was suspended with 2 M HCl (1:15) for 30 minutes at room temperature. Deproteination: demineralized product was soaked with 2 M NaOH for 90 minutes at 55°C. Decolorization: decolorized with 0.315% NaOCl during 5 minutes	Chitin has a cytotoxic effect against Hep2 cells with IC50 = 400 µg/ml and total toxicity at 2,000 µg/ml and RD cell lines with IC50 = 200 µg/ml; total toxicity is not reached even at 300 µg/ml	The interaction between positive charged group of chitin and derivative with negative charge group of tumor cells	(Bouhenna *et al*., 2015)
2.	Shrimp (*P. longirostris*)	Demineralization: the shrimp shell was suspended with 1.5 M HCl (1:15) for 30 minutes at room temperature. Deproteination:demineralized product was soaked with 2 M NaOH for 120 minutes at 45°C. Low molecular chitin preparation: chitin was hydrolyzed with 7N HCl at 70°C for 3 hours	Chitin has the potential to be a specific anticancer on the human monocytic leukemia cell line, THP-1. However, the potential is not greater than the low molecular chitin	Possible mechanism based on the presence of chitin-binding protein (maybe YKL-40), also known as chitinase. This bond is shown by the expression of THP-1 cells and the unexpression of MRC-5 cells due to binding to chitin. This interaction will inhibit the growth of tumor cells	(Salah *et al*., 2013)
3.	Alpha chitin	α-Chitin with diameter particle average 11.0 µm	Giving food containing 0.2% α-chitin in mice can reduce peanut allergy in mice better than β-chitin and β-chitosan at the same dose. Chitin has IgE protection which will mediate anaphylaxis. Additionally, chitin inhibits the Th2 response thereby reducing the incidence of inflammation	Increase levels of IL-12, where IL-12 will stimulate Th1 cells to produce IFN-gamma, thus producing IL-12 which will strongly suppress the production of IgE	(Bae *et al*., 2013)
4.	Shrimp	Shown in Table 3 no. 9	Chitin was tested on fibroblast cell lines from mouse and human and osteoblast cell lines for 4 days using tetrazolium colorimetric based (3-[4,5-dimethylthiazolyl-2]-2,-diphenyl tetrazolium bromide assay) and neutral red uptake assays. The results show that there are no acute cytotoxicty and proproliferation effects	–	(Teng *et al*., 2001)
5.	Prawn	Kitin (MW 1.08 × 10^5 KDa; purity 97.2%)	Giving food containing 2% chitin in rats for 30 days showed effectiveness in preventing gastric ulcer induced by HCl + ethanol	Chitin administration will reduce lipid peroxidation and increase the activity of antiperoxidation enzymes and glutathione-dependent antioxidant enzymes	(Anandan *et al*., 2004)

CONCLUSION

There are several methods to extract α-chitin from crustaceans, that is, chemical, microbiological, chemical–enzymatic combination, using NADES, IL solvent, and sonication. The best α-chitin extraction method from crustaceans is the chemical l–enzymatic combination method. This method was able to provide more efficient extraction time and is environmentally friendly with quality parameters, such as very good and consistent deproteination and demineralization. In addition, the chitin yield was better than that of other methods. Followed by successive recommended methods were the microbiological methods, NADES, chemical methods, and IL, respectively. α-Chitin has proven to possess anticancer and anti-inflammatory potential and could accelerate wound healing. The mechanism of action of α-chitin anti-inflammatory activity is still interesting to be further explored.

CONFLICTS OF INTEREST

All the authors declare that they have no conflicts of interest for this work.

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