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...
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.
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          | HCl (w/v) | Time  | T(°C) | NaOH (w/v) | Time  | T(°C) | Note                      | Chitin yield (%) | Reference             |
|-----|---------------------|-----------|-------|-------|------------|-------|-------|---------------------------|-----------------|----------------------|
| 1.  | Chilean crab        | 2 N HCl   | 1 hours| Room  | 1 M NaOH   | 3 hours| 100   | Decolorization: H2O2 10% at 80°C | 10.4            | (Bernabé et al., 2020) |
| 2.  | Lobster             | HCl 6%    | 2.5 hours| Room  | NaOH 10%   | 3 hours| 90    |                          | 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 | 6 hours| Room  | 1.25 M NaOH| 4 hours| –    |                          | 20±2            | (Manni et al., 2010)  |
| 5.  | Shrimp (Parapeneaeopsis stylifera) | 0.25 M HCl | 15 minutes| Room  | 1 M NaOH   | 24 hours| 70   | DP: 98%                   | –               | (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 | (Marathiah and Palavesam, 2017)              |
|     | Crab                        | *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)                         |
|     | Lobster                     |                                  |                                                                                      | –    | 40.18 |                                                  |
| 2.  | Shrimp                      | *Bacillus subtilis*              | Fermentation process                                                                 | 83.07 | 96.0 | (Shrimp, 2009)                               |
|     | Crab                        | *Bacillus mojavensis*            | Crab shell was fermented into medium with 5% glucose, at 37°C for 5 days using protease-producing bacteria | 76.7 | 81.6 | (Haji et al., 2015)                          |
|     | Lobster                     | *Bacillus pumilus*               |                                                                                      | 73.2 | 80.4 |                                                  |
|     | Lobster                     | *Bacillus licheniformis*         |                                                                                      | 83.07 | 95   |                                                  |
| 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*                    |                                                                                      | 79.8 | 85.3 |                                                  |
|     | Crab                        | *Bacillus mojavensis*            |                                                                                      | 89   | 92   |                                                  |
|     | Lobster                     | *Bacillus licheniformis*         |                                                                                      | 81.8 | 85.1 |                                                  |
|     | Lobster                     | *Bacillus cereus*                |                                                                                      | 81.8 | 85.1 |                                                  |
| 5.  | Shrimp (Metapeneaus monoceros) | *B. pumilus*                    | Using Placket–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)               |
|     | Shrimp (Metapeneaus monoceros) | *B. mojavensis*                  |                                                                                      | 78.7 | 88   | (Ghorbel-Bellaaj et al., 2012)               |
|     | Lobster                     | *B. licheniformis*               |                                                                                      | 55.55 | 90.8 |                                                  |
|     | Lobster                     | *B. cereus*                      |                                                                                      | 77.3 | 88.6 |                                                  |
|     | Lobster                     | *B. amyloliquefaciens*           |                                                                                      | 66.05 | 90.8 |                                                  |
|     | Lobster                     | *B. subtilis*                    |                                                                                      | 79.9 | 91.25|                                                  |
| 7.  | Shrimp (Penaeus vannamei)    | *Lactobacillus 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 Placket–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)                    |
| 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 deproteinization (Bajaj et al., 2015). Deproteinisation 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 (Ramirez-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 deproteinisation process (Haji 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.
Crawfish shell was fermented with 10% dextrose at 30°C.

36.67 ± 1.33

Huang. (2007)

Reference
Crustacean A. niger0576
Shrimp (Panulirus virginalis)
Brown crab (Cancer pagurus)
Red crab (Chironomecus japonicus)
Shrimp (Penaeus vannamei)
Crab (Callinectes bellicosus)
Shrimp (Panulirus monodon)
Shrimp (Crangon crangon)
Crawfish (Procambarus clarkii)
Shrimp

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 kamchatkensis (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 after 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% MgSO4·7H2O, and 0.1% KH2PO4. 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) |
| 3.  | Brown crab (Cancer pagurus) | Exiguobacterium spp., B. licheniformis, B. subtilis + Lactobacillus spp., B. cereus + Pseudomonas spp., B. cereus + Arthrobacter luteus | 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% 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) |
| 4.  | Red crab (Chionomeces japonicus) | L. paracasei, S. marcescens | Crab leg shells with 10% glucose sol and inoculated microalgal 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 | 14.5 ± 0.99 | (Jabeur et al., 2020) |
| 5.  | Shrimp (Penaeus vannamei) | Deproteinization: S. marcescens, Demineralization: Lactobacillus plantarum | Deproteinization: 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 days. Demineralization: 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) |
| 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 (Panulirus monodon) | Deproteinization: Bacterium HP1 (culture GM), Shrimp (Crangon crangon) | Deproteinization: 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) |
| 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, A. niger0307, A. niger0474 | Shrimp shell was fermented with fungi inoculum at 30°C for 4 days. Fungal mycelia decanted from shrimp shell. Then, soak the shrimp shell in 5% lithium chloride-N, N-dimethylacetamide solvent (1:150) for 48 hours | 22 ± 2 | (Teng et al., 2001) |

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 hydrochlorodurea 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.
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).

### Ionic liquid

Sonication

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

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

| No. | Crustacean                      | Demineralization               | Deproteinization               | Note                                                                 | Chitin yield (%) | Reference                   |
|-----|---------------------------------|---------------------------------|---------------------------------|----------------------------------------------------------------------|------------------|------------------------------|
| 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,         |                                | 11.88                        | (Ding et al., 2020)           |
|     |                                 |                                 |                                 |                                                                      |                  |                              |                              | and continued to soak        |                                |                              |                              |                               |                               |
|     |                                 |                                 |                                 |                                                                      |                  |                              |                              | in 3% oxalic acid sol       |                                |                              |                               |                               |                               |
|     |                                 |                                 |                                 |                                                                      |                  |                              |                              |process in 5% (w/v) potassium|                                |                              |                               |                               |                               |
|     |                                 |                                 |                                 |                                                                      |                  |                              |                              | permanganate solution, 30 |                                |                              |                               |                               |                               |
|     |                                 |                                 |                                 |                                                                      |                  |                              |                              | minutes, and continued to  |                                |                              |                               |                               |                               |
|     |                                 |                                 |                                 |                                                                      |                  |                              |                              | soak in 3% oxalic acid sol |                                |                              |                               |                               |                               |
|     |                                 |                                 |                                 |                                                                      |                  |                              |                              | process in 5% (w/v) potassium|                                |                              |                               |                               |                               |
| 2.  | Crayfish (Procambarus clarkii)  | 10% inoculation                 | 48 hours                        | 50                                                                   | Proteinase K     | Demineralization and          | DP: 93%                      | 94                           | (Dun et al., 2019)           |
|     |                                 | of Bacillus coagulans with      |                                 |                                                                      | 1,000 U/g       | deproteination processes are  |                              |                              |                                |                              |                               |                               |                               |
|     |                                 | 5% (w/v) glucose added          |                                 |                                                                      |                  | carried out simultaneously    |                              |                              |                                |                              |                               |                               |                               |
| 3.  | Blue crab (P. segnis)           | Proteinase from Portunus segnis| 3 hours                         | 50                                                                   | 0.55 M HCl      | 30 minutes                    | 4                            | 19.06 ± 1.65                 | (Hamdi et al., 2017)         |
|     | Shrimp (P. kerathurus)          | viscera                         |                                 |                                                                      |                  |                              |                              | 22.23 ± 0.94                 |                                |                              |                               |                               |                               |
| 4.  | Shrimp (Metapenaeus monoceros)  | Proteinase from B. majavensis   | 3 hours                         | 50                                                                   | 1.5 M HCl       | 6 hours                       | 25                           | 77% ± 3%                    | (Younes et al., 2014)        |
|     |                                 | Proteinase from Bacillus        |                                 |                                                                      |                  |                              |                              | –                           |                                |                              |                               |                               |                               |
|     |                                 | capricus                       |                                 |                                                                      |                  |                              |                              | –                           |                                |                              |                               |                               |                               |
| 5.  | Shrimp (Metapenaeus monoceros)  | A2 crude enzyme produced by P.  | 3 hours                         | 40                                                                   | 5% HCl (1:10)   | 6 hours                       | 25                           | 85%                         | (Ghorbel-Belhaj et al., 2011)|
|     |                                 | aeruginosa                     |                                 |                                                                      |                  |                              |                              | –                           |                                |                              |                               |                               |                               |
| 6.  | Shrimp (Metapenaeus monoceros)  | SV1 crude enzyme by B. ceras    | 3 hours                         | 40                                                                   | 1.5 M HCl       | 6 hours                       | 25                           | 88%                         | (Manni et al., 2010)         |
|     |                                 |                                 |                                 |                                                                      |                  |                              |                              | 16.55±1.5                   |                                |                              |                               |                               |                               |

*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: -         | (Huang et al., 2018)         |
|     |                  |                                                                            | DM: 99% DP: 93.8%|                              |                              |                               |                               |
| 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) H2O2 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)       |
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) 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 | Demineralization | Deproteination | Chitin yield (%) | Reference               |
|-----|-----------------------------|-------|------------------|----------------|------------------|-------------------------|
| 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) | 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 | 10 l | 37 | 27 | (Bajaj et al., 2015) |
| 2.  | Shrimp (Penaeus vannamei)   | 0.20 l| Shrimp shell: GYC medium containing inoculum (1 kg:1 l) with 9 l tap water | The deproteination results were fermented with 11 of lactobacilli lactic acid with 9 l of tap water | 300 l | 40 hours | 37 | 23 | (Sorokulova et al., 2009) |
| 3.  | Shrimp (Penaeus vannamei)   | 0.30 l| Shrimp shell: GYC medium containing inoculum (39 kg: 29 l) with 261 l tap water | The deproteinated product was fermented with 29 l lactobacilli lactic acid with 271 l tap water | 930 l | 40 hours | DP: 90.3 | (Duan et al., 2012) |
Conclusions

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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Table 9. Pharmacological activities of α-chitin.

| No. | Crustacean                  | Chitin obtain          | Pharmacological activities                                                                 | Mechanism                                                                 | Reference                        |
|-----|---------------------------|------------------------|-------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------|----------------------------------|
| 1   | Shrimp (Parapeneaus longirostris) | Demineralization: the shrimp shell was suspended with 2 M HCl (1:15) for 30 minutes at room temperature. Deproteinization: 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. Deproteinization: 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 cytotoxicity and proproporation effects | – | (Teng et al., 2001) |
| 5   | Prawn                     | Kitin (MW 1.08 × 10^4 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 antioxidant enzymes and glutathione-dependent antioxidant enzymes | (Anandan et al., 2004) |
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