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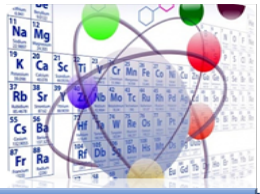

Isolation of Chitin from the Shell of the Blue Crab (*Portunus pelagicus*) and a Review of Its Potential as a Cholesterol - Lowering Agent

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ABSTRACT

The swimming crab (Portunus pelagicus) is a fishery commodity that produces large amounts of shell waste and has the potential to be used as a source of chitin. This study aims to isolate chitin from swimming crab shells through demineralization and deproteinization processes and then characterize it using FTIR, accompanied by a literature review regarding the potential of chitin as a cholesterol-lowering agent. The demineralization stage produced a yield of 35.07%, while the yield of chitin after deproteination reached 87.10%. FTIR analysis showed typical chitin absorption bands, namely N–H stretching (3255 cm^{-1}), Amide I (1621 cm^{-1}), Amide II (1552 cm^{-1}), C–O–C asymmetric stretching (1154 cm^{-1}), and β-(1→4) glycosidic bonds (872.8 cm^{-1}), which confirmed the formation of the chitin structure. Based on a literature review, chitin and chitosan have the potential to lower cholesterol levels through bile acid binding and reduced lipid absorption. Therefore, chitin from crab shell waste is suitable for development as a raw material for bioactive chitosan, although further biological testing is needed.

Keywords: chitin, cholesterol, Portunus pelagicus.

1. INTRODUCTION

Hypercholesterolemia is a condition characterized by elevated cholesterol levels in the blood, which may result in atherosclerosis, a major underlying cause of cardiovascular disease. This condition is associated with high intake of saturated fats stemming from unhealthy dietary patterns [1]. Excess cholesterol accumulation in arterial walls causes vascular stiffness and increases the risk of coronary heart disease and stroke [2]. Data from the 2018 National Health Survey reported that 54.4% of the Indonesian population aged 15 years and above had abnormal cholesterol levels, based on the NCEP ATP III criterion of ≥200 mg/dL, with a higher prevalence observed in women than in men [3]. Pharmacological agents such as statins are widely used as lipid-lowering therapy by inhibiting the hepatic HMG-CoA reductase enzyme, which leads to

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decreased cholesterol biosynthesis. Statin treatment is known to reduce LDL-C levels by approximately 18–55%, increase HDL-C by 5–15%, and decrease triglyceride levels by 7–30% through the suppression of CETP (Cholesteryl Ester Transfer Protein) activity. Despite their effectiveness, prolonged statin use may be associated with adverse effects, including muscle pain and metabolic disturbance [4]. Increasing awareness of the potential side effects associated with long-term pharmacological therapy has spurred interest in the use of natural substances as altern ative anti hypercholesterolemic agents. One such substance is chitosan, a natural biopolymer derived from chitin, which contains positively charged amine groups that allow it to bind bile acids and subsequently inhibit cholesterol absorption in the gastrointestinal tract [5].

Chitin is the second most abundant structural biopolymer after cellulose and is found in the exoskeletons of crustaceans such as shrimp and crabs. The shells of the swimming crab are often produced as waste from the fishery processing industry [6]. Chitin polymers consist of the monomer 2-acetamide-2-deoxy-D-glucose (N-acetylglucosamine), with intermonomer bonds at the β-(1-4) position [7]. The process of isolating chitin from the shells includes demineralization steps to remove minerals (such as $CaCO_3$) and deproteinization to remove residual proteins, thus obtaining pure chitin. This process is important because chitin is a starting material for the synthesis of bioactive derivatives such (*Portunus pelagicus*) are reported to be an abundant source of chitin, with a content of around 20–30% of the shell weight, and as chitosan and can be further characterized using infrared spectroscopy (FTIR) to determine the structure of its functional groups [6].

Various studies have shown that chitin and its derivatives, particularly chitosan and chitooligosaccharides, possess biological activities related to lipid metabolism. These compounds are reported to interact with bile acids and lipids in the gastrointestinal tract, thereby inhibiting cholesterol absorption and contributing to lowering total cholesterol levels [8]. Another experimental study also reported that chitin isolated from crustacean shells has the potential to lower cholesterol levels. Chitin extracted from mud crab (*Scylla spp*.) shells was reported to reduce cholesterol levels in beef viscera by up to 82.04%, demonstrating its effectiveness as a cholesterol-lowering agent [9]. However, most research still focuses on chitosan, so studies on the potential of chitin as a cholesterol-lowering agent are still limited. Nevertheless, preliminary evidence suggests that chitin also has the potential to play a role in controlling cholesterol levels. Based on this, this study aims to isolate chitin from crab shell waste (Portunus pelagicus), characterize its structure using FTIR, and review chitin's potential as a cholesterol-lowering agent through a literature review. The results of this study are expected to support the use of shell waste as a functional biomaterial and a natural alternative source for controlling hypercholesterolemia

2. EXPERIMENTAL

2.1. Chemicals, Equipment and Instrumentation

The chemicals used in this study were 200 g of crab shells (*P. pelagicus*), distilled water, Hydrochloric Acid (HCl) 1M solution, sodium hydroxide (NaOH) 1M, solution, sodium hydroxide (NaOH) 60%, Aquades (H_2O). The equipment used in this study included a blender (Miyako), a 100mesh sieve, analytical balance, a pH meter, a stirrer bar, a drying oven, Fourier Transform Infrared Spectroscopy (FT-IR) a buchner funnel, a muffle furnace, a desiccator, and standard laboratory glassware.

2.2. Research Procedure

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Preparation of blue crab shells (P. pelagicus)

100 g of blue crab shells were cleaned. The shells were then rinsed with running water to remove any attached dirt, and then dried under the sun for 48 hours. After drying, the shells (*P. pelagicus*) were ground using a blender and then sieved using a 100 mesh sieve [10].

Chitin isolation from blue crab shells (P. pelagicus)

Isolation of crab shells was carried out using a modified version of a previously reported method [11]. Chitin was isolated from the shells of the Rajungan crab (*P. pelagicus*) through two main stages, namely demineralization and deproteinization. The crab shell sample, weighing 71 g, was subjected to demineralization by treatment with 1M HCl at a ratio of 1:10 (v/v). The mixture was heated at 80 °C for 1 hour under continuous stirring. Afterward, the sample was washed with distilled water until a neutral pH (pH 7) was reached, filtered, and then dried in an oven at 85 °C for 4 hours [11]. The demineralized samples were then deproteinized by treatment with 1M NaOH at a ratio of 1:10 (v/v), followed by heating at 80 °C for 1 hour with continuous stirring. The samples were then washed with distilled water until the pH was neutral (pH 7), filtered using a Büchner funnel, and dried in an oven at 85 °C for 4 hours [11]. The chitin obtained from the deproteinization process was then weighed and characterized using an FT-IR spectrophotometer to identify its functional groups [12]. This characterization was carried out to ensure the successful isolation of chitin from *Portunus pelagicus* shells.

3. RESULTS AND DISCUSSION

3.1. Results of Chitin Isolation Crab Shells (P. pelagicus)

The results of chitin extraction from crab shells are presented in Table 1. The raw material for making chitosan is crab shells (*P. pelagicus*) obtained from crab trader waste in Medan city, namely the MMTC market.

Table 1. Chitin and chitosan extraction results (*P. pelagicus*)

Treatment	Yield (g)	Physical Appearance
Powder preparation (*P. pelagicus*)	71 g	Pale white
Demineralization	24, 9 g	Brownish white
Deproteinazation (Chitin)	21,69 g	Yellowwish white

In this case, the Demineralization process was carried out with the aim of removing mineral content, especially calcium carbonate ($CaCO_3$), which is the main component of crustaceans [13]. The mineral separation process is indicated by the formation of CO_2 gas in the form of gas bubbles when HCl solution is added in the mineral separation process. The use of HCl is carried out to dissolve Ca^{2+} ions in the form of $CaCO_{3(s)}$ to produce $CaCl_{2(aq)}$ which is soluble in water with CO_2 gas and water as byproducts. $CaCO_{3(s)}$ in the crab shell powder will react with HCl to form bubbles indicating the presence of CO_2 gas, thus the mineral

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group ($CaCO_3$) is lost from the structure, while the organic fraction in the form of chitin remains [14]. The demineralization process with HCl will produce the following reaction:

$$CaCO_{3(S)} + 2HCl_{(aq)} \rightarrow CaCl_{2(aq)} + H_2O_{(l)} + CO_{2(g)}$$
$$Ca_3(PO_4)_{2(S)} + 4HCl_{(aq)} \rightarrow CaCl_{2\,(aq)} + Ca(H_2PO_4)_{2\,(aq)}$$

The crude chitin obtained after the demineralization stage is washed with an excess of distilled water to remove any remaining HCl. This washing is done to prevent damage to the chitin during the deproteinization stage [14]. The demineralization results in this study experienced a shrinkage from the initial weight of 71 g of powder to 24.9 g of coarse chitin with a yield of 35.07% and produced a brown residue. This is in accordance with previous studies, which stated that demineralization functions to remove inorganic salt content, producing a brown coarse chitin powder that settles as a residue, which is a general characteristic of isolated chitin in the demineralization stage [15].

The deproteination stage aims to break the bonds between protein and chitin by adding NaOH to produce purer chitin [14]. In the deproteination reaction, protein bonds are broken, which is indicated by the solution changing to a thick, reddish-brown color. This is consistent with previous research. The thickening of the extractor solution is caused by the protein content in crude chitin that is released and binds with Na^+ ions in the solution, forming sodium proteinate. During the deproteination process, the ends of the protein chains containing polyamides and having a negative charge react with a base (NaOH), forming amino salts. This stage produces chitin with a yield of 87.10%, indicating that most of the protein has been successfully removed without damaging the chitin structure [11]. The results of the *P. pelagicus* chitin obtained from the deproteinization process can be seen in Figure 1

Figure 1. Chitin Produced from the Deproteinization Stage of *Portunus pelagicus* Shells

3.2 Fourier Tansform Infrared Analysis (FT-IR) Chitin

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Based on the results of the FT-IR analysis obtained, the spectrum shows the presence of functional groups in the crab shell (*P. pelagicus*) samples. Functional groups were identified from the infrared absorption patterns at specific wavenumbers, as presented in the following figure

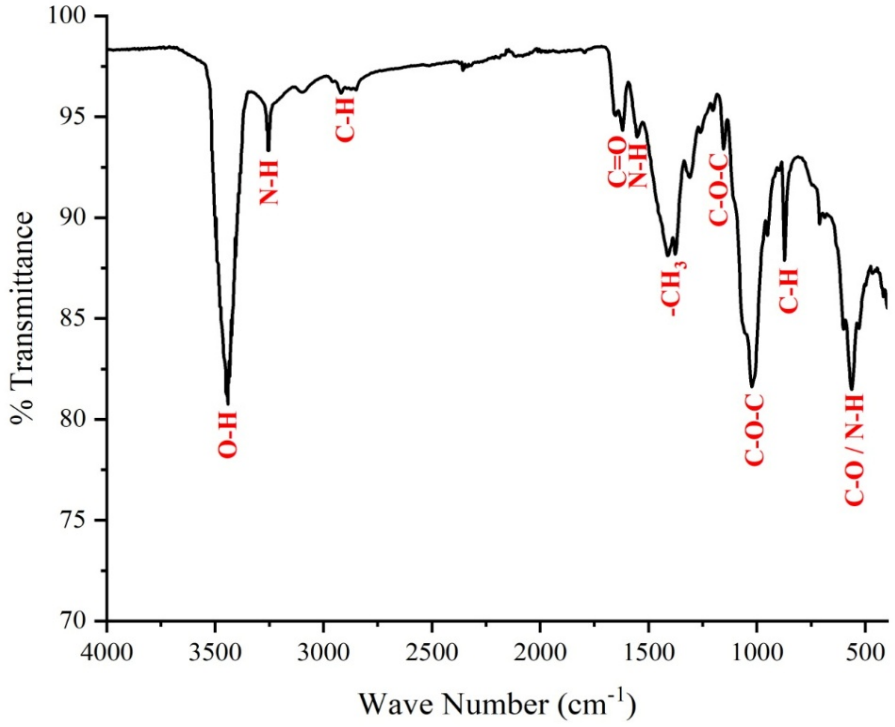

Figure 2 shows the Fourier Transform Infrared (FTIR) absorption peaks. Analysis was conducted to confirm the functional groups of Chitin isolated from blue swimming crab shells (*P. pelagicus*). The FTIR spectrum exhibited characteristic absorption bands of chitin. A broad absorption band at approximately 3255 cm^{-1} corresponded to N–H stretching vibrations, indicating the presence of amide groups. The absorption band observed at 1621 cm^{-1} was assigned to C=O stretching (Amide I), while the band at 1552 cm^{-1} represented N–H bending (Amide II), which are typical functional groups of Chitin. Additionally, absorption in the region of 1000–1150 cm^{-1} indicated C–O–C stretching vibrations, confirming the polysaccharide backbone, while the band around 890 cm^{-1} was attributed to β-(1→4) glycosidic linkages, characteristic of chitin structure.

The following table presents the characteristic FTIR absorption bands and corresponding functional groups of Chitin isolated from (*P. pelagicus*).

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Table 2. Characteristic FTIR Spectral Bands of Chitin (*P. pelagicus*)

Fuctional Group	Spectral Region (cm^{-1}) [16]	Wavenumber (cm^{-1})
N–H stretching	3250-3300	3255
Amide I (C=O stretching)	1640-1680	1621
Amida II (N–H bending)	1530-1560	1552
C–O–C asymmetric stretching	1000–1150	1154
β-(1→4) glycosidic bond	870-890	872,8

The chitin structure of *Portunus pelagicus* shells, confirmed through a characteristic FT-IR absorption band, specifically the presence of a β-(1→4) glycosidic bond at 872.8 cm^{-1}, indicates that the chitin obtained has structural characteristics consistent with pure chitin. The β-(1→4)-N-acetyl-D-glucosamine structure is reported to play a role in chitin's biological activity, primarily through its ability to interact with bile acids and lipids in the gastrointestinal tract. Literature studies have shown that chitin has the potential to lower cholesterol levels through a non-covalent bile acid binding mechanism, primarily through hydrogen bond formation, thereby inhibiting enterohepatic reabsorption of bile acids. This condition leads to increased bile acid excretion and stimulates the liver to utilize endogenous cholesterol for new bile acid synthesis, ultimately contributing to lower serum cholesterol levels. Chitin from crustacean shells has been reported to have a higher bile acid binding capacity than some conventional dietary fibers [8]. Furthermore, several in vivo studies also reported that chitin supplementation in hypercholesterolemic animal models was able to reduce plasma total cholesterol levels by 25–35% and LDL by up to 40%, while increasing HDL fractions without exhibiting hepatotoxic side effects at specific dose ranges [17] .

Previous research also supports the findings that surface-deacetylated chitin nanofibers (SDACNF) are capable of lowering serum total cholesterol, chylomicrons, and very-low-density lipoprotein (VLDL) levels in a mouse model of hypercholesterolemia. This effect is associated with increased fecal excretion of lipids and neutral sterols due to inhibited intestinal cholesterol absorption. This mechanism is thought to originate from the partially deacetylated chitin framework, resulting in positively charged amino groups, which enhance interactions with bile acids and disrupt the formation of cholesterol micelles, thus demonstrating chitin's potential as a cholesterol-lowering agent [18]. Thus, the FT-IR characterization results in this study confirm that chitin isolated from (*P. pelagicus)* shells has a typical structure of natural chitin with maintained structural integrity. This structure, which is composed of repeating units of β-(1→4)-N-acetyl-D-glucosamine, has been widely reported to play a role in the biological activity of chitin, especially in its interaction with bile acids and lipids in the gastrointestinal tract, so it has the potential to be developed as a natural cholesterol-lowering agent. Although further in vivo and clinical studies are still needed, the structural evidence obtained in this study and support from literature studies indicate that chitin has the potential to be used as a natural functional material for controlling cholesterol levels.

4. CONCLUSION

Chitin was successfully isolated from the shells of blue swimming crab (*Portunus pelagicus*) through demineralization (yield 35.07%) and deproteinization (yield 87.10%) processes, producing chitin with characteristic FTIR bands such as NH stretching at 3255 cm^{-1}, Amide I at 1621 cm^{-1}, Amide II at 1552 cm^{-1},

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C-O-C asymmetric stretching at 1154 cm^{-1}, and β-1,4 glycosidic bond at 872.8 cm^{-1}. Literature review supports the potential of this chitin as a cholesterol-lowering agent through bile acid binding and inhibition of lipid absorption in the gastrointestinal tract, with in vivo studies showing reductions in total cholesterol by 25-35% and LDL by up to 40%. Therefore, chitin from blue swimming crab shell waste has promising potential for development as a raw material for bioactive chitosan to address hypercholesterolemia, although further in vivo and clinical biological tests are needed to validate effectiveness and optimal dosage.

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