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REVIEW ARTICLES; UTILIZATION OF CRAB SHELLS FOR CHITIN

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ABSTRACT

This article aims to study the method of isolation of chitin from crab shells. Based on the literacy study above, it can be concluded that the isolation of chitin from crab shells can be done by chemical methods, fermentation methods, and enzymatic methods. Of the three methods actually have the same steps as demineralization and deproteinization, the demineralization process aims to remove minerals in the crab shell, while deproteinization aims to remove the protein content in chitin.

Keywords: method, isolation, deproteination, demineralization, application

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PRELIMINARY

Indonesia is a maritime country that has the largest number of islands in the world, based on a decision determined by the United Nations Conferences on the Standardization of Geographical Names (UNCSGN) and the United Nations Group of Experts on Geographical Names (UNGEGN) which took place on August 7-18 2017 in New York. , the United States, stated that there are 16,056 islands in Indonesia, with a coastline of 99,093 km^2, and an ocean area of about 5.8 million km^2. With the wealth of this vast ocean area, of course the potential for natural resources is very abundant, but until now the processing of marine products in Indonesia is still very small in scale, for example in the processing of crustaceans, most people only take the meat for direct consumption, as a mixture for making crackers. , shrimp paste or

animal feed and consider the shell waste even though crustacean shells contain chitin and chitosan which are of course useful and needed by many people

. Chitosan is an extract from the skin of hard-skinned animals such as shrimp, crabs, lobsters, etc. (Pratiwi 2011). Sources of chitosan are very abundant in nature, especially from crustaceans such as shrimp and crabs. Moreover, Indonesia has a lot of mangrove forests which are a habitat for crab crustaceans to live. Crustaceans are commonly used for various processing commodities such as crackers, shrimp paste, petis, fish feed as well as for export commodities so that they produce a lot of skin waste in very large quantities and are not utilized properly.

Seeing the potential utilization of chitin from crab shells that have not been utilized properly and the form of reducing crab processing waste, it is deemed necessary to conduct a study on how to extract chitin from crab shells, so that with this study it is hoped that this study can become material for understanding and insight for the community in the future so that they can utilize the potential of crabs to the maximum so that in the end it can increase the important economic value of crabs.

Chitin

Chitin comes from the Greek chitin, which means nail skin. Chitin is the main component of the exoskeleton of invertebrates, crustaceans and insects where this component functions as a supporting and protective component. Chitin compound is a polymer of polysaccharide group, which formally can be considered as a cellulose derivative compound in which the hydroxyl group on the C-2 atom is replaced by an acetamido group (Taufan & Zulfahmi 2010). These derivative compounds are obtained by deproteination and demineralization (Sanjaya & Yuanita, 2007). Through the deacetylation process, chitin will turn into chitosan (poly(1,4)-2-amine-2-deoxy-ID-glucose or poly-(/-1,4-glucosamine) (purnawan et 01.,2008). Another compound of chitin is 2-acetamide-2-deoxy-D-glucopyranose.

Chitin is the top three of the most abundant polysaccharides in addition to cellulose and starch, besides that chitin is also ranked second after cellulose as the most abundant organic component in nature (Pratiwi 2014). Cellulose and starch are important substances for plants to form their food (carbohydrate substances) and the formation of cell walls (Pratiwi 2014).

Chitin is also found in the exoskeleton of marine zoo-plankton including types of coral and jellyfish. Types of insects, namely butterflies, beetles have chitin substances, especially in the outer cuticle layer. While in the cell walls of yeast, mushrooms, and other types of fungi, chitin is also found (Taufan & Zulfahmi 2010). Chitin is a natural polymer that can be found in nature differently depending on the source.

Chitin ($C_8H_{13}NO_5$) is the second most abundant polysaccharide after cellulose, in the form of an amorphous or white crystalline solid, which is biodegradable. The main difference between cellulose and chitin is the source of the two materials taken. Cellulose is obtained from plants while chitin is obtained from marine invertebrates and fungi (Rout 2001). Chitin is insoluble in water, dilute organic acids, organic acids, concentrated alkalis and organic solvents but soluble in concentrated acids such as sulfuric acid, nitric acid, and phosphoric acid (Junianto 2008).

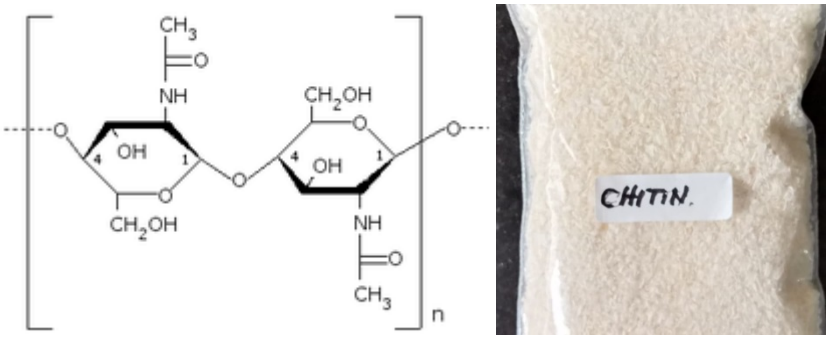

Figure 1. Chitin (Sourcehttps://elicyl-oligotech.com/chitin-/231-chitin-polysaccharide.html)

Mangrove crab

Figure 2. Mangrove Plate (sourcehttps://www.riau.go.id/home/kepiting-bakau-scylla-serrate-skala.html)

According to Stephenson and Campbell 1960), mud crabs can be classified as follows:

Class : Crustaceans

Order : Decapoda

Family: Portunidae

Genus : Scylla (de Han)

Species: Scylla serrata

The potential of mangrove crabs (Scylla spp.) in Indonesia is quite large, because these crabs have a very wide distribution and are found in almost all Indonesian waters. This group of crabs live mainly on beaches overgrown with mangroves, shallow waters near mangrove forests, estuaries and muddy beaches, so they are often called mud crabs or mangrove crabs.

Mangrove crab is also one of the community's favorite food commodities, apart from its delicious taste, mangrove crab also has a high protein content high protein content (62.72%) and easily digestible meat (Fujaya et al., 2001). (Alfrianto & Liviawaty 1992) stated that every 100 grams of mud crab meat (fresh), contains 13.6 grams of protein; 3.8 grams of fat; 14.1 grams hydrate charcoal and 68.1 g water. (Siahainenia 2008) also stated that mud crab meat and eggs (in dry weight) contain high protein (67.5%) with relatively low fat content (0.9%). The delicacy and high nutritional value puts mangrove crabs as an exclusive type of seafood with a

fairly expensive price, so that an understanding develops within the community, that consuming this type of food is a prestige for its consumers (Siahainenia 2008). Mangrove crabs, in addition to playing a role in the cycle of the food chain, also play other ecological roles.

The crab exoskeleton is composed of the main component in the form of chitin(Khoushab and M. Yamabhai, 2010: 1989), crab shells contain a fairly high chitin compound, which is about 70% of other crustaceans and about 20-30% of the weight of dry skin (Hendri 2008). Given the potential of crabs in the production of chitin from shell waste, this is a good prospect to be developed in Indonesia.

Functional properties of chitin and its derivatives

Kitin Crystalline form, insoluble in ordinary solvents but soluble in strong acid solutions. Chitin is easily biodegradable, non-toxic, insoluble in water, dilute inorganic acids and organic acids but soluble in dimethyl acetamide and lithium chloride solutions. Chitin has a good texture, whiter color, protein and minerals that are not too high. One of the properties of chitin is that it can bind metal ions (chelates metal ions) such as Fe, Cu, Cd, Hg, and has adsorption properties. Chitin is difficult for the body to digest because it is a glucose polymer, but it can bind to toxins and glucose in the body. Glucose contained in chitin does not turn into blood glucose so it does not increase the production of cholesterol. Some of the functional properties of chitin and its derivatives are:

1. Anti Microbial

Chitin is a hydroponic biopolymer, chitin can be acetylated into chitosan. Chitosan has a strong positive charge so that it can bind to the negative charge of other compounds or can detoxify, inhibit bacterial growth, and is easy to degrade biologically and is non-toxic (Kaho 2006). The mechanism is that chitosan will form a porous membrane that can absorb water in food, so that it can inhibit the growth of microbes in the food. In addition, chitosan has an amine functional group (-NH2) which has a very strong positive charge which can attract negatively charged amino acid molecules that form proteins in microbes.

2. Antioxidant

Chitin can be applied as an antioxidant (Wiyarsi and Priyambodo, 2009). Antioxidants are chemical compounds that can donate one or more electrons to free

radicals, so that these free radicals can be quenched. In chitosan, the amine group, NH2, plays a role in scavenging free radicals (Xie et al., 2002). Based on the above, the authors are interested in conducting research on the antioxidant potential of mud crab shell chitosan (Scylla serrata) with the addition of different NaOH.

3. Cholesterol Lowering

Chitin can function as cholesterol lowering through electrostatic interaction mechanism between chitin and cholesterol. In vitro, the mechanism of action of chitin when interacting with cholesterol is that chitin will bind cholesterol from brain viscera contained in ethanol solution and cholesterol will be precipitated with chitin after centrifugation. According to Einbu, 2007 in Hayes, (2012), cholesterol and chitin when mixed will form a binding reaction (electrostatic interaction), so that cholesterol is no longer free. This is because chitin has a positively charged acetamide group which binds to a cholesterol molecule which has a negative charge, namely hydroxyl. The mechanism of lowering cholesterol in the body occurs when chitin and chitosan capture and dissolve fat in the stomach. Chitin and chitosan fiber which has bound fat into a large mass which the body cannot absorb and increase its excretion with feces (Xu, 2007, in Pratiwi, 2014: 38). Wei and Wenshu (2015: 1404) also explained that when chitosan enters the intestine, the chitosan molecule will lose its positive charge and experience precipitation. Fat will be trapped into the precipitation which causes a decrease in absorption.

Chitin Extraction Method From Crab Shell

Chemical Method

1. Material preparation

   The crab shell waste was boiled for 15 minutes, then washed with water to remove the adhering dirt, then dried in an oven at a temperature of 110-120 ^{o}C for approximately one hour, then put in a desiccator, and weighed until a constant weight was obtained. After drying, it was ground and then sieved through an 80 mesh sieve.

1. Mineral removal (demineralization)

   The crab shell powder that has been mashed up to 80 mesh size is added with 1.5 M HCl solution in a ratio of 1:15 (w/v). The mixture was heated at a

temperature of 40-50^{o}C for 4 hours while stirring at a speed of 50 rpm and then centrifuged for 15 minutes at a speed of 2000 rpm, so that it was obtained in the form of a supersenate. The solid obtained was washed with distilled water to remove the remaining HCl. The final filtrate obtained was tested with AgNO3 solution, if no white precipitate was formed, the remaining Cl- ions contained were lost. Furthermore, the solids were dried in an oven with a temperature of 80^{o}C for 24 hours and obtained shrimp shell powder without minerals which was then cooled in a desiccator.

2. Protein loss (deproteination)

The crab shell powder obtained from the demineralization results was added with a 3.5% NaOH solution with a ratio of 1:10 between the solvent and the sample. The mixture was heated at a temperature of 40-50^{o}C for 4 hours while stirring at a speed of 50 rpm and then centrifuged for 15 minutes at a speed of 2000 rpm, in order to obtain a solid in the form of a supersenate. The last filtrate obtained was tested with the PP indicator, if there was no brick red color change, the remaining OH- ions contained were lost. Furthermore, the solids are filtered and cooled to obtain chitin which is then washed with distilled water. The solid obtained was dried in an oven at 80^{o}C for 24 hours and then cooled in a desiccator.

3. Deacetylation

The results obtained from the deproteination process were followed by a deacetylation process by adding 60% NaOH in a ratio of 1:20 (w/v). The mixture was stirred and heated at a temperature of 40-50^{o}C for 4 hours with a stirring speed of 50 rpm and then centrifuged for 15 minutes at a speed of 2000 rpm, in order to obtain a solid in the form of a supersenate. The solids obtained were neutralized with distilled water until the pH was neutral. The solids were then dried in an oven at 80^{o}C for 24 hours. The chitosan obtained was then characterized.

Fermentation Method

Chemical processes in chitin extraction can cause hydrolysis of chitin polymers, resulting in inconsistency of the final product. The use of chemicals in the chitin extraction process can also cause environmental pollution problems that require

costs to overcome. Therefore, fermentation is an alternative to the chitosan production process that is environmentally friendly because it utilizes the activity of microorganisms. The steps that can be taken to carry out fermentation for chitin extraction are as follows;

1. Washing process

   Washing is done by using water to remove dirt that is still attached to the crab shell. Drying was carried out in an oven at a temperature of 55^{o}C-65^{o}C until a constant weight was obtained. After that, the process of size reduction and sieving was carried out at a size of 0.5 mm.

2. Inoculum preparation

   The inoculum preparation stage begins with the process of making media by mixing PDB (Potato Dextrose Broth) with distilled water into an Erlenmeyer then heated on a hot plate and homogenized with a magnetic stirrer. After the mixture boils, the PDB media is poured into a test tube and then proceeds with the sterilization process using an autoclave at 121^{o}C for 15 minutes.

3. Fermentation

   The fermentation process is carried out using 100 grams of crab shell flour added with nutrients, namely KH2PO4 0.09 gam, MgSO4 0.045 grams, and (NH4)2SO4 0.36 grams, dissolved with some water to achieve a moisture content of crab shell flour of 40%, 50%, and 60% after the addition of the inoculum, then the fermentation process is carried out. The fermentation process was carried out for 72 hours with sampling every 24 hours and the pH of the sample was measured using a pH meter. After the fermentation process ends, product recovery is carried out starting with centrifugation, washing, filtering, and drying. Centrifugation was carried out to separate the filtrate and precipitate containing chitin. The sediment was washed using aquadest until it reached a neutral pH.

Enzymatic Method

In this isolation method, there are 3 stages consisting of a deproteination stage, a demineralization stage, and a decolorization stage (Purwatiningsih 2009).

1. Demineralization Stage

Weighed 100 grams of crab shell powder and then dissolved in an acid solution (HCl 1.0 M) with a ratio of 1:10 (sample: solvent), then stirred with a stirrer for 1 hour at a temperature of 75^{o}C. Furthermore, it was filtered through a Büchner filter and the resulting residue was washed using distilled water to a neutral pH, then dried in an oven at 80^{o}C for 24 hours to proceed to the next stage.

2. Decolorization Stage

   The results from step (1) were then weighed and dissolved into 0.5% NaOCl with a ratio of 1:10 (sample: solvent), then put into an erlenmeyer and stirred with a stirrer for 1 hour at a temperature of 75^{o}C. Furthermore, it was filtered through a Büchner filter and the resulting residue was washed using distilled water to a neutral pH, then dried in an oven at 80^{o}C for 24 hours.

3. Deproteination Stage

   The sample from step (2) was continued by dissolving it into the protease enzyme in a ratio of 1:10 (sample: solvent), then put into an incubator shaker and incubated for 1, 2, 3 and 24 hours, at 50^{o}C. Furthermore, it was filtered through a Büchner filter, and the residue was washed with distilled water until the pH was neutral, then dried in an oven at 80^{o}C for 24 hours. Then the samples were characterized.

Chitin Product Quality

The quality of the chitin product produced is based on the high and low degree of distillation obtained, the higher the degree of distillation, the better the chitin product obtained. The higher the degree of deacetylation of chitin, the better the quality of the chitin produced (Josua et all 2015), furthermore (fitri 2005) explains that the degree of distillation >50% is called chitosan.

From several previous studies it is known that chitin products produced from crab shells have different qualities depending on the extraction process carried out and the level of accuracy, but in general the quality of chitin produced by crab shells is quite good, this is explained by (Agustina et al. all 2015),

Parameter	Nilai dari kitosan yang diperoleh	Nilai dari standar internasional
Kadar air	1,55 %	≤ 10 %
Kelarutan dalam asam asetat 2%	Larut	Larut
Tekstur	Serbuk	Serbuk
Warna	Putih krem	Putih sampai kuning pucat
Uji dengan larutan ninhidrine	Positif bewarna ungu	-

Table 1. Table of results of crab shell chitin extraction according to (Agustina et all 2015)

The resulting chitosan has a low water content of 1.55%. The amount of water content in chitosan is undesirable in various fields of use, because it will affect the resistance to attack by microorganisms (Rochima et al., 2004). The moisture content of chitosan is influenced by the process at the time of drying, drying time, the amount of chitosan being dried and the surface area where the chitosan is dried. The solubility of chitosan in acetic acid is one of the parameters that can be used as a standard for assessing the quality of chitosan. The higher the solubility of chitosan in 2% acetic acid, the better the quality of chitosan produced (Rochima et al., 2004; Mukherjee, 2001). The resulting chitosan has perfect solubility in 2% acetic acid.

Chitin Applications And Its Derivative Products

Chitin can be transformed into chitosan, a biopolymer product that has a wider application in the industrial world because of its natural, biodegradable, biocompatible and non-toxic nature. Chitosan is a type of polysaccharide obtained from the deacetylation of chitin which has the molecular formula C6H11NO4. Chitosan is a chitin derivative product obtained by chemical deacetylation using bases or enzymatic deacetylation using lipase and phospholipase enzymes (Vargaz and Martinez 2010).

Chitin and chitosan have very broad benefits in everyday life, for example as adsorbents for heavy metal waste and dyestuffs, preservatives, anti-mildew, cosmetics, pharmaceuticals, flocculants, anti-cancer, and anti-bacterial (Prashantb & Tbaranatban, 2007). has several benefits for humans, so it is a trade material that has a high economic value. The benefits of chitosan include;

1. agriculture field,

   Chitosan offers a natural alternative in the use of chemicals that are sometimes harmful to the environment and humans. Chitosan creates defense mechanisms in plants (such as vaccines for humans), stimulates growth and stimulates certain enzymes (phytoalexin chitinase, pectinnase, glucanase and lignin synthesis): This new organic controller offers an approach as a biocontrol tool;
2. water treatment field,

chitosan can be used as a raw material for the manufacture of ultrafiltration gel;

3. food field,

   chitosan has been widely used in food composition in Japan, Bropa and the United States, as a fat catcher which is a breakthrough in the field of diet; and

4. health field,

   Chitosan is used for bacteriostatic, immunological, anti-tumor, cicatrizant, homeostatic and anti-coagulant, ointment for wounds, malignancy, orthopedics and suture swelling due to surgery (Kusumawati 2009).

Seeing the potential use of chitin and chitosan as a very important intermediate product, of course the chitin industry targets the export market share as its sales goal, moreover, the use of chitin in the country is still very common and rarely done due to technological factors and minimal utilization.

2.6 Commercial products from chitin/chitosan

No	Commercial products	Product
1	Organic food preservative	 **Source:** https://tokopedia.com/chitosan-pengawet.html

2	Weightmanagement supplements	 **Source:** deepbluehealtz.co.nz
3	Plant Organic Fertilizer	 **Source:** https://shopee.co.id/chifarm.html
4	Cosmetics	 Source : http://aquatonale.rs/en/chitosan-cellulaire/.htmll

CONCLUSION

Based on the literacy study above, it can be concluded that the isolation of chitin from crab shells can be done by chemical methods, fermentation methods, and enzymatic methods. Of the three methods actually have the same steps as demineralization and deproteinization, the demineralization process aims to remove minerals in the crab shell, while deproteinization aims to remove the protein content in chitin.

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