Global Scientific JOURNALS

GSJ: Volume 9, Issue 5, May 2021, Online: ISSN 2320-9186
www.globalscientificjournal.com

Article Review: CHITIN EXTRACTION FROM SHRIMP SHELLS

By

Junianto[1], Ivanna Shelma Taofani[2], Muhammad Rama Sukmadhani[2] and Intan Ukhti Fitriana[2]

1) Lecturer Staff of the Department of Fisheries - Faculty of Fisheries and Marine Sciences, Padjadjaran University, Indonesia. junianto@unpad.ac.id
2) Student of Fisheries Study Program - Faculty of Fisheries and Marine Sciences, Padjadjaran University, Indonesia; Corresponding Author: ivanna18002@mail.unpad.ac.id

Keywords

Deproteination, demineralization, decolorization, method, type.

ABSTRACT

The purpose of this review article is to examine the potential of shrimp shells in Indonesia for chitin raw material, chitin definition, chitin extraction method and chitin characteristics from shrimp shells. Based on the results of the study, it can be concluded that Indonesia has the potential for shrimp shells which is quite potential for chitin raw material. Chitin is β- (1,4) -2-acetamide-2-deoxy-D-glucose or β- (1,4) -N-acetylglucosamine. The chitin extraction method can be carried out chemically and enzymatically. The two methods in extracting chitin have two stages, namely deproteination-demineralization and decolorization. Shrimp shell chitin characteristics vary widely in value, depending on the type of shrimp and the method of extraction.

INTRODUCTION

Indonesia is the largest archipelagic country in the world, has a total of 17,499 islands with an area of 3.25 million km^2 of Indonesian waters consisting of a territorial sea area of 0.30 million km^2 and an archipelagic sea area of 2.95 million km^2. The area of Indonesia's Exclusive Economic Zone (ZEE) is 2.55 million km^2. The length of the coastline recorded as part of Indonesia's territory reaches 81,791 km (Pushidrosal 2015). This makes Indonesia has enormous marine potential, both biological resources and other resources that exist below sea level. Particularly in the fisheries sector, which is one of the marine fields covering activities of catching, hatching, cultivating all types of fish and other aquatic biota found in coastal areas and in the oceans (Kusumastanto 2003).

Shrimp is one of the mainstay commodities in the Indonesian fisheries sector which produces a lot of waste. The shrimp waste has the potential to become an environmental pollutant. However, shrimp waste which contains a lot of chitin can be used as raw material for making chitosan (Wulandari 2008).

Crustacean processing waste such as skin, head, and feet is one of the problems that must be faced by crustacean processing plants. Processing the waste into chitin can increase the added value which is quite high. The chitin content in crab waste reaches 50-60%, while in shrimp waste it reaches 25-30% (Gildberg and Stenberg 2001). However, because the raw material that is easily obtained is shrimp waste, the process of making chitin and chitosan usually makes more use of shrimp waste (Anonymous 2007). The purpose of this review article is to examine the potential of shrimp shells in Indonesia for chitin raw material, chitin definition, chitin extraction method and chitin characteristics from shrimp shells.

POTENTIAL OF SHRIMP SHELLS IN INDONESIA

Indonesia has enormous marine potential, both biological resources and other resources that exist below sea level. In particular, the fisheries sector, which is one of the marine fields, includes activities of catching, hatching, cultivating all types of fish and other aquatic biota found in coastal areas and in the oceans, and industrial processing of products from the coast and the oceans (Kusumastanto 2003).

Indonesian territorial waters have many sources of shells of hard-skinned marine invertebrates (crustaceans) which contain abundant chitin. The chitin contained in crustaceans is in high enough levels ranging from 20-60% depending on the species. Currently in Indonesia, around 56,200 tons of chitin-containing waste is produced per year (Ministry of Marine Affairs and Fisheries 2000).

Picture 1. Shrimp (KKP News, 2018)

Shrimp is one of the mainstay commodities in the Indonesian fisheries sector which produces a lot of waste. The shrimp waste has the potential to become an environmental pollutant. However, shrimp waste which contains a lot of chitin can be used as raw material for making chitosan (Wulandari 2008).

DEFINITION OF CHITIN

Chitin is one of the biopolymers which is the second most abundant ingredient in nature after cellulose and can be found as a component of the skeleton of crustaceans (hard-skinned animals) such as shrimp, crab, crab, and squid (Khan *et al.* 2002). Chitin is a substance that is widely useful for human life and has been in the spotlight of researchers as well as entrepreneurs to develop its production on an industrial scale (Jaworska *et al.* 2003).

Figure 2. Shrimp shell waste (KKP News, 2015)

Crustacean skin waste contains 14-35% chitin. Shrimp waste contains 74% organic material (mainly protein and chitin tissue), 26% minerals and only 0.4% fat (Gildberg and Stenberg 2001). Apart from crustaceans, chitin can also be found in shellfish (mollusca), insects and fungi.

CHITIN EXTRACTION METHOD

Chitin is obtained from shrimp shells through a deproteination process using sodium hydroxide (NaOH) and demineralization using hydrochloric acid (HCl) (Steven *et al.* 1998). Chemical processes have caused corrosion of equipment and depolymerization of ı-glucoside chains, (Toan *et al*. 2006).

Extraction of chitin can be done biologically, by utilizing lactic acid bacteria for the demineralization process, and proteolytic bacteria in the deproteination process. Several studies of chitin extraction through batch fermentation systems have been carried out, including Jung *et al*. (2005), using L. Paracasei subsp. Tolerance of KCTC-3074 and Serratia marcescens, demineralization rate 97.2%. and deproteination 52.6%. Rao and Stevens (2006), using L. Plantarum, demineralized 81.4%, and deproteinated 52.2% - 59.8%. Using B. subtilis can reduce 72% minerals and 84% protein. Jung *et al*. (2007) using Serratia marcescens FS-3 can reduce protein 68.9%. The subsequent-batch fermentation system using L. acidophilus FNCC 116 and B. licheniformis F11.1, can reduce minerals 95.69% and protein 92.42%.

The level of mineral and protein removal using the batch fermentation system, as described above, is still relatively low at around 95%. Based on these facts, research on chitin extraction with a continuous fermentation system is a new innovation to overcome deficiencies in batch fermentation systems and chemical processes.

The process of making chitin from shrimp shells begins with a reduction in the size of the shrimp shells, followed by a process of removing minerals (demineralization process). This mineral removal process is carried out by dissolving the shrimp shells in hydrochloric acid. Because the protein in shrimp shells binds to the chitin to be taken, to obtain chitin, the protein removal process is carried out, namely the process of separating the chitin bonds from the proteins contained in the shrimp shell (deproteination process). Minerals in shrimp shells range from 30-40% while the protein content is approximately 35% (Prasetyaningrum *et al*. 2007).

According to Fernandez-Kim (2004), the demineralization process can be carried out by extracting using a 1N hydrochloric acid solution at room temperature for 30 minutes with the ratio of the skin or shell processed with HCl solution is 1:15 (gram / mL). The effectiveness of mineral removal in the demineralization process can be seen using the ash content parameter. In his research, the demineralization process produced a product with an ash content of 31 - 36%.

According to No and Meyers (1995), the process for removing protein in crustacean animal shells can be done by dissolving it in a NaOH solution with a concentration of 1–10% at a process temperature of between 65–100^{0}C for 0.5–12 hours. Comparison between the

processed shell and the alkaline solution used, the optimum value is obtained at 1:10 (g / mL) or 1: 15-10 (g / mL). The process is carried out with sufficient stirring so as to maximize the deproteination process. Meanwhile, from the research results obtained by Fernandez-Kim (2004), 3% NaOH solution can be used to carry out the protein removal process (deproteination).

In general, the sequence of demineralization and deproteination processes can be carried out sequentially or not, namely the deproteination process is carried out first and then followed by the demineralization process using the acidic decalcification procedure, as well as a process with a sequence of demineralization processes followed by a deproteination process (Fernandez-Kim 2004; No *et al*. 2000).

The process of isolation or production of chitin consists of 3 stages, namely the deproteination stage, the demineralization stage, and the decolorization stage (Purwatiningsih 2009). The isolated chitin was characterized against several parameters such as: moisture content, ash content, protein content and degree of deacetylation. The characteristic values of isolated chitin were compared with standard chitin to determine whether the chitin approached or conformed to the characteristics of standard chitin.

DEPROTEINATION STAGE

Deproteination is the stage of removing protein found in shrimp waste. At this stage, a protease enzyme was used isolated from the bacterium Bacillus licheniformis HSA3-1a. At this stage the protease enzyme is more likely to be used because basically the enzyme works specifically on the protein substrate to be catalyzed. In addition, the use of enzymes is more environmentally friendly and produces a uniform degree of chitin deacetylation compared to the use of chemicals in the deproteination process which tends to be random for the results of the degree of deacetylation.

The principle of the deproteination process is to release the bonds between protein and chitin. The deproteination process is carried out by treatment using hot NaOH solution for a relatively long time where with this treatment the protein will be released and form dissolved sodium proteinate (Suhardi 1993). The protein content in the shell will covalently bind with chitin but there are also proteins that are physically bound, namely proteins from meat scraps attached to the shell matrix which vary in number (Suhardi 1993). This indicates that the protein content after the reduction in the deproteination stage continues to decrease and is marked by a change in color. The loss of protein content is indicated by the reduction in the color intensity of the solution to become clearer (colorless) in the filtrate at the end of the treatment (Abdou *et al.* 2008; Rhazi *et al.* 2000; Tolaimate *et al*. 2003).

DEMINERALIZATION STAGE

Demineralization is a step that aims to remove inorganic compounds in shrimp waste. According to Johnson and Peniston (1982), crustacean skin generally contains 30-50% minerals based on dry weight, with the most mineral being $CaCO_3$. In addition, there is also $Ca_3(PO_4)^2$ with levels of 8- 10% of the total inorganic material. This mineral content can be removed by reacting the shrimp waste sample with a 1.0 M HCl solution, where the reaction of CaCO3 and HCl is as follows:

$$CaCO_3\ (s) + 2HCl\ (l)\ CaCl_2\ (l) + H_2O\ (l) + CO_2\ (g)$$

The reaction between $CaCO_3$ and HCl causes the formation of CO_2 gas which is indicated by the presence of air bubbles when the HCl solution is added to the sample. This shows that there has been a mineral separation process in the shrimp waste. According to Johnson and Peniston (1982), demineralization is generally carried out with a solution of HCl or other acids such as H_2SO_4 under certain conditions. The effectiveness of HCl in dissolving calcium is 10% higher than H_2SO_4. The optimum can be obtained by extraction using 1.0 M HCl which is incubated at 75^{o}C for 1 hour (Bahariah 2005 & No *et al.* 1989).

The results of the demineralization process showed a decrease in weight from the initial weight. This indicates the degradation of minerals contained in the sample during the demineralization process. This demineralization stage is a stage that plays an important role in the isolation of chitin. The results of this stage greatly affect the quality of chitin, especially in terms of ash content. The lower the ash content of the chitin obtained, the better the quality of the chitin produced.

DECOLORIZATION STAGE

Decolorization is the stage of removing pigments (dyes) in shrimp waste. The dark colored pigment in shrimp waste is called crustacyani which is a lipoprotein compound, where the lipid group is a caratenoid compound known as astaxanthin. According to Kasmas, E in Hamsina *et al.* (2002) decolorization aims to provide an attractive appearance to the chitin product that is obtained later. The decolorization process was carried out by dissolving the demineralized sample in 0.5% NaOCl solution for 1 hour at 75^{o}C. In this process, the color of the chitin product is produced, which was brownish white to pure white.

CHARACTERISTICS OF CHITIN

Chitin is a natural polymer which on each repetition of the polymer unit has an N-acetyl group attached to the C2 atom. Chitin is composed of acetyl glucosamine monomers

which bind to each other by (1-4) β-glycosidic bonds. Another name for chitin is β- (1,4) -2-acetamide-2-deoxy-D-glucose or β- (1,4) -N-acetylglucosamine.

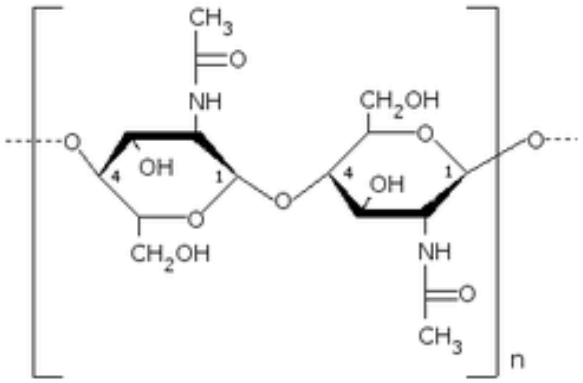

Figure 3. Chitin structure (Yurnaliza, 2002)

Chitin is insoluble in water, alcohol and dilute acids and alkalis, but chitin can dissolve in strong acids, fluoroalkohols and NN-dimethylacetamide in lithium chloride (Gagné 1993). The properties of chitin such as solubility, molecular weight and completeness of acetyl groups vary depending on the source of chitin and the method of isolation applied.

According to Komariah (2013) chitin is insoluble in water so its use is limited. However, by modifying the chemical structure, a chitin derivative compound will be obtained which has better chemical properties. One of the chitin derivatives is chitosan. Naturally chitin is often incomplete acetylation while chitosan also usually still contains acetyl groups with various levels.

In nature, chitin is a compound that does not stand alone but combines with other compounds such as proteins, minerals and pigments. Chitin forms white crystals, tasteless, odorless and insoluble in water (Rahayu and Purnavita 2007), organic solvents such as alcohol, acetone, hexane and in dilute and concentrated bases. Chitin can dissolve in concentrated mineral acids, for example HCl, HNO_3 and H_2SO_4 (Savitri *et al*. 2010).

Based on the results of research by Kurniasih and Dwiasi (2007), chitin characterization derived from white shrimp (Litophenaeus vannamei) has a moisture content of 5.39%, ash 2.66%, 1.54% fat and 36.16% protein. The moisture content in chitin is highly dependent on the drying process and also the packaging so that the water content depends on the water content in the environment (Suhardi 1992). The ash content of chitin is influenced by the conditions of the demineralization process, namely the duration of the process, the process temperature and the concentration of HCl. The more concentrated the HCl is used, the faster and more mineral salts that can be removed (Suhardi 1992).

CONCLUSION

Based on the results of the study, it can be concluded that Indonesia has the potential for shrimp shells which is quite potential for chitin raw material. Chitin is β- (1,4) -2-acetamide-2-deoxy-D-glucose or β- (1,4) -N-acetylglucosamine. The chitin extraction method can be carried out chemically and enzymatically. The two methods in extracting chitin have two stages, namely deproteination-demineralization and decolorization. Shrimp shell chitin characteristics vary widely in value, depending on the type of shrimp and the method of extraction.

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