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Probiotic enriched *Daphnia* sp: the nutritional profile and enzymatic activities

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Effect of different carbon doses of tapioca (*Manihot esculenta*) flour on vegetative cells and spore production of *Bacillus megaterium*

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Enrichment of feed for growth of cantang grouper (*E. fuscoguttatus* x *E. lanceolatus*) in floating cages

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Addition of Lemuru Fish Oil to Protein Retention and Feed Utilization Efficiency of silver barb *Rasbora argyrotaenia*

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Response of kutuklin hemagglutinin protein adhesion in koi fish (*Cyprinus carpio*) infected by *Myxobolus* sp.

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Effect of different filter media use on aquaponics system on ammonium (NH₄⁺), nitrite (NO₂) and nitrate (NO₃) concentrations of catfish (*Clarias* sp.) aquaculture

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Preliminary study: the effect of cryopreservation on the gastrula-staged embryo of African catfish (*Clarias gariepinus*)

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Marine litter distribution in Ampana Beach Tojo Una-Una Regency Central Sulawesi Province

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Provision of bacteria from shrimp pond sediment towards N/P ratio, plankton abundance, and total bacteria in the culture media of white shrimp (*Litopenaeus vannamei*)

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Correlation between water quality and prevalence on Koi (*Cyprinus carpio*) which infested by *Argulus* in Mungkid Subdistrict and Muntilan Subdistrict, Magelang Regency, Central Java

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Effect of Mengkudu's (*Morinda citrifolia*) distillation with differential fruit ripeness to control *Argulus* on *Carassius auratus auratus*.

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Hypoxic Preconditioning Effect on the Expression of Intracellular Heat Shock Protein (HSP) 27, HSP 70 and HSP 90 on Cultured Adipocyte-Derived Mesenchymal Stem Cells (AMSCs)

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Correlation between Wall Motion Score Index (WMSI) and Anatomical M-mode (AAM) Systolic Thickening with Functional Capacity in Heart Failure among Post-myocardial Infarction Patients

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The effect of hydrochloric acid concentration and temperature demineralization on characteristics of chitin from penshell (*Atrina pectinata*)

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Abstract. This study aims to determine the effect of HCl concentration and temperature affect chitin characteristics as the result of demineralization process from penshells. This study uses Completely Randomized Design (CRD) factorial consisting. The method based on two steps, there are demineralization and deproteination. This study aims to determine the effect of HCl concentration and temperature on chitin characteristics as the result of demineralization process for penshells. This study used Completely Randomized Design (CRD) with two factors, including HCl concentration (2N, 4N, and 6N) and temperature (33°C and 60°C) which consists six combination treatments and three replications. Data was analyzed by analysis of variance (ANOVA) and followed by Duncan's Multiple Range Test to determine differences between treatments. The results showed that interaction of HCl concentration and temperature has significant effect ($p < 0.05$) to ash content of chitin. The use concentration of 6N and 33°C produces low ash content. Characteristics chitin resulted from the treatment of 6N and 33°C produces ash content $25.33\% \pm 6.82$, moisture content $3.67\% \pm 1.10$, yield $0.72\% \pm 0.12$ and protein content 5.86 %.

1. Introduction

Shellfish belong to invertebrate animals [1]. Shells are included in the bivalve class which has two shells. The body part of the shell is soft and protected by a hard shell [2]. The only part of shellfish that is usually consumed are the meat, so the shell can be an environmental problem because it becomes waste. Based on data on exports of Indonesian fishery products in 2003 and 2004, waste generated from shells was around 2,752 tons [3]. The clam shell is a self-protection tool. The shell consists of a layer of carbonate (Crystalline Calcium Carbonat) separated by a thin layer of protein between the shell and body parts (muscles and flesh) [1]. In addition, shells contain chitin. One example of the type of clam that is included in the classis bivalvia is the ax axle or the so-called manuk shell. Axles (*Atrina pectinata*) are among the sea shells of the pinnidae family. Axles are a popular food source with high commercial value in a number of Asia Pacific regions. At present the use of axle shells has not been much studied.

Chitin is a group of carbohydrate compounds that can be produced from marine waste originating from shrimp, crabs, and shellfish [4]. Chitin is a natural polymer that has an acetamide group (NHCOCH₃) with a molecular formula (C₈H₁₃NO₅)_n [5]. Chitin is quite abundant in nature. Chitin is



also the second largest compound after cellulose [16]. Chitin is a natural polysaccharide that has many uses, such as chelating agents, adsorbents, and emulsifiers [6].

The stages of making chitin through two processes, namely demineralization and deproteination. Demineralization functions to eliminate mineral content, especially calcium carbonate (CaCO_3) in the shell. Seashell shells contain as much as 3% -4% protein and contain quite high minerals [7]. Therefore, it is necessary to demineralize the shells to reduce the mineral content contained in the shells.

Hydrochloric acid (HCl) is most widely used in the demineralization process because it is more effective and produces chitin with a lower remaining mineral content [8]. The use of temperature in the demineralization process is to speed up the reaction between the solvent and the minerals contained in the shell. An increase in temperature can accelerate the reaction whereas a decrease in temperature can slow the reaction [9]. The increase in temperature in the demineralization process can increase the penetration of HCl solution into the matrix of the shell so that it can help reduce the minerals contained in the shell of the shell.

This study aims to determine the concentration of HCl and temperature that affect the characteristics of chitin demineralization process of axle shells (*Atrina pectinata*).

2. Material and methods

2.1. Method

This research was conducted in April 2016-June 2016 in the Education Laboratory, Faculty of Fisheries and Maritime, Universitas Airlangga, Surabaya for the manufacture of chitin shells of axles, testing of water content and ash content. The research was also conducted at the Laboratory of Fisheries Product Quality Control and Testing (LPPMHP) in Surabaya for testing protein content and the Laboratory of Material Characterization Division of the Department of Material and Metallurgical Engineering, Institut Teknologi Sepuluh November (ITS) for FTIR testing.

2.2. Tools and material

The tools used in this study are analytical scales, beaker glass, water baths, filters, pH pens, glass stirring rods, thermometers, measuring cups, measuring pipettes, dropper pipettes, porcelain cups, cruss pliers, ovens, desiccators, electric furnaces. The materials used in this study were the axle shell (*Atrina pectinata*) and chitin extraction solution, namely technical HCl and technical NaOH.

2.3. Work procedure

a. Material Preparation

Shells are obtained by collecting axles (*Atrina pectinata*) shells from Kenjeran Beach, Surabaya. The shells obtained are put in a plastic bag. The shells obtained are then washed and cleaned from impurities by brush. Shells that have been cleaned are then dried in the sun to dry and crushed by pounding them into small pieces.

b. Chitin Extraction

The making of chitin in this study refers to the method of making chitin [10] which has been modified for a long period of time use. Demineralization is carried out by inserting the axle shell into the beaker glass then adding the concentration of HCl treatment (2N, 4N, 6N) with a ratio of 1:10 (g powder / ml HCl), then heated and stirred at the treatment temperature of 33 ° C and 60 ° C for 3 hours. The precipitate obtained is filtered and washed with tap water until it reaches a pH close to neutral. Then the precipitant is being dried in the oven using a temperature of 60°C. The dried sludge is then blended after deproteination. Deproteinination is done by inserting the shells into a glass beaker with a ratio of 1:10 (g of powder / ml NaOH), and being stirred at a temperature of 70°C for one hour. The chitin that has been heated is then filtered and washed using tap water until it reaches a pH close to neutral.

2.4. Test procedure

a. Ash Level Testing

Ash content testing was carried out using gravimetric method referring to the [5]. Ash content testing is done by means, the empty cup is first put into the oven with a temperature of 105 ° C for one hour then put in a desiccator for 30 minutes and the cup is weighed (A). A sample of 0.5-1 g was put into a porcelain cup that had been weighed and put in an electric furnace with a temperature of 600 ° C for approximately six hours. Samples inside the furnace are removed after the sample has cooled, after which the cup is put into the desiccator and then weighed (B). Ash content can be calculated based on the formula:

$$\% \text{ Ash} = \frac{B - A}{B - C} \times 100\%$$

A = Weight of an empty cup, expressed in g

B = Cup weight + ash, expressed in g

C = Weight of plates and empty cups, expressed in g

b. Moisture Testing

The method used in testing water content is the gravimetric method refers to the [5]. Calculation of water content is done by means of an empty cup put in the oven for one hour at a temperature of 105 ° C. The cup that has been put in the oven is transferred to the desiccator about 30 minutes then the weight of the cup is weighed (A). Chitin samples of 0.5-1 g were put into an empty cup and weighed (B). The cup which has been filled in the sample is put into an oven at 105°C for three hours then the cup is transferred using cruss pliers into the desiccator for 30 minutes after it is weighed (C). Calculation of water content can be calculated using the formula:

$$\% \text{ water content} = \frac{B - C}{B - A} \times 100\%$$

A = Weight of an empty cup, expressed in g

B = Cup weight + initial sample, expressed in g

C = Cup weight + dry sample, expressed in g

c. Yield of Chitin

Yield is the amount of chitin produced from the chitin extraction process after the demineralization and deproteination stages. The yield of chitin can be calculated by comparing the weight of chitin obtained with the weight of the starting material. The yield calculation is used to determine the amount of chitin that can be produced after the extraction process. The yield is calculated using the following formula:

$$\text{Yield of Chitin} = \frac{A}{B} \times 100\%$$

A = Chitin weight in g

B = Initial weight of material in g

d. Testing Protein Levels

Protein content testing is carried out referring to the [11]. Protein content testing is done by weighing a sample of 2 g on weigh paper, folded and put into a destruction flask, then adding two catalyst tablets and several concentrated boiling stones H₂SO₄ (95% -97%) as much as 15 ml and H₂O₂ as much as 3 ml is added slowly and let it sit for 10 minutes in the acid chamber. Destruction is carried out at 410°C for two hours or until the solution is clear, the solution is allowed to cool down to reach room temperature. After it cooled down, the solution is added with 50-75 ml of distilled water. Erlenmeyer flasks containing 25 ml of 4% H₃BO₃ solution containing indicators are prepared for the distillate container. A flask containing the results of destruction on a series of steam distillation apparatus is installed and 50-75 ml of sodium hydroxide-thiosulfate solution is added. Distillation is carried out and the distillate is accommodated in Erlenmeyer until the volume reaches a minimum of 150 ml (the distillate will turn yellow). The distillate is titrated using 0.2 N HCl until the color changes from green to neutral gray (natural gray). Blanks are also carried out workmanship such as sample stages. Calculation of N content is calculated by the formula:

$$\% N = \frac{(VA-VB)HCl \times N HCl \times 14.007 \times 6.25 \times 100\%}{W \times 1000}$$

in which :

- VA : ml HCl for sample titration
- VB : ml HCl for blank titration
- N : The standard HCl normality used
- 14.007 : Nitrogen atomic weight
- 6.25 : Protein conversion factor for fish
- W : Sample Weight (g)

2.5. Data analysis

This research uses factorial completely randomized design (RAL) which consists of two factors with six treatment combinations and three replications. The treatment in this study was the use of concentrations of HCl (2N, 4N, and 6N) and temperature (temperature 33°C and temperature 60°C) for demineralization of axle shells (*Atrina pectinata*). Data analysis in this study used Analysis of Variance (ANOVA) and continued with Duncan's Multiple Range Test to determine differences between treatments.

3. Result and discussion

a. Chitin ash content

The results of chitin ash produced from the treatment combination are shown in Figure 1. The result of this study indicate that the higher the concentration of HCl and the temperature used, the lower the ash content. The initial ash content of the scallop shell material was 94.8%. Calculation of ash content in the process of making chitin is an important parameter. Ash content can indicate the effectiveness of the demineralization process carried out in the manufacture of chitin. Based on the results of the Analysis of Variance (ANOVA) the interaction of HCl concentration and temperature has a significant effect ($p < 0.05$).

The use of higher concentrations can increase the reactivity rate because the greater the number of reagent particles results in many chances of collision between the reacting particles and minerals contained in the shell. The increase in temperature in the demineralization process can cause the particles contained in the solution to move faster so that the kinetic energy increases. Increased kinetic energy can cause collisions that produce reactions [9]. Based on Duncan's analysis, the treatment that has an effect on decreasing the ash content of this study is A3B1 (concentration of 6N and temperature 33 ° C). This is due to the lowest ash content produced when compared to other treatments.

b. Chitin water content

The results of chitin water content resulting from the treatment combination are shown in Figure 2. Water content is the amount of water that still exists in a material that has been evaporated. Water content is one important parameter. Water content can determine the durability of the product [12]. The lower the water content produced, the more durable the product. Based on the analysis of variance (ANOVA) showed that the concentration of HCl had a very significant effect on chitin water content ($p < 0.05$). But there is no influence of temperature and interaction between HCl concentration and temperature ($p < 0.05$). This statement is consistent with [13] research saying that the increase in chitin water content can be influenced by the increased concentration of HCl used. The higher the concentration of HCl used during demineralization, the chitin produced will have a stronger binding to water and hygroscopic. This is due to the high levels of minerals and protein that are lost so that chitin is purer and the chitin binding capacity to water is stronger. Other factors that can affect chitin water content according to [12] are drying process, drying temperature, drying time, cross-sectional area of drying place and the amount of material dried. Temperature has no significant effect ($p < 0.05$) on chitin water content because chitin water content depends on the relative humidity of the air around the storage area because chitin is hygroscopic [13].

c. Yield of chitin

The chitin yield produced from the combination is provided in Figure 3. The yield produced shows that the higher HCl and the temperature used, the lower the chitin produced. Results of Analysis of Variance (ANOVA) showed the difference between HCl and temperature of chitin yield ($p < 0.05$). However, the interaction of HCl concentration and temperature did not affect the yield of chitin ($p < 0.05$). This is caused by the increase of temperature in the demineralization process, which also increase the rate of reaction between hydrochloric acid and calcium carbonate. Resulting in the increasing of mineral content lost and causing low yield [14]. Meanwhile, increasing the efficiency of HCl can increase the speed of HCl reaction in reducing minerals.

The decrease in yield is in line with the decrease in ash content using increasing concentration and temperature. The yield correlates with the amount of shell material that is lost when going through the stages of demineralization and deproteination [12]. The more material constituents are lost, the lower the yield. In addition, the decrease in yield can be caused by the infiltrated chitin while filtering resulting in decrease of yield.

d. Supporting parameters

Deproteination in the manufacture of chitin aims to reduce protein content in the shell. At the time of deproteination, the protein contained in the shell is converted into water-soluble sodium proteinate salt [5]. The deproteination results from the A3B1 treatment (concentration of 6N and temperature 33 ° C), showed that the protein that was still present in the shell was 5.86%. The lower the levels of protein and minerals contained in chitin, the chitin produced is purer.

Chitin analysis of axle shells using FTIR showed functional group information contained in the material. The results of FTIR spectrophotometric analysis can be seen in Figure 4. Chitin extracted from axle shells showed an absorption patterns at wavelengths of 3649.73 cm^{-1} and 3270.04 cm^{-1} . Chitin absorption pattern at a wavelength of 3649.73 cm^{-1} indicates the presence of a -OH functional group and a wavelength of 3270.04 cm^{-1} indicates the presence of a -NH function group. This is consistent with the statement [15] which states that chitin has a unique absorption pattern. The pattern represents the functional groups contained in chitin. Chitin has an absorption pattern at a wavelength of 3446.26 cm^{-1} which shows the -OH group and has an absorption at a wavelength of 3200 cm^{-1} which shows a functional group -NH on the NHCOCH_3 amide. This is also reinforced by the appearance of absorption at wavelength 1623.03 cm^{-1} (at feather shells 1651.07 cm^{-1}) showing the amide band I (C = O), absorption at wavelength of 1395.43 cm^{-1} (at 1373.32 cm^{-1} feather shells -1) shows methyl CH_3 and wavelength 1507.36 cm^{-1} (in feather shells 1527.62 cm^{-1}) shows amide II (buckling -NH) and 1373.32 cm^{-1} (in shell feathers 1373.32 cm^{-1}) shows amide III (CN stretching) is evidence of the presence of acetyl. This is in accordance with the research of [17] on chitin shell shells showing wavelength uptake in the absorption area.

4. Conclusion

The increase in HCl concentration and demineralization temperature had an effect on the characteristics of chitin produced. The use of 6N concentration and temperature of 33°C produces the lowest ash

content. The characteristics of chitin produced from the 6N treatment and 33°C temperature produced an ash content of $25.33\% \pm 6.82$, a moisture content of $3.67\% \pm 1.10$, yield of $0.72\% \pm 0.12$, and content of protein by 5.86%. Based on the research that has been done, it is recommended to be able to characterize the minerals found in the shells of axes. In addition, further research is needed for the utilization of shell wastewater demineralization products and effective ways of neutralization to wash chitin after the demineralization and deproteination process.

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