

# Antioxidant Activity of Chitosan from the Waste of Green Mussels Shell (*Perna Viridis* L)

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**Abstract** — Green Mussels (*Perna viridis* L) is consumed by a lot of people, but the waste of green mussels shells is still not used optimally. This study aims to determine the antioxidant activity of waste of green mussels shell using DPPH method. Chitosan obtained through deproteination, demineralization and deacetylation process. Antioxidant activity is determined by DPPH method. The yield of chitosan produced in this study was 59.3% with the degree of deacetylation of 49%. Antioxidant activity of chitosan as indicated by the IC50 has a value of 170.03 ppm which is classified as a weak category. Therefore, chitosan needs to be modified in order to increase its antioxidant activity.

**Keywords:** *antioxidant activity, green mussels (Perna viridis L), chitosan*

## I. INTRODUCTION

Degenerative disease is a chronic disease that affects the quality of life and productivity of a person. Examples of degenerative diseases are cardiovascular disease (heart and blood vessels) including hypertension, diabetes mellitus, and cancer. Degenerative diseases that have a fairly high mortality rate is coronary heart disease [1]. This is consistent with the data which states that approximately 17.5 million Indonesians died because of heart disease. One of the degenerative diseases is caused by free radicals.

Free radicals are atoms or molecules that contain one or more unpaired electrons in their outermost orbitals. Free radical compounds arise due to various complex chemical processes that occur in the body, for example, side products from the oxidation or burning of cells that occur during breathing, cell metabolic processes, inflammation or when the body is exposed to environmental pollution such as motor vehicle fumes, smoke cigarettes, pollutants, and solar radiation. Free radicals in the body are highly reactive and interact destructively through oxidation reactions with certain cells composed of fat, protein, carbohydrates, DNA and RNA, so antioxidants are needed to overcome these free radicals [2], [3].

Antioxidants are substances that function to prevent the body's biological system from adverse effects due to excessive oxidation processes or reactions. Various methods are used to measure antioxidant activity including DPPH. The advantages of DPPH method are simple, easy, fast, sensitive and only require a small sample [4]. The activity of antioxidant compounds was determined by the presence of

free hydroxyl and carbon double bonds functional groups. Antioxidants in the body can be obtained from synthetic materials and natural ingredients. The weakness of synthetic antioxidants is that it can increase the occurrence of carcinogenesis so it is recommended to use natural ingredients. One of the antioxidants from nature is Chitosan [5], [6].

Chitosan is an abundant, yellowish-white amorphous solid, non-toxic and biodegradable polymer. It can be applied in various fields including agriculture, health, biotechnology, and food industry. In the food industry, chitosan is used as an antioxidant [7]. Chitosan consisted of three reactive functional groups, namely amino groups, primary and secondary hydroxyl groups. The amino (NH<sub>2</sub>) and hydroxyl (OH) groups in chitosan are functional groups of antioxidant activity [8]. Chitosan is derived from chitin compounds produced from the deacetylation process. Dompeipen (2016)[9] has been successfully synthesized chitosan from shrimp shell waste with a yield of 63%. According to M Victor (2016)[10] research, chitosan produced from snail shells is 45.02%. But chitosan from green mussels shell has not been applied optimally.

Green mussels (*Perna Viridis* L) is one of the shells that often consumed by the community. But the shells of green mussels have not been used optimally so that they are still waste in the environment. Waste of green mussel shells can be used as a source of chitosan through the deacetylation process, so the economic value of green mussel shells can be improved. Based on the background, it is necessary to research the use of chitosan from green mussel shell waste as an antioxidant.

## II. MATERIAL AND METHOD

### A. Materials

The waste of green mussels was obtained from Tasikmalaya, West Java, Indonesia. The other materials are NaOH (Bratachem®), HCl (Bratachem®), CH<sub>3</sub>COOH (Bratachem®), Metanol p.a (Bratachem®), Vitamin C (Bratachem®), 1,1-diphenil-2-pikrilhidrazil (DPPH) (Sigma Aldrich®), aquadm, aquadest.

### B. Instrumentations

FTIR spectrometer (Simadzu) used to identify functional group of chitin and chitosan, UV-Vis spectrometer used to

determine the antioxidant activity of chitosan from green mussels shell.

### C. Preparation of Green Mussels Shell

Green Mussels Shell was taken from Tasikmalaya, West Java, Indonesia. It was dried and milled until becoming powder. The determination of green mussels was carried out at the Animal Taxonomy Laboratory, Department of Biology, Universitas Padjadjaran Bandung, West Java, Indonesia.

### D. Isolation of Chitin

#### Deproteinization

The mashed sample was put into a 500 mL beaker, then 3% NaOH solution was added with a ratio of 3:1 (mL NaOH/g shell) and stirred with a magnetic stirrer for an hour. After that, it was heated at 80 °C for 30 minutes. Then, it was filtered and neutralized by washing using aqua dm. The yield of deproteinization was dried in an oven at 60 °C.

#### Demineralization

The result of deproteinization was added with 1.25 N HCl at a ratio of 3:1 (mL HCl/g of the shell). Then, it was heated at 75 °C for an hour. The solution was filtered and neutralized, then it was dried in an oven at 60 °C.

### E. Synthesis of Chitosan

#### Deacetylation

Chitosan was made through the deacetylation process of chitin by Knorr method using a 60% NaOH solution. The ratio used was 20:1 (mL NaOH/g chitin). The mixture was heated at 90-100 °C while stirring for 60 minutes. After it is cooled, it was filtered and the residue was neutralized. Then, it was dried in an oven at 60 °C [9].

### F. Determination of Antioxidant Activity

As much as 1 mL of chitosan (100, 120, 140, 160 and 180 ppm) from green mussel shell was mixed with 1 mL of 100 ppm of DPPH solution. The mixture was shaken and incubated at room temperature for 45 minutes. Then, it was measured by using UV-Vis spectrophotometer at 516 nm of wavelength.

Data obtained from antioxidant activity test is absorbance value, then the percentage of antioxidant is calculated using the following formula:

$$\text{Inhibition(\%)} = \frac{\text{Absorbance of blank} - \text{Absorbance of sample}}{\text{Absorbance of Blank}} \times 100\% \quad (1)$$

The IC<sub>50</sub> (Inhibition Concentration) value can be calculated using linear regression equation  $y = bx+a$  with  $x$  is concentration of test solution and  $y$  is the percentage of antioxidant activity [11].

## III. RESULTS AND DISCUSSION

### A. Synthesis of Chitosan from Green Mussels Shell

Chitosan from green mussels shell waste is produced through deproteinization, demineralization and deacetylation process. Deproteinization is the process of removing protein found in the shells of green mussels using NaOH solution through the hydrolysis process. In this process, the protein will be released and become Na-proteinate which can dissolve in water and will be lost during washing and filtering [9]. Demineralization aims to remove the minerals contained in the green mussels shell. Minerals will react with HCl to form a water-soluble mineral salt. One of the examples of minerals contained in shells of green mussels is calcium.

The last process is deacetylation which is the transformation chitin into chitosan. The aim of this process is to break the acetyl group (-COCH<sub>3</sub>) which is bound to nitrogen in the structure of chitin and form amine (-NH<sub>2</sub>) in chitosan. The yield of chitosan was determined based on the percentage of the weight of chitosan produced compared to the weight of chitin from the green mussels shell. The yield of chitosan produced in this study was 59.3%.

Identification with FTIR is used to show the structural change from chitin to chitosan. The result of F (a) identification can be seen in figure 1.

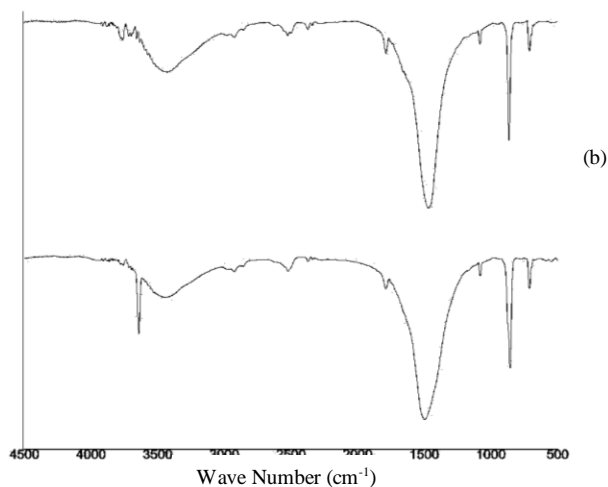


Fig. 1. FTIR Spectrum of chitin (a) and chitosan (b)

The difference in peak is clearly seen between chitin and chitosan at 3641.60 cm<sup>-1</sup> which is -NH functional group. This shows that chitosan has been successfully synthesized. The C=O functional group indicated at wave number 1784.15 cm<sup>-1</sup>, in chitosan, there is still a C=O group this is caused by the imperfect deacetylation process.

The determination of the deacetylation degree of chitosan aims to determine the formation of chitosan from chitin. Ray 2011 states that the value of the degree of deacetylation for chitosan that can be applied is 40-

98% [12]. Based on research, the value of the deacetylation degree of chitosan from green mussels is 49%. This indicates that chitosan obtained can be used because it has a deacetylation degree of more than 40%. The degree of deacetylation is influenced by several factors including NaOH concentration, reaction time and temperature. The amount of OH<sup>-</sup> in the solution causes more acetyl groups to be released so that the value of the deacetylation degree will be greater.

### B. Antioxidant Activity of Chitosan

Antioxidant activity testing was performed using DPPH method, the principle of this method is an interaction between antioxidants from a sample with DPPH either by transfer of electron or hydrogen radicals to DPPH. If all the electrons in DPPH are paired then it becomes stable. The reaction between antioxidants marked by the change in color of DPPH solution from purple to yellow. The change in DPPH color intensity is proportional to the number of electron transfers followed by a decrease in DPPH absorbance. In principle, this method is a measurement of residual absorbance of DPPH that has decreased due to reacting with samples that have antioxidant activity [13].

The result of antioxidant activity of sample showed in Fig. 2.

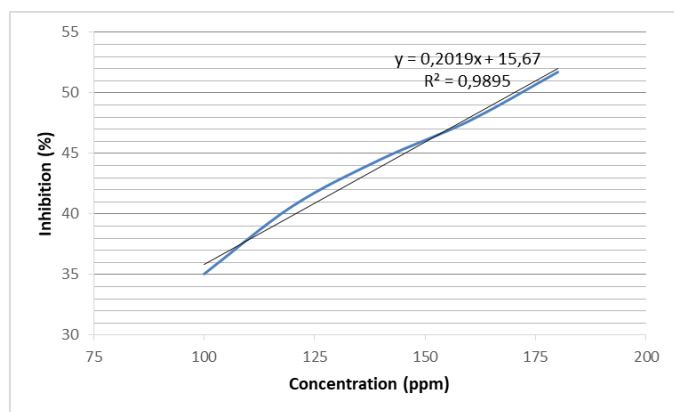


Fig 2. Antioxidant Activity of Chitosan from Green Mussels Shell

The parameter used for DPPH method is IC<sub>50</sub> value. The smaller IC<sub>50</sub> value means a higher antioxidant activity. From the result of the determination of antioxidant activity obtained IC<sub>50</sub> value for chitosan from green mussels shell is 170.03 which is classified as a weak category. This is related to the value of the deacetylation degree of chitosan because the amine group (NH<sub>2</sub>) in chitosan has a role in the capture of free radicals [3].

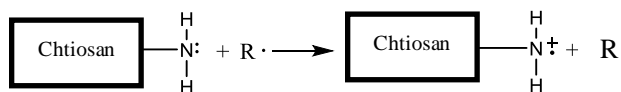


Fig. 3 Reaction Free Radical with Chitosan

In this study, the value of the deacetylation degree of chitosan from green mussels shell obtained 49% so the

deacetylation process does not take place perfectly and the number of NH<sub>2</sub> groups in chitosan is still low, this causes the antioxidant activity of chitosan to be weak.

## IV. CONCLUSION

Based on the results of research, it can be concluded that chitosan from green mussels shells have weak antioxidant activity with IC<sub>50</sub> values of 170.03 ppm. So, it is recommended for further research to optimize the deacetylation process so that the degree of deacetylation increases or modifies the chitosan produced.

## ACKNOWLEDGMENT

This work has supported by research centre and community service (P3M) STIKes Bakti Tunas Husada.

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