Effect Of Microwave Heating On The Antioxidant Activities And Physicochemical Properties Of Sea Cucumber (Holothuria Scabra) Powder

Ansharullah Ansharullah, Andi Besse Patadjai, Asranudin

Abstract: This study was aimed to characterize the antioxidant activity of sea cucumber (Holothuria scabra), which were processed into powder with various processing methods. The processing methods used were smoking, steaming and microwaving. The powder were then measured its antioxidant activity using 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. Other characteristics tested were their chemical content, morphological properties with Scanning Electron Microscope (SEM), and FTIR spectrum of the sea cucumber flour obtained. The results showed that the sea cucumber flour production with the microwaving method produced a powder which had stronger antioxidant properties compared to smoking and steaming methods. Based on IR spectroscopy data, it was shown that the compounds contained in samples which have vibrations of secondary amine groups (N-H), aromatic C-H groups, aliphatic C-H groups, C = O groups and aromatic groups indicated as alkaloid compounds. The sea cucumber powder produced with microwave method had a higher protein content, and its morphological appearance indicated a more physical damage. The method of sea cucumber powder preparation with microwave appeared to be likely more efficient in time, and would have a better nutritional values.

Index Terms: antioxidant, microwave heating, physicochemical properties, scanning electron microscope, sea cucumber, FTIR, alkaloid compounds.

1. INTRODUCTION

Sea cucumber is a natural source of food and medicine that contains various new functions and antioxidants with biological activities that can be used in the food and biomedical industries. Sea cucumbers are soft-bodied marine invertebrates of the Holothuroidea class, and have long been used as food and traditional medicine in Asian countries [1,2]. The sea cucumber species of Holothuria scabra has been studied considering that this species was edible, having medicinal effects, and low toxicity [3-7]. This species has a high-value sea cucumbers in Asia, and any new functional ingredient obtained from this sea cucumbers could be used for further development of new products in food and pharmaceuticals industries [2]. These organisms are potential sources that contain many therapeutic ingredients such as triterpene glycosides, carotenoids, bioactive peptides, vitamins, minerals, fatty acids, collagen, gelatin, chondroitin sulfate, and amino acids [1,8,9]. In recent years, the health benefits of sea cucumbers have been validated through scientific research and have provided drug assessments such as wound healing, neuro-protective, antitumor, anticogulant, antimicrobial, and antioxidants [8-13]. In the coastal areas of Southeast Sulawesi, Indonesia, sea cucumbers of the Holothuria scabra have been widely used by local communities as one of the economic commodities [14]. However, fresh sea cucumbers are highly perishable, so they need to be processed further in order to increase its shelf life and the added value. Processing sea cucumbers into flour would improve and widen their use in various functional food formulations. In the process of flour making, the material would be changed its physicochemical characteristics and antioxidant activity, especially if high temperatures are employed. Therefore, this study was intended to examine the effect of various processing methods, namely smoking, steaming and microwaving in the process of powder production of sea cucumbers on its antioxidant activity, and its physicochemical properties.

2 MATERIALS AND METHOD

2.1 Materials

The materials used in this study were freshly harvested sea cucumber of Holothuria scabra, obtained from the coastal area of Wawonii, Konawe Kepulauan, Southeast Sulawesi, Indonesia. The processing preparation was carried by: evisceration, washing with distilled water, and removing the lime layer on the surface of the skin by adding the tuber extract of Dioscorea hispida. About 100 g sample was further heated in three different method; namely smoking, steaming and microwave heating each for 10 minutes. The temperature of the smoking and steaming was 80°C; while the microwave heating using SHARP Model R-21DO was set to high. The samples were then dried in an air oven for 6 hours at 60°C, and grounded into powder for further analysis.

2.2 Analysis of Antioxidant Activities

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The assay of antioxidant activity was carried out using 1,1-diphenyl-2-picrylhydrazyl (DPPH) as free radicals according to Molyneux [17]. Samples were extracted using ethanol. A 5 gram wet sample is cut into small pieces (± 1 cm) then mashed and put into erlenmeyer and soaked with 100 ml of methanol for 1 hour on top of the shaker. The immersion solution was filtered and the filtrate was evaporated using a rotary evaporator at 40°C. The dried extract was then put into a vial for testing. The test begins with the making of a series of concentrations, namely 50, 100, 150, 250, 500, 750 ppm. Each series of concentration piped 1 mL was added 1 mL of DPPH 0.25 mM solution and 3 mL of ethanol. Next, the sample and DPPH were mixed with cortex for 1 minute then incubated for 30 minutes in the dark room and room temperature. The absorbance was measured using the Shimadzu Mini-UV U-1240 Spectrophotometer at a wavelength of 513 nm. Inhibition Concentration (IC50) values were recorded as the number of sample concentrations to reduce the DPPH concentration by 50%. Percentage inhibition was calculated by formula (1):

\[
\% \text{ Inhibition} = \frac{\text{Absorbance of blank} - \text{Absorbance of DPPH}}{\text{Absorbance of blank}} \times 100\%
\]

(1)

2.3 Physicochemical Analysis
The chemical content of sea cucumber including moisture, protein, fat, and ash were measured according to the protocol published by the Association of Official Analytical Chemists [15]. Carbohydrates were calculated as the difference between 100 and the amount of water, fat, protein, and ash. While the morphology and microstructure of the powder were tested using a scanning electron microscope (SEM) TESCAN 20 KV.

2.4 Characterization with FTIR spectrophotometer
One milligram of sample was taken, mixed with KBr, and grounded thoroughly until homogeneous. The mixture was put into a pellet maker with a pressure of 74 mmHg and within 5 minutes to obtain a pellet of ± 1 mm thickness. The plate was placed on a container then measured its absorption with FTIR Spectrophotometer.

3 RESULTS AND DISCUSSION

3.1 Antioxidant Activity
The results of this study indicate that the method of processing sea cucumber into flour greatly influences its antioxidant activity. As shown in Fig. 1, the microwave treatment method produces the product with the strongest antioxidant activity (IC50 of 89.89). While the processing method that uses steaming and smoking has moderate antioxidant activity. A compound was said to have the ability as an antioxidant is very strong if the IC50 value was less than 50 ppm (category 1), strong if the IC50 value was between 50-100 ppm (category 2), moderate if the IC50 value was between 100-150 ppm (category 3), weak if the IC50 value was between 150-200 ppm (category 4) and very weak if the IC50 value was more than 200 ppm (category 5) [16]. Various studies have identified the types of antioxidants found in sea cucumber, namely from the phenolic group [17, 18], steroids and alkaloids [19, 20]. High-performance liquid chromatographic analysis of the sea cucumber mixed extract was revealed in the presence of some phenolic components, such as chlorogenic acid, pyrogallol, routine, coumaric acid, catechin, and ascorbic acid [21].

Antioxidants were easily damaged by high temperature treatment. With conventional heating processes, such as smoking and steaming, the possibility of antioxidant damage was higher than microwave heating. It can be explained that microwave heating involves three energy conversions, namely the conversion of electrical energy into electromagnetic energy, electromagnetic energy into kinetic energy, and kinetic energy into heat energy. In conventional heating, heating occurs through a temperature gradient, whereas in the microwave method, heating occurs through direct interaction between microwave materials. This results in faster energy transfers, and has the potential to improve product quality [22, 23].

3.2 Physicochemical Analysis
The method of processing of sea cucumber flour had affected their proximate composition, as shown in Table 1. In general, the microwave method had an impact on higher protein level; the smoking methods produced a higher fat level, and the steaming method produced a more water content. All processing methods produced sea cucumber flour which contained similar amount of ash.

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>PROXIMATE COMPOSITION OF SEA CUCUMBER POWDER PROCESSED WITH DIFFERENT METHODS OF HEATING</th>
</tr>
</thead>
<tbody>
<tr>
<td>Content</td>
<td>Smoking</td>
</tr>
<tr>
<td>Moisture (%)</td>
<td>13.49 ± 0.46</td>
</tr>
<tr>
<td>Protein (%)</td>
<td>63.18 ± 1.15</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>8.51 ± 0.54</td>
</tr>
<tr>
<td>Ash (%)</td>
<td>3.89 ± 0.14</td>
</tr>
<tr>
<td>Carbohydrate (%)</td>
<td>10.93 ± 0.28</td>
</tr>
</tbody>
</table>

In the steaming process, sea cucumber meat which had a lot of muscle tissue was thought to be still strong enough in binding water due to physical conditions with a minimal damage. With the smoking and microwave methods, the muscle tissue had more damages and decomposition, so the ability to bind water was low and resulted in a lower water content.

Product protein content was higher in the microwave heating
method, compared to conventional heating methods. This was thought to be due to the microwave method, heating occurs through direct interaction between components in the flour material, and results in faster energy transfer, so that protein damage can be minimized [23].

Meanwhile, the fat content of products that experience the smoking process has a higher fat content. This was due to changes in the water content which was greatly reduced during the smoking process, which had affected the increase in fat content.

### 3.3 Electron Scanning Micrograph Properties

Observations on the morphology of sea cucumber flour were carried out by testing it with a Scanning Electron Microscope (SEM). This test was aimed to see the effect of the heating treatment method on the morphological structure of the flour produced. As shown in Fig. 2c, most structural damage occurred in the microwave heating process; while the steaming method shows minimal structural damage.

More structural damage to flour produced from the microwave process will have the potential to facilitate if the product is applied in food product formulations [23]. More physical damages of the structures would be easier for various components to be interacted and mixed each other in processing, if the materials would be used in the formulation of food products.

### 3.4 FTIR Spectrum Characteristics

FTIR spectrophotometer was a device used to obtain the infrared spectrum of absorbance in a sample that was used to determine the type of functional group in an isolated animal or plant tissue. Based on the results of the interpretation shows that the functional groups determined from the results of the IR wavelength is a functional group contained in flavonoid compounds (Fig. 3). With the area of the spectra that read ranging from 3500-700 cm⁻¹ and included in the middle IR, so the antioxidant compounds obtained in the results of the study could be thought to be a secondary metabolite compound of flavonoid type, which was characterized by the presence of the bound -OH functional group, -CH aliphatic,

![Fig. 2a. Scanning electron micrograph of 2000 magnification of the sea cucumber powder processed with the method of smoking.](image)

![Fig. 2c. Scanning electron micrograph of 2000 magnification of the sea cucumber powder processed with the method of microwaving.](image)

![Fig. 3. FTIR Spectrum of the antioxidant of the sea cucumber powder.](image)

Based on IR spectroscopy data, it was shown that the compounds contained in samples which have vibrations of secondary amine groups (N-H), aromatic C-H groups, aliphatic C-H groups, C = O groups and aromatic groups indicated as alkaloid compounds

### 4 CONCLUSION

The sea cucumber powder production with the microwaving method produced a powder which had stronger antioxidant properties compared to smoking and steaming methods. The sea cucumber powder produced with microwave method had a higher protein content, and its morphological appearance indicated a more physical damage. This indicated a potential for a wider application of the powder in the formulation of various food products. The method of sea cucumber powder preparation with microwave appeared to be likely more efficient in time, and would have a better nutritional values.

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