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Production and Composition Identification of Sea Cucumber Jelly from *Holothuria Scabra*

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Abstract. Based on research conducted by various researchers showing that sea cucumbers are high in nutrients needed by the body such as proteins, polysaccharides, fats, amino acids, and show anti-bacterial activity, anti-fungi and antioxidant which are good for the body. From the information above, supplement with sea cucumber-based ingredients can produce supplements that rich in nutrients. In this study, the authors has done the production of supplements with the main ingredients is sea cucumber that most common found in Indonesia which is *Holothuria scabra* in the same form as commercial products and has conducted a comparative test, composition identification and test of anti-bacterial, anti-fungi and antioxidant activity. The result of the composition obtained are jelly made from 35% hydrolysate, 45% water, 15% gelatin and 5% sugar. The results of sample jelly protein 5.1% and commercial jelly protein 0.175%, result of sample jelly fat 0.03% and commercial jelly fat 0.06%, and result of sample jelly carbohydrate 2.6% and commercial jelly carbohydrate 2.9%. Antioxidant and anti-bacterial tests also show that artificial sea cucumber jelly has a higher activity. It can be concluded that sea cucumber jelly has good nutrients and good antioxidant activity and good anti-microbial activity.

INTRODUCTION

Indonesia, which is known for its rich marine biodiversity, has many potentials that have not yet been discovered and utilized. In the field of food and health, sea cucumbers are known as organism that contain very good nutrients and have active components such as bioactive peptides, anti-microbial agents, inflammatory agents and anticancer agents [1]. Sea cucumbers are one of the marine animals that contain many good sources of nutrition for humans. Sea cucumbers are an important component for marine ecosystems, they are commonly found in areas that have coral, rocks or seaweed in shallow water [2].

This type of biota known as sea cucumber has a high economic value because of its high content or nutritional content. From the results of the study, the nutritional content of sea cucumbers in dry conditions consisted of 8.2% protein, 1.7% fat, 8.9% water, 8.6% ash, and 4.8% carbohydrate [3]. Sea cucumber has begun to show its ability as a basic ingredient as a source of good nutrition and bioactive ingredients for a long time. Since hundreds of years ago,

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ethnic Chinese have used sea cucumbers as the basic ingredients of traditional medicines and foods with high value because they believe that sea cucumbers have good content [4]. Then a few years ago until now our predecessors guessed evidenced by the many studies conducted on sea cucumbers to prove the benefits of bioactive content such as chondroitin sulfate which is able to strengthen bones again to prevent bone loss, the super oxide dismutase (SOD) enzyme as an anti-cancer and anti-inflammatory, and cell growth factor (CFG) that is capable trigger new cell regeneration [5]. Plenty of marine organisms in the ocean has potential secondary metabolites being used as medicine such as antiviral, antioxidant, antimicrobial [6,7].

However, commercial use of sea cucumbers in Indonesia so far only uses parts of the meat which are considered to have high nutritional content as the basic ingredients of cooking. Marketing of sea cucumbers as a source of nutrition, active ingredients and important agent agents has not been too developed. The reason why the production of sea cucumber-based supplements made in Indonesia has not yet been carried out is because of the unclear view of the Indonesian population living on the coast of the sea. They are still lack knowledge about the properties possessed by marine biota. Many of them are still doubtful because they are not used to processing sea cucumbers into other forms of products such as jelly. With the enlightenment of how easy and cheap the making of sea cucumbers to a jelly-shaped supplement, they are expected to have a high desire to be more innovative in processing sea catches. In this discussion, it is expected that sea cucumbers can be a supplement ingredient in the form of jelly which has the same or better content than foreign supplement products.

The purpose of this study was to make sea cucumber jelly using sea cucumbers from the Indonesian sea, conduct comparative test of proteins, polysaccharides, and fats of sea cucumber jelly from Indonesian and Malaysian sea cucumbers, and to test the anti-microbial activity, and antioxidant of sea cucumber jelly from Indonesian and Malaysian sea cucumbers.

MATERIALS AND METHODS

Formulation of Sea Cucumber Jelly

The experiment jelly was made with a composition of 35% (w/w) sea cucumber hydrolysate, 45% (w/w) water, 15% (w/w) gelatin, and 5% (w/w) sugar. Soaking is done to clean sea cucumbers from dirt and also to soften sea cucumber meat. Based on these tests, it can be concluded that the length of immersion time is 2-3 days. Before the formulation, cucumber and hydrolysate were counted with the help of papain enzyme as much as 5% (w/w) for 4 hours at 70^{o}C.

Antimicrobial Activity Assay

The anti-microbial activity test was done with the disc diffusion method. Bacterial strain *Pseudomonas aeruginosa*, *Escherichia coli*, *Micrococcus luteus*, and *Bacilus subtilis;* fungi *Candida albicans* and broad-spectrum standard antibiotic chloramphenicol was used in this research given by Research center for Biology, Indonesian Institute of science, Bogor, West Java, Indonesia. Antimicrobial activity assayed by using Disc Diffusion Method based on method described by Fera Ibrahim et al., (2013) with slight modification [8]. Bacterial inoculums were spread onto sterile NA agar plates (90-mm diameter), then it allowed till surface medium were solid and dry. Furthermore, sterile paper discs (7mm diameter) were placed onto the nutrient agar surface and addition of 40 µl of sea cucumber jelly sample, chloramphenicol (30 µg/disc) as positive control and commercial gamat jelly sample as normal control, was performed. Hereinafter, prior to used, Sea Cucumber Jelly sample, chloramphenicol and commercial sample were filtered (0.22 µm). These plates were incubated at 37°C for 24 hours, followed by measured and calculated of diameter of inhibition zones minus the disc diameter. This assay activity was performed to obtain triplicated results.

Test of Antioxidant Activity

The Assay of antioxidant activity will be carried out by the method of alloxan analysis. We used the sea cucumber jelly sample (S) and commercial gamat jelly sample (G). Both samples proved to contain GSH in the form of antioxidant enzymes. Alloxan was used as standards. The calibration curve of standards (alloxan) was measured by the absorbance from UV Vis Spectrophotometry. The equation formula was $Y = 0.00007x - 1,656$ and $R^2 = 0,96023$. All determinations were carried out in triplicate.

Test of Protein Composition

The identification of proteins will be carried out using the Kjeldahl method. This analysis can be divided into three stages, namely the process of destruction, distillation and titration. To obtain the results of the protein content calculation is needed by the following formula in Eq.1:

$$Protein\ Content\ (\%) = \frac{(V_1 - V_2) \times N \times 0.014 \times f.k \times f.p}{W} \quad (1)$$

Where,
W : Weight of sample
V_1 : Volume of HCl 0.01N for example titration
V_2 : Volume of HCl for sample titration
N : HCl normality
f.k : Protein constant
f.p : Dilution factor

Test of Fatty Acid Composition

The fat identification will be carried out by the Soxhlet method. The principle of this method is to extract free fat with non-polar solvents in the form of hexane or other fat solvents. The formula used based on Eq.2:

$$Fatty\ Acid\ Content\ (\%) = \frac{W - W_1}{W_2} \times 100\% \quad (2)$$

Where,
W : Weight of sample (gram)
W_1 : Weight of flask before extraction (gram)
W_2 : Weight of flask after extraction (gram)

The weight of the flask before extraction is 45 grams and after extraction it is 45.0027 grams.

Test of Carbohydrate Composition

Testing of carbohydrate content is done by elimination method. This method can be done because testing of water, ash, protein and fat content has been done, so that the rest is in the form of carbohydrates.

RESULTS AND DISCUSSION

Test of Anti-microbial Activity

The results obtained from the anti-microbial test for 5 different types of microbes are as follows in Table 1.

TABLE 1. Anti-microbial test activity result

Microbes	Diameter (mm)		
	Positive Control	Own Sample	Commercial Sample
P. aeruginosa (Gram -)	30	11	0
E. coli (Gram -)	23	11	0
M. luteus (Gram +)	49	40	0
B. subtilis (Gram +)	40	14	0
C. albicans	25	11	0.7

From the table above, it can be said that almost all tests are in the strong category, except for *M. luteus* which is in the very strong category. It can also be seen that greater barriers to testing use gram-positive bacteria. This is in accordance with the literature which says that gram-positive bacteria are easier to absorb antibiotics because gram-

positive bacteria do not have a lipid bilayer layer that has permeable selective properties making them more susceptible to antibiotics.

Based on previous testing, it was said that sea cucumbers have a higher anti-fungal ability because their bodies have adapted to have a stronger effect on fungi that are more commonly found in their environment than bacteria [9]. Based on Farouk, et al. which has isolated several bacterial strains from the tissues of several sea cucumber species and seen that the secretions and extracts from these bacteria have anti-bacterial activity with moderate to high activity [10]. It is said that bacteria that can produce antimicrobial agents require foods that contain high nutrients (10% glucose w/v). The result of this study was similar to Hing, H L (2007) that observed crude extract using methanol extraction from sea cucumber species *Stichopus chloronotus* and *Holoturia edulis* has antifungal activities against *Candida albicans* using the well diffusion method [11].

Test of Antioxidant Activity

The following are the results of the standard calibration curves performed with GSH as a standard.

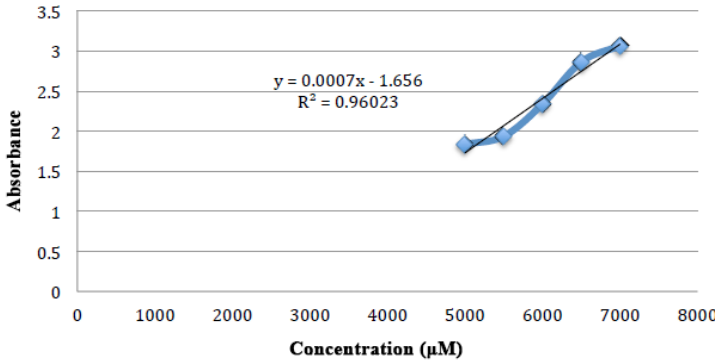

FIGURE 1. The standard curve of the alloxan method using GSH

For sample sea cucumber jelly sample (S), the absorbance value of 2.285 was obtained and for commercial gamat jelly sample (G) was 1.843. The negative control obtained is 0.086. Using the equation of the line from the standard curve above in Figure 1, the concentration of antioxidant content can be known by the formula. Both samples proved to contain GSH in the form of antioxidant enzymes [12]. however, samples of sea cucumber jelly appear to have higher GSH concentrations of 5630 µM per 1 gr/l alloxan. Commercial samples contain only 4998.57 µM per 1 gr/l alloxan. The result was equal with previous study revealed that sea cucumber from *H. edulis* and *S. horrens* contain promising levels of antioxidant and cytotoxic natural products that might be used for cancer prevention and treatment [13].

Test of Protein Composition

For white sea cucumber jelly, a protein content of 5.1% was obtained and 0.175% for commercial products. If the two results are compared, white sea cucumber jelly has a higher content. Based on previous research, the percentage of the content protein in the wet *H. scabra* [14], which is the base material for the S sample is 9.94%. While the protein content in *S. variegatus* in wet conditions is 8.2% [15]. With a basic difference in the main raw materials, it is not surprising that the value is different.

But the difference is quite far can be caused by the treatment of the base material when the product is formed. In sample S, the process of making jelly is only done for 1-2 days under normal conditions. While making sample G is carried out for months at a temperature that is not necessarily maintained. In addition, the reason why the protein content in the sample G is much lower is due to the length of time the sample is stored. Sample G in the form of commercial gamat jelly which is not known exactly how long the storage time before testing. Too long storage time and other factors such as storage environment conditions such as temperature and humidity can also affect the condition of proteins.

Test of Fatty Acid Composition

In sample S, the sample used is 10.0417 grams. The results are 0.03%. In sample G, the sample used was 7.0173 grams. The results are 0.06%. It showed that the fat content in the sample S is lower compared to sample G. However, both samples had relatively low-fat content. This is because sea cucumbers generally contain low fat. Based on previous research, the reason why the fat content in sample G is higher when compared to sample S, because the base material of sample G in the form of golden sea cucumber (*Stichopus variegatus*) has a higher fat content compared to the base material sample S in the form of sand sea cucumber (*Holothuria scabra*).

Test of Carbohydrate Composition

Water content obtained for Sample S was 91.1% and for sample G was 96.6% while for ash content sample S was 1.24% and for sample G was 0.67%. By using the method by difference, the carbohydrate content in sample S is 2.6% and for sample G is 2.9%. The carbohydrate values contained by both are not much different, but look higher in commercial samples. This is because the basic ingredients of the sample in the form of *S. variegatus* contain higher carbohydrates compared to *H. scabra*. Comparison of these two sea cucumbers is indeed very far, but the end result is almost similar due to the freshness of the sea cucumber itself. The sample of sea cucumber jelly counts fresher than the commercial sample whose manufacturing process takes months. Long treatment times and improper storage can change the carbohydrates they contain.

CONCLUSION

Sea cucumber jelly have a higher anti-microbial activity than the commercial sample in all microbes. The sample of sea cucumber jelly looks to have a higher concentration of GSH than commercial samples. The protein content of artificial sea cucumber jelly samples is higher compare than commercial samples. The fat and carbohydrate content in the two samples was not too different. Differences are due to differences in basic ingredients and treatments when making products. White sea cucumber jelly is considered suitable for supplemental ingredients because it has good content.

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