Artificial rice from *Gracillaria* sp. as functional food to prevent diabetes

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Abstract. Seaweed *Gracillaria* sp. was one of the local potentials of West Java-Indonesia, this seaweed was easily cultivated, but currently underutilized. Therefore, processing was very important to increased value added of seaweed. The purpose of this research were characterize and determine antialphaglukosidase activity of *Gracillaria* sp. seaweed as raw material for artificial rice. The research method used a Complete Randomized Design (CRD) of two factors with continued testing of Duncan and Kruskal Wallis. The addition of agar flour influenced the water content, ash, fat and protein content on artificial rice. The result of organoleptic test showed that addition of agar flour for artificial rice gave significant effect on color, odor and texture. The resulting artificial rice has a moisture content of 9.9-13.7%, ash content of 1.1-4.3%, fat 0.3-3.7%, protein 2.7-5.9%, carbohydrate 77.7-80.3%, fiber 0.11-0.95%, and tannin 0.11-0.20%.

Keywords: agar flour; antidiabetic; artificial rice; *Gracillaria* sp.; seaweed.

1. Introduction

In China, *Gracilaria* originally were utilized as food and as binding material in the preparation of lime for painting walls. The use of seaweed as food spreads to several Asian countries, until the content of agar was discovered by the western countries and the Japanese [1].

Seaweed is one of the potential commodities from Indonesian waters to be developed. Based on data from the Ministry of Maritime Affairs and Fisheries [2], seaweed production in Indonesia's at 2015 amounted 11.68 million tons or about 66.87% of the total aquaculture production. The potential of Indonesian seaweed has opportunities to be utilized in food, pharmaceutical, cosmetic and textile industries.

One of the industries that use seaweed is a gelatin/agar industry. Agar used as thickening, emulsifying, stabilizing and other functions in the food sector [3]. The seaweed group producing agar was *Gracilaria* sp. This type of seaweed has been cultivated and has important economic value, it was easily obtained and easier to process [4].

The results of research from Yusasrini and Damayanti [5] showed that *Gracilaria* sp. contains of crude fiber 20.48% and tannins, which were many health benefits. The results of Prabha *et al*. [6]...
showed that red seaweed contains tannins which have health benefits. The results of the research by Setiawati [7] reported that artificial rice with addition seaweed can reduce the density of kamba and blood sugar levels in rats (*Mus musculus*) strains ddY by 35%. Agusman *et al.* [8], research about used *E cottonii* seaweed and addition mocaf flour on analog rice. Dewi and Halim [9] studied on producing analog rice with arrowroot tubers and seaweed flour as a staple food.

The research related addition agar flour from *Gracilaria* sp. to make artificial rice and its characteristics for diabetes were important to conduct, since it has rich fiber and tannin.

2. Materials and Methods

2.1. Materials

The samples used in this study were *Gracillaria* sp. seaweed collected from Muara Gembong, Bekasi, West Java; rice starch and corn starch from Dramaga, Bogor, West Java. The material were prepared and made artificial rice with addition *Gracillaria* sp. seaweed powder and then analyzed.

2.2. Research treatments

The first steps were identification of species, proximate, heavy metal, and active component analysis of raw material seaweed. The next step preparation raw material that was seaweed powder, rice starch and corn starch, and then mixed, and then put in to the extruder machine and then analyzed. The artificial rice analyzed includes proximate, heavy metal, total fiber, tannin, organoleptic, and alpha-glucosidase inhibitors activities. The last stage was to determine IC$_{50}$ value in the treatments for *in vitro* test.

2.3. Chemical Analysis

The chemical analysis which comprised proximate analysis [10] on raw material and artificial rice; active component [11] and alpha-glucosidase inhibitors activities [12] on seaweed powder and artificial rice, heavy metal [13] on seaweed powder and artificial rice; organoleptic [14], total tannin [10] and fiber [10] on artificial rice.

2.4. Statistics Analysis

The experimental design for the determination of the best concentration method used a completely randomized design (CRD). If the ANOVA F test on different effects, then followed by Duncan's test.

3. Results

3.1. Characteristics of raw materials

The Chemical content of dried seaweed *Gracillaria* sp. were moisture, ash, fat, protein, and carbohydrate. The results test of chemical content from dried seaweed were presented in Table 1.

| No | Parameters (content) | Concentrations |
|----|----------------------|----------------|
| 1  | Moisture (%)         | 8.25 ± 0.33    |
| 2  | Ash (%)              | 6.36 ± 1.76    |
| 3  | Fat (%)              | 2.95 ± 0.13    |
| 4  | Protein (%)          | 9.95 ± 0.14    |
| 5  | Carbohydrate (%)     | 71.56 ± 1.26   |

Information: n = 3; Values are mean ± standard deviation

Heavy metal content in foodstuffs and processed foods must conform to BPOM standards. The results heavy metal test of dried seaweed *Gracillaria* sp. were presented in Table 2.
Table 2. Metal content of raw material from *Gracillaria* sp..

| No | Metal | Concentrations | Standard BPOM |
|----|-------|----------------|---------------|
| 1  | Cu    | 0.902 ± 0.42   | 0.043         | 10.0          |
| 2  | Cr    | <0.004         | <0.004        | -             |
| 3  | Cd    | 0.038 ± 0.36   | 0.010         | 0.2           |
| 4  | Pb    | 0.20 ± 1.26    | <0.003        | 0.5           |
| 5  | Hg    | 0.12 ± 0.67*   | 0.010         | 0.03          |

Information: n = 3; Values are mean ± standard deviation.

Secondary metabolites screening of agar-agar powder from dried seaweed *Gracillaria* sp. was presented in Table 3. Based on Table 3, the agar-agar powder from dried seaweed *Gracilaria* sp. contained alkaloids, sponins, flavonoids, tannins, and, triterpenoids.

Table 3. The secondary metabolites screening of dried seaweed *Gracilaria* sp..

| Test          | Parameter |
|---------------|-----------|
| Alkaloids     | +         |
| a. Dragendorff| +         |
| b. Meyer      | +         |
| c. Wagner     | +         |
| Saponins      | +         |
| Flavonoids    | +         |
| Tannins       | +         |
| Triterpenoids | +         |

Information: n = 3.

3.2. Characteristics of Artificial Rice

The chemical content of artificial rice from agar-agar seaweed *Gracillaria* sp. were moisture, ash, fat, protein, carbohydrate, fiber, and tannins. The results test of the chemical content artificial rice from agar-agar seaweed *Gracilaria* sp. were presented in Table 4.

Table 4. The results chemical content of artificial rice.

| No | Type of test | Formula A          | Formula B          | Formula C          |
|----|--------------|-------------------|-------------------|-------------------|
| 1  | Moisture (%) | 13.38 ± 1.02 a    | 10.53 ± 1.04 a    | 11.35 ± 1.01 ab   |
| 2  | Protein (%)  | 2.79 ± 0.42 a     | 4.77 ± 0.55 c     | 3.42 ± 0.35 b     |
| 3  | Fat (%)      | 2.56 ± 0.32 a     | 2.85 ± 0.32 a     | 2.87 ± 0.12 a     |
| 4  | Ash (%)      | 1.88 ± 0.04 a     | 2.53 ± 0.12 ab    | 2.64 ± 0.22 ab    |
| 5  | Carbohydrate | 79.52 ± 2.12 a    | 79.32 ± 1.82 a    | 79.73 ± 1.42 a    |
| 6  | Fiber (%)    | 0.12 ± 0.02 a     | 0.35 ± 0.02 b     | 0.53 ± 0.02 c     |
| 7  | Tannins (%)  | 0.11 ± 0.01 a     | 0.12 ± 0.01 a     | 0.14 ± 0.01 ab    |

Information: n = 3; Values are mean ± standard deviation; a–b values with different superscripts within the same line were significantly different.

Sensory analysis (five senses testing) was done by quantitative methods, that was the preference test (hedonic test) [14]. Organoleptic testing on artificial rice was carried out including color, odor, and texture. The results organoleptic test of artificial rice from agar-agar seaweed *Gracilaria* sp. were presented in Table 5.
Table 5. The results organoleptic test of artificial rice.

| Type      | Formula A        | Formula B        | Formula C        |
|-----------|------------------|------------------|------------------|
| Color 1)  | 3.92 ± 0.32 a    | 5.61 ± 0.42 b    | 6.92 ± 0.52 c    |
| Odor 2)   | 5.82 ± 0.81 b    | 7.13 ± 0.62 c    | 6.63 ± 0.42 b    |
| Texture 3) | 6.13 ± 0.53 b    | 7.08 ± 0.92 c    | 6.37 ± 0.52 b    |

Information: n = 3; Values are mean ± standard deviation, a–b values with different superscripts within the same line were significantly different.

1) The value is between neutral to like
2) The value is between ordinary to like
3) The value is between ordinary to like

3.3. Alphaglucosidase Inhibitors Activity

Antidiabetic activity can be measured in vitro using \( \alpha \)-glucosidase enzyme inhibiting method. The \( \alpha \)-glucosidase (\( \alpha \)-D-glucoside glucohydrolase, EC 3.2.1.20) is an enzyme that catalyzed the breakdown of bonds 1,4 \( \alpha \)-glikosida at the tip non reducing agent from maltooligosakarida by releasing \( \beta \)-D-glucose. This enzyme can also slowly hydrolyzed the 1,6-\( \alpha \)-D-glucosidic bond so that it can continue the action of \( \alpha \)-amylase, that is, advanced hydrolysis of \( \alpha \)-limit dextrin to glucose [15]. The screening result of antidiabetic activity was presented in Table 6.

Table 6. The results of antidiabetic activity test.

| No | Type of Material                      | Value IC\(50\) (ppm) |
|----|--------------------------------------|-----------------------|
| 1  | Dried seaweed flour                   | 32 ± 2.32             |
| 2  | Agar-agar seaweed flour               | 58 ± 2.55             |
| 3  | Artificial rice from seaweed flour    | 156 ± 3.52            |

Information: n = 3; Values are mean ± standard deviation.

4. Discussion

The results of proximate analysis showed that \textit{Gracillaria} sp. dried seaweed contains very low moisture content that is 8.25\%, high protein content, so does ash content, while fat content is moderate.

The moisture content is an important criterion in determining the quality and shelf-life of processed seaweed meals, where high moisture can accelerate the growth of microorganisms. In addition, with drying and storage of seaweeds will likely to affect the moisture content of seaweed [16].

The high ash content of \textit{Gracillaria} sp. may be caused by the high ash content from habitat. That was according with Padidela and Thummala [17] were explains that ash content caused by habitat and environmental differences. Rasyid \textit{et al.} [18] explains the nutrient composition of dried red seaweed \textit{Gracilaria gracilis} of moisture (19.05\%), protein (10.86\%), ash (6.78\%), fat (0.18\%), carbohydrate (63.13\%) and dietary fiber (27.48\%) basis on the dry weight.

Purwaningsih \textit{et al.} [19] explains that the various of moisture content, ash content, lipid content, and protein content of matah merah snail was affected of many factors. The factors are species, size, gonado somatic index, temperature, various of feed and sampling location.

The data showed that high ash content seaweed powder of \textit{Gracillaria} sp. correlated with heavy metal levels that was exceeding BPOM (2014) standard, that was Hg, even though the washing process has been carried out.

Seaweed powder of \textit{Gracillaria} sp. couldn't used as raw material in artificial rice because it doesn’t comply with BPOM standards, i.e. the heavy metals content, especially Hg. The next treatment was reduced levels of heavy metals, and then make seaweed powder/agar-agar. The result of metal content agar-agar seaweed powder of \textit{Gracillaria} sp. accordance with the BPOM standard (2014) (Table 2).
Marine organisms have ability to synthesize chemical, that can help them to survive from changes of temperature, extreme salinity and pressure, prevent from predators, paralyze prey, and prevent poisoning and infection. Agar-agar powder from dried seaweed *Gracilaria* sp. contained alkaloids, sponins, flavonoids, tannins, and, triterpenoids (Table 3). Research on raw materials from sea by Purwaningsih *et al.* [20] showed that active components of *Telescopium* sp. were saponin, flavonoids, steroids, and, triterpenoids.

Several studies have reported the antidiabetic activity from secondary metabolites. Alkaloids may decrease the activity of transaminases and creatinine production in diabetic mice [21]. Flavonoids are compounds known as $\alpha$-glucosidase inhibitors. The mechanism of flavonoids inhibition against $\alpha$-glucosidase enzyme is through bond hydroxylation and substitution on the ring $\beta$. This inhibitory principle produces hydrolysis of carbohydrates and delays glucose absorption and inhibits the metabolism of sucrose into glucose.

Flavonoids have been reported to have antiplatelet aggregation and aldose reductase inhibitory activities, which may have helped to reduce the severity of the diabetic syndrome. Flavonoid and triterpenoid could be responsible for the good clinical effects on type 2 diabetes through targeting oxidative stress and postprandial hyperglycemia [22].

### 4.1. Characteristics of Artificial Rice

The results of various analyses showed that concentration of seaweed flour had significant effect on water, protein, and fiber levels ($\alpha = 0.05$). Duncan’s test results showed that protein on artificial rice with addition of seaweed flour at concentration of 15% and 20% had significantly different with addition of 10%. The results showed that artificial rice has protein content with range of 2.56-4.77%. The other study conducted by Setiawati *et al.* [7] showed that artificial rice from seaweed has a protein content with a range of 8.01-8.39%.

Duncan’s test result showed that fiber on artificial rice with addition of seaweed flour at concentration of 15% and 20% had significantly different with addition of 10%. The crude fiber content of artificial rice increased with increasing concentration of seaweed flour, which caused seaweed to become a source of fiber. Yusasrini and Darmayanti [5] explained that crude fibre content of seaweed *Gracilaria* sp. was 20.48%.

The results of organoleptic test using Kruskal-Wallis method showed that concentration of agar-agar flour had significant effect on color, smell, and texture ($\alpha = 0.05$). The Multiple Comparisons test results on color, smell, and texture showed that artificial rice with addition agar-agar at concentration of 10% was different significantly with addition of 15%, and also different from addition of 20%. The best treatment was added agar-agar flour from seaweed *Gracilaria* sp. with concentration at 15%.

The test results of antidiabetic activity showed that artificial rice from seaweed flour had lower antidiabetic activity compared to agar-agar seaweed flour and dried seaweed flour (Table 6). Antidiabetic activity of dried seaweed flour ($IC_{50} = 32$ mg/ml) was higher/stronger than Matah merah snails (*Cerithidea obtusa*) extract examined by Cahyani *et al.* (2014), where the value of $IC_{50}$ is 36.4 mg/ml. This indicated that dried seaweed flour at concentrations of 32 mg/ml can inhibited $\alpha$-glucosidase activity at 50%, while the snail extracts at concentrations of 36.4 mg/mL.

### 5. Conclusions

The seaweed of *Gracilaria* sp. could made into a agar-agar flour for artificial rice raw materials, and that has been in accordance with BPOM standards as a raw material for processed products. Dried seaweed *Gracilaria* sp. had contained active components: alkaloids, saponins, flavonoids, tannin, and, triterpenoids. Antidiabetic activity of artificial rice had IC50 value of 156 ppm. The best treatment was the addition of agar-agar flour from seaweed *Gracilaria* sp. with a 15% concentration.
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