Characteristics of green seaweed salt as alternative salt for hypertensive patients

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Abstract. Seaweeds from seashore contain high nutrient and various bioactive compounds that have various beneficial roles such as antioxidant property. Antioxidants can improve health and play a role in preventing the emergence of chronic diseases. This study was aimed to characterize green seaweed salt antioxidant activity. The study consisted of analysis of the mineral, and NaCl content, as well as determination of the antioxidant activity of green seaweed salt by CUPRAC and FRAP method. Different factors affecting salt quality and antioxidant activity including types of seaweed (Caulerpa lentillifera and Halimeda opuntia), temperatures (40°C, 55°C and 70°C) and extraction times (10 and 30 minutes) were evaluated. The results showed that the interaction between seaweed, time and temperature did not significantly affect the level of salt content, but significantly affected the Na:K ratio and NaCl content. Salt of C. lentillifera treated at 40°C for 30 minutes showed the highest antioxidant activity measured with FRAP method (137.07 μmol trolox/g), while salt of H. opuntia treated at 40°C treatment for 30 minutes gave the highest antioxidant activity as measured by CUPRAC method (58.14 μmol trolox/g).

Keywords: characteristics, hypertensive patients, salt, seaweed

1. Introduction

Seaweed is a food ingredient with high nutrient content and is a source of water-soluble vitamins (B1, B2, B12, C) and fat soluble vitamins (β-carotene with the activity of vitamin A and E). Vitamin E is a fat soluble vitamin and prove to have antioxidant activity [1]. More than 555 species consisting of red, brown, green and blue green seaweed grow in Indonesian waters [2]. Local community commonly uses some of the seaweed species as food. Seaweed species such as Caulerpa racemosa, Caulerpa lentillifera, Ulva sp. of Chlorophyta and Eucheuma cottonii, E. spinosum, Gracilaria sp. of Rhodophyta are found in large quantity. Eucheuma sp. is the main seaweed commodity in Indonesia. Although some species of seaweed have been used for direct consumption or as source of active compounds, species from chlorophytes (green seaweed) are considered underutilize. Seaweeds from the Chlorophyta species have not been used optimally by the community.

Green seaweed and brown seaweed produce various bioactive compounds such as antibacterial compound, antioxidant compound, and can be used as sources of pigment (food coloring/dye) [3-13]. The utilization of seaweed as a source of antioxidants has been previously evaluated in cosmetics [14-20]. Seaweed can also be utilized as salt source, which can be used for hypertensive patients [21]. Antioxidants can improve health and play a role in preventing the emergence of chronic diseases, it can act in preventing cell damage [22]. The potential of seaweed antioxidants can be applied in
various fields, especially in the pharmaceutical industry. There were 739,820 cases of hypertension from low to high risk categories [23]. The increased of hypertension cases was caused by various factors including the result from lifestyle changing and consumption patterns of people who prefer fast food. Fast food contains high saturated fat, salt and sugar and low in dietary fiber as well as contains preservatives, coloring and flavoring [24]. Excess salt consumption is known as a high risk factor for people with hypertension. Salt has an important role for the human body but it can be dangerous if it is consumed excessively. Reducing sodium salt intake can reduce blood pressure by 2–8 mmHg [25]. There have been a number of low sodium salt products (<95% NaCl) which are claimed to help keep hypertensive patients’ blood pressure in normal condition. Sodium ions in salt can result in water retention increasing blood volume and peripheral vascular resistance. Seaweed has antioxidant activities in various forms such as vitamins and minerals that can counter free radicals and reduce cell and tissue damage. Red and brown seaweed extract can act as a natural ACE inhibitor, inhibit oxidation of LDL, and a source of α-amylase and α-glucosidase [26, 27]. Salt production from seaweed is one way to use natural ingredients that have antioxidant activity, mineral and low in sodium content. Information on seaweed salt from C. lentillifera and H. opuntia production for hypertensive patients is still very limited, thus research is needed to determine the characteristics and antioxidant activity of seaweed salt as an alternative salt for hypertensive patients.

2. Materials and Methods

2.1. Seaweed preparation
Green seaweeds (C. lentillifera and H. opuntia) were obtained from Pramuka Island Kepulauan Seribu. Both C. lentillifera and H. opuntia were harvested in September 2016. Seaweed samples were identified in the Marine Hydrobiology Laboratory, Department of Marine Science and Technology IPB University. Seaweed samples were cleaned from carried over sand and debris. Samples were further washed using sea water, stored in a container and dried for 3-5 days.

2.2. Seaweed salt production
Production of the seaweed salt followed previously published method [28]. In brief, the dry seaweeds were grinded with a blender and were sifted using 30-mesh sieve. Subsequently, the seaweed juices were mixed with water (1:10) and heated at various temperatures (40°C, 55°C and 70°C) and different duration times (10 and 30 minutes). During heating process, seaweed juices were constantly stirred. The seaweed mixtures were then filtered using a 500 micron mesh cloth and filter paper number 4 and dried at 60°C for 24 hours.

2.3. Yield measurement
Yield was a percentage of the ratio of the final weight to initial weight of seaweed before undergoing treatment [29]. Yield of the seaweed was calculated using the equation (1):

\[
yield = \frac{\text{final weight}}{\text{start weight}}
\]

2.4. Antioxidant activity analysis using Cuprac and FRAP method
The FRAP assay was carried out according to the previously described method [30] with some modifications. FRAP work solutions were initiated by mixing 300 mM acetate buffer (pH 3.6), 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) (Sigma-Aldrich) in 40 mM HCl and 20 mM FeCl3. 6H2O (10:1:1). A 25 µL sample solution was mixed with 3 ml of FRAP reagent, and incubated for 30 minutes at 37°C. The absorbance was then measured at 595 nm. The absorbance values were converted into antioxidant activity and expressed in µmol trolox/g. The FeSO47H2O was used as standard for the assay.

The CUPRAC test was carried out following previously published method [31]. Salt extract 0.4 mL which has been dissolved in ethanol 99.9% was mixed with 1 mL of CuCl2.2H2O 0.01 M; 1 mL ethanolic neukoprin 0.0075 M; 1 mL of ammonium acetate buffer pH 7.1 M and 0.7 mL aquades. The
The mixture was subsequently incubated at room temperature in dark conditions for 30 minutes and the absorbance was measured at 450 nm. The absorbance value is expressed in the μmol trolox/g.

2.5. Seaweed mineral content analysis
The Analysis was started with sample preparation based on the following method [29]. Ten grams of the sample was inserted into erlenmeyer and was added with 5 mL HNO₃. The mixture was heated on a heat plate with temperature of 120°C for four hours. Additional acid H₂SO₄ 0.4 mL was added and heated until the solution was more concentrated. Subsequently, 2-3 drops of mixed solution HCl and HNO₃ was added and the sample was kept heated until the color changed from brown to dark yellow and turned into light yellow. After cooled down, 2 mL of distilled water and 0.6 mL of HCl were added. Demineralized water was added until the final volume was 100 mL. The mineral content was further analyzed using Atomic Absorption Spectrophotometer (AAS).

2.6. NaCl content analysis
The analysis of NaCl followed the previously described method [32]. The analysis was carried out by putting the sample into furnace until turning into ashes. Two hundred fifty mg of samples were washed with 10 mL of distilled water and were transferred to 250 mL Erlenmeyer flask. Three mL of 5% potassium chromate (K₂CrO₄) solution were then added and the mixture was then titrated with silver nitrite (AgNO₃) 0.1 M. The endpoint of the titration was reached when the orange color arises. NaCl level was calculated by the following formula:

\[
\text{salt (NaCl) (\%)} = \frac{(T \times M \times 58.4)}{W \text{ (mg)}} \times 100\%
\]

T = Standard solvent volume AgNO₃ 0.1 M
M = Molarity of silver nitrite
W = Sample weight

2.7. Statistical analysis
The study used factorial complete randomized design using analysis of variants (ANOVA) at 95% credence interval for data analysis. The treatment that affected the response was further analyzed with the Duncan test.

3. Results and Discussion

3.1. Raw material characterization
The results of identification through morphological observations were carried out at the Marine botanical laboratory, Research Center for Oceanography-LIPI and Laboratory of Marine Hydrobiology at the Department of Marine Science and Technology IPB. Identification shown that the seaweeds used for this research were C. lentillifera and H. oponentia, both were phylum Chlorophyta and class Caulerpaceae and Halimedaceae, respectively.

3.2. Yield and seaweed salt mineral
The analysis of the yield and mineral content on the seaweed salts were aimed to compare the effect of the treatment given. The yield and mineral analysis results were presented in table 1. Table 1 shows that the types of seaweed, time and temperature did not give significant effects at p <0.05 on the yield but gave a significant effect on the Na:K and NaCl ratios. The yield of both seaweed salts ranged from 20.13%-25.67%. The treatment of seaweed variation, time and temperature did not affect the yield because all seaweeds used as the raw material for salt production were purified and blended until reaching homogenous size, and similar amount of the seaweeds was used for all treatments to have the salt produced. It was suspected that the solubility of sodium and chloride which were the main structure of salt from both seaweed were the same, resulting in the yields that were not significantly different.
Table 1. Yield and mineral of the seaweed salt.

| Sample    | Temperature (°C) | Time (Minutes) | Yield (%) | Na:K     | NaCl (%) |
|-----------|------------------|----------------|-----------|----------|----------|
|           |                  |                |           |          |          |
| C. lentillifera | 40               | 10             | 24.67±1.15| 14.65e   | 59.36±0.98c |
|           |                  | 30             | 24.22±1.17| 13.78f   | 55.35±0.04f  |
|           | 55               | 10             | 24.78±1.64| 19.95e   | 56.46±1.18e  |
|           |                  | 30             | 25.67±1.86| 7.50i    | 58.05±0.26d  |
|           | 70               | 10             | 22.94±1.73| 19.08b   | 61.85±0.26a  |
|           |                  | 30             | 23.22±1.20| 17.87c   | 60.78±0.50b  |
| H. opuntia | 40               | 10             | 20.13±0.61| 9.45f    | 54.23±0.30g  |
|           |                  | 30             | 21.33±1.89| 9.15g    | 50.66±0.25i  |
|           | 55               | 10             | 23.20±1.20| 7.94h    | 49.68±0.22j  |
|           |                  | 30             | 23.47±1.89| 7.59i    | 45.21±0.02k  |
|           | 70               | 10             | 23.07±1.29| 7.29j    | 55.94±0.37ef |
|           |                  | 30             | 22.40±1.74| 7.12k    | 51.93±0.14h  |

* The numbers in the same column followed by the same letters are not significantly different at the 5% test level (Duncan multiple range test)

Duncan multiple range test results showed that the interaction between seaweed species, time and temperature had a significant effect (p <0.05) on the Na: K ratio. The Na:K ratio from the three seaweed salts ranged from 7.12 to 19.95 g/kg. The Na: K ratio is important for controlling blood pressure and control the excess of fluid that contains abundant number of K on hypertensive patients. Potassium increases cell growth and helps keep blood pressure normal while sodium could be used to maintain the balance of osmotic fluid and acid base [33].

Seaweed types, time and temperature had also a significant effects (p <0.05) on the resulting NaCl level. NaCl levels of seaweed salts ranged from 45.21 to 61.85%. The difference of NaCl levels could be related to different types of seaweed resulting in different amounts of NaCl from each type while temperature and time could also optimize the extraction of NaCl from seaweed. NaCl levels in from H. opuntia decreased with the increasing temperature but the other way around with C. lentillifera. The increased amount of NaCl levels extracted from C. lentillifera could be caused by the high ability of distilled water to extract Na. All components in seaweed have the ability to dissolve in water with different portions [34]. The water volume used for extraction at a certain temperature was expected to improve the quality of the extract because the heat can accelerate the transfer between molecules. There was a 22-fold increased of Na in U. ohnoi and U. leachate leaching which used as raw materials for the manufacture of seaweed salt [28].

3.3. Antioxidant activity on seaweed salt

Antioxidant activity analysis in seaweed salts was carried out using three methods that have different working procedures. The used of these three methods was aimed to detect the activity of seaweed salts in trapping the free radicals, in order to investigate the other potential advantages when hypertensive patients are using the salts, besides of their low NaCl levels. The test results of antioxidant activity were presented in table 2.

The interaction between seaweed species (C. lentillifera and H. opuntia), heating time (10 and 30 minutes), and temperature (40 °C, 55 °C and 70°C) had significant effects on the FRAP antioxidant activity (p <0.05). The best antioxidant activity was found in the salt extracted from C. lentillifera treated at 40°C for 30 minutes which categorized as a strong antioxidant. A compound is categorized into a strong antioxidant compound when their antioxidant activity is 100–500 µmol Fe2+/g [35]. Bioactive compounds from seaweed are phenol and flavonoid groups similar to those found in high plants [36]. Active components and antioxidants are compounds that has a role in reducing hypertension [26]. The interaction between seaweed types (C. lentillifera and H. opuntia), times (10 and 30 minutes) and temperature (40 °C, 55 °C and 70°C) also had significant effects on the CUPRAC
antioxidant activity method (p <0.05). The best antioxidant activity was found in the salt extracted from *H. opuntia* treated at 40°C for 30 minutes.

Table 2. Antioxidant activity of the seaweed salts.

| Source       | Temperature (°C) | Time (minute) | FRAP (µmol Fe²/g) | CUPRAC (µmol trolos/g) |
|--------------|------------------|---------------|-------------------|------------------------|
| *C. lentillifera* | 40               | 10            | 109.00±0.00<sup>a</sup> | 19.02±0.229<sup>g</sup> |
|              |                  | 30            | 138.75±0.00<sup>a</sup> | 18.69±0.270<sup>g</sup> |
|              |                  | 70            | 129.75±0.00<sup>b</sup> | 17.74±0.229<sup>h</sup> |
|              | 55               | 10            | 128.50±0.00<sup>c</sup> | 17.07±0.071<sup>i</sup> |
|              |                  | 30            | 122.25±0.75<sup>ef</sup> | 14.45±0.164<sup>j</sup> |
| *H. opuntia*  | 40               | 10            | 127.00±0.00<sup>d</sup> | 22.12±0.164<sup>f</sup> |
|              |                  | 30            | 109.75±0.50<sup>e</sup> | 47.64±0.327<sup>d</sup> |
|              |                  | 70            | 123.13±0.13<sup>c</sup> | 58.14±0.142<sup>a</sup> |
|              | 55               | 10            | 121.50±0.00<sup>f</sup> | 48.02±0.322<sup>d</sup> |
|              |                  | 30            | 120.25±0.75<sup>e</sup> | 52.67±0.109<sup>c</sup> |
|              | 70               | 10            | 120.50±0.01<sup>g</sup> | 55.71±0.397<sup>b</sup> |
|              |                  | 30            | 118.63±0.38<sup>h</sup> | 36.05±0.109<sup>e</sup> |

* The numbers in the same column followed by the same letters are not significantly different at the 5% test level (Duncan multiple range test)

4. Conclusion

The interaction between seaweed types, time and temperature did not have a significant effect against the yield of seaweed salts (p <0.05) but significantly affected the Na: K ratio and NaCl levels. The best antioxidant activity based on FRAP method was found from salt extracted from *C. lentillifera* heated at 40°C for 30 minutes, while based on CUPRAC method was from *H. opuntia* heated at 40°C for 30 minutes.

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