Mineral content, heavy metals and amino acid profiles of Halimeda opuntia seaweed from several waters in Indonesia

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Abstract. Halimeda opuntia is a type of calcareous seaweed which is including in the Chlorophyta division. In Indonesia, this type of seaweed is available abundantly, but the market value and the benefits are still very low. This research aims to study the characteristics of H. opuntia seaweed especially to assess the mineral content, heavy metals, and amino acid profiles from several waters in Indonesia. Sampling was carried out in four waters locations in Indonesia, namely the Binuangeun waters, Banten; South Lampung; East Lombok, NTB; and South Konawe, Southeast Sulawesi. Mineral analysis showed that H. opuntia contained K minerals (16.50 - 92.40 mg/100g), Ca (18.60 - 24.37 g/100g), Mg (35.20 - 510.50 mg/100g), Fe (30.34 - 470.00 mg/kg), Zn (2.13 - 5.43 mg/kg), Mn (3.60 - 23.45 mg/kg), and P (90.40 - 142.00 mg/kg). Heavy metals analysis showed that Lead (Pb) content in H. opuntia seaweed from four water locations was still low <0.04 mg/kg so that Cadmium (Cd) <0.05 mg/kg. Based on the analysis of amino acids, there were 12 amino acids detected, namely Alanine, Glycine, Valine, Leucine, Isoleucine, Proline, Aspartic Acid, Phenylalanine, Glutamine, Lysine, and Tyrosine. Based on mineral content, heavy metals and amino acid profiles, the direction of utilization of this type of seaweed is pharmaceutical ingredients, fertilizers, and feed.

1. Introduction

Indonesian waters are overgrown with various types of seaweed that have benefits both as ingredients for agar, carrageenan, and alginate. However, there is still seaweed that has not been used, including Halimeda opuntia. This seaweed generally thrives in tropical waters, including Indonesia. H. opuntia has thallus in the form of small sheets with a rough, stiff, greenish-white surface, and shaped like a branched kidney [1]. In this thallus found extracellular aragonite deposits containing mostly CaCO₃, MgCO₃ 1% and SrCO₃ 1.3%. This high carbonate level makes H. opuntia referred to as the largest carbonate contributor in the ocean [2, 3].

Research of amino acid profile of H. opuntia seaweed is still very limited. The research still limited to bioactive compounds of H. opuntia and their biological activities. Yanuhar and Wahyuningtyas (2017) [4] reported that the crude protein of Halimeda can produce an adaptive immune response. Another research from Ahmad et al. (2014) [5] shows that the protein fraction from Halimeda macrobola can be a good alternative antioxidant because it has high antioxidant activity.

Time harvesting, tall and morphological of thallus, depth, occurrence in water, and geochemical condition of environment affect mineral composition in seaweed [6]. In 2006, Santoso et al.[7] have reported the mineral content of 9 species of seaweed from Indonesia, include Halimeda macroloba. Its
concluded that seaweed from Indonesia was rich in macrominerals while micronutrients were low. This content and profile are almost similar to seaweed from Japan and Spain [7].

This research was conducted to study the characteristics of Halimeda opuntia seaweed from four different Indonesian waters locations. The research results obtained are expected to be used to determine the potential for the development of its use as food and non-food.

2. Materials and methods

2.1. Sampling

Seaweed was taken from four water locations in Indonesia, namely 1). South Lampung (S5°52,637'; E105°25,520'); 2). Binuang, Banten (S6°50,708'; E105°52,815'); 3). East Lombok, NTB (S8°55,048'; E116°31,752'), and 4). South Konawe, Southeast Sulawesi (S4°25,845'; E112°48,732'). Halimeda opuntia seaweed obtained from the sampling location was sorted and washed using seawater, then put in a plastic bag. Furthermore, seaweed was stored in a coolbox that fills with ice, then taken to the laboratory of the Research and Development Centre for Marine and Fisheries Product Processing and Biotechnology. While in the field, ice was always replaced to maintain the freshness of seaweed. While in the laboratory, seaweed was stored in cold storage before analysis.

2.2. Mineral and heavy metal content analysis of Halimeda opuntia

Analysis of Halimeda opuntia seaweed (K, Ca, Mg) seaweed was carried out regarding AOAC-985.35/40.1.14-2005 [8]; minerals (Fe, Zn, Mn) referring to SNI-01-2896.5-1998 [9]. Phosphorus (P) minerals using spectrophotometric methods. Analysis of heavy metals (Pb and Cd) was carried out regarding AOAC-999.11/9.1.09-2005 [8].

2.3. Amino acid profile analysis of Halimeda opuntia

Analysis of the amino acid profile was carried out by following the procedure from EZ: Phenomenex amino acid testing kit. Halimeda opuntia seaweed was dried using a freeze dryer, and then hydrolysis was carried out according to the method i.e., a sample of 20 mg in a bottle was added with 1 ml of HCl 6 N, then heated for 60 minutes at 180°C in the Microwave. The next step was 100 µl of the hydrolysis sample prepared using the EZ-Faasttm GC-FID testing kit hydrolyzed amino acids analysis kit - Phenomenex (KG0-7167). The amino acid composition of the sample was reading using Gas Chromatography - Flame Ionization Detector (GC-FID), and the standard used was essential and non-essential amino acids from Phenomenex (AG0-7184) [10].

3. Results and discussions

3.1. Mineral content

The minerals observed in Halimeda opuntia seaweed include Potassium (K), Calcium (Ca), Magnesium (Mg), Iron (Fe), Zinc (Zn), Manganese (Mn) and Phosphorus (P).

| Mineral | Sampling location of Halimeda opuntia |
|---------|--------------------------------------|
|         | Lampung | Binuang, | Kendari | Lombok |
| K (mg/100 g) | 16.50±0.14 | 41.80±0.57 | 92.40±1.70 | 31.35±0.64 |
| Ca (mg/100 g) | 22880.00±0.00 | 18601.50±41.72 | 19078.00±165.46 | 24367.50±62.93 |
| Mg (mg/100 g) | 35.20±0.28 | 510.50±0.71 | 42.70±1.13 | 126.00±1.41 |
| Fe (mg/kg) | 30.35±0.35 | 470.00±0.00 | 316.50±2.12 | 148.50±0.71 |
| Zn (mg/kg) | 3.68±0.03 | 2.13±0.02 | 5.43±0.11 | 3.19±0.01 |
| Mn (mg/kg) | 23.45±0.21 | 8.56±0.00 | 7.33±0.01 | 3.60±0.15 |
| P (mg/kg) | 142.00±0.00 | 125.50±4.95 | 110.00±0.00 | 90.40±0.14 |
From the seven types of minerals observed, calcium has a much higher level than other minerals, which is 18.6 - 24.4 g / 100g dry weight (table 1). Calcium levels are greater than the calcium levels of seaweed in general, as reported by Dharmananda (2002) [11] that seaweed calcium levels, in general, are about 4-7% of the dry weight or about 4-7 g / 100g dry weight. Based on the average calcium levels, Halimeda opuntia is a source of calcium. High levels of calcium parallel with high levels of ash in Lombok waters. According to Venugopal (2010) [12] different types of minerals depend on the habitat of each seaweed. The amount of variation in the number of minerals and organic components on the bottom of the water and the nature of the depth of the water, the distance from the soil and the environment affect the number of minerals present in seaweed. Barton (1928) in [13] reported Halimeda opuntia contained 90.16% calcium carbonate, 0.54% calcium sulfate and silica, and important organic matter 3.80%.

3.2. Heavy metal contamination
Metal contamination observed in H. opuntia from the four locations was only Lead (Pb) and Cadmium (Cd) (table 2). The results of the analysis showed that the levels of Pb and Cd were within safe limits according to SNI 2690: 2015, namely Cd of 0.1 mg/kg and Pb of 0.3 mg/kg [14] Types of heavy metals which are compounds that endanger health include mercury (Hg), lead (Pb), cadmium (Cd), Arsenic (As), chromium (Cr), nickel (Ni). The sequential levels of heavy metal toxicity are Hg, Cd, Pb, As, Cu and Zn respectively.

Table 2. Levels of heavy metal contamination in H. opuntia seaweed

| Levels of heavy metal | unit | Lampung | Binuageun | Lombok | Konawe |
|----------------------|-----|---------|-----------|--------|--------|
| Lead (Pb)            | mg/kg | < 0.04  | < 0.04    | < 0.04 | < 0.04 |
| Cadmium (Cd)         | mg/kg | < 0.05  | < 0.05    | < 0.05 | < 0.05 |

3.3. Amino acid profile
In this study, 12 amino acids were identified, including six essential amino acids (Valine, Leucine, Isoleucine, Phenylalanine, Lysine, Tyrosine) and six non-essential amino acids (Alanine, Glycine, Proline, Aspartic Acid, Glutamine) (table 3). The two highest amino acids in this study are phenylalanine and leucine. Amino acids play an important role because they help the formation of protein as a basic ingredient in the formation of cells, muscles, and the immune system. Based on the results of proximate analysis, H. opuntia taken from various territorial waters of Indonesia is known to contain proteins composed of amino acids.

Phenylalanine is an essential amino acid contained in foods such as breast milk, fish, cheese, cottage, peanuts, lentils, sesame seeds, meat, and poultry. Humans can metabolize phenylalanine but some people do not have the ability to metabolize phenylalanine or phenylketonuria phenylalanine which accumulates in the body, is converted to phenylpyruvate, excess phenylpyruvate can cause seizures, brain damage, and mental retardation. Supplements with phenylalanine are considered to have antidepressant effects because structurally, phenylalanine is closely related to dopamine, epinephrine (adrenaline), and tyrosine [15]. While Leucine helps reduce the breakdown of muscle protein [16].

Table 3. Amino acid profiles of Halimeda opuntia in four waters in Indonesia

| Types of amino acids | Concentration (mg/g) |
|----------------------|----------------------|
|                      | Lampung  | Binuangeun | Lombok  | Konawe  |
| Alanin               | 0.14     | 0.07       | 0.08    | 0.08    |
| Glisin               | 0.11     | 0.05       | 0.07    | 0.07    |
### 3.4. Potential utilization of *H. opuntia*

The results of the analysis carried out above can be used as a basis for determining the potential use of *H. opuntia*. Plants need enough macro and micronutrients to grow optimally. Macronutrient elements include N, P, K, Mg, Ca, and S. While microelements include B, Cu, Zn, Fe, Mo, Mn, Na, Cl, Si, and Ni. Based on the analysis results, *H. opuntia* contains relatively high K, Ca, Mg, and the presence of other minerals needed by plants such as Fe, Zn, Mn, and P. Another possible use of *H. opuntia* is as feed ingredients. Ca and P minerals play a role in the body of laying hens as constituents of the body's skeleton (bone) and eggshell. Based on SNI for chicken feed, the requirements for calcium and phosphorus are respectively 3.25-4.25% and 0.6-1% [17]. Thus, *H. opuntia* can function as a source of Ca, and P. Utilization as feed has also been observed by Subagiyo [18] who reported that Halimeda sp. can be used as functional feed on shrimp which functions to modulate non-specific defense systems. The amino acid content of *H. opuntia* can increase the nutritional value of feed.

One of the basic ingredients of toothpaste is a scrubber / abrasive, which works to clean teeth and scrub the surface of teeth. The abrasive material used must have a maximum cleaning effect with minimum abrasion — generally more than 50% of the toothpaste content. Materials commonly used as an abrasive are calcium carbonate, tricalcium phosphate, calcium sulfate, calcium pyrophosphate, insoluble sodium metaphosphate, and anhydrous alumina. Calcium carbonate (CaCO₃) is a water-soluble scrubber. Abrasiveness and the ability to clean from abrasive materials is determining by the size, shape, and hardness of the material. *Halimeda opuntia* seaweed contains high calcium carbonate, which is around 90.16% [19-21]. This potency can use as a basic ingredient in toothpaste formula replacing calcium carbonate from other sources. Sources of calcium carbonate include rocks, mollusc shells.

Calcium deficiency can cause several diseases including osteoporosis and periodontal [22], diabetes [23], cancer [24]. A good source of natural calcium comes from milk, but Indonesian people's milk consumption is still low at 14 g/day or with calcium contribution of 20 mg/day [25]. Another source of calcium that consumed today is in the form of supplements. Increased intake of calcium in food is safer than supplements because indigestion high calcium concentrations will suppress bone remodeling [26]. Therefore, calcium fortification in food products is a very appropriate choice. *Halimeda opuntia* seaweed has the potential as a source of natural calcium that can use for fortification.

### 4. Conclusion

*H. opuntia* contains relatively high K, Ca, Mg, and the presence of other minerals needed by plants such as Fe, Zn, Mn, and P. Heavy metals analysis showed that Lead (Pb) content in *H. opuntia* seaweed from four water locations was still low <0.04 mg/kg so that Cadmium (Cd) <0.05 mg/kg. The content of heavy metals (Pb and Cd) is still below the threshold allowed by BSN. Based on the analysis of amino acids, there were 12 amino acids detected, namely Alanine, Glycine, Valine, Leucine, Isoleucine, Proline, Aspartic Acid, Phenylalanine, Glutamine, Lysine, and Tyrosine. The highest amino acid content in several regions in Indonesia is dominated by Phenylalanine, Leucine,
and acid aspartate, with the highest amino acid composition derived from Lampung waters. Based on mineral and amino acid content, *Halimeda opuntia* seaweed can be used as an alternative food ingredient, while in terms of Ca mineral content can be used as an alternative cosmetic ingredient (toothpaste as a source of lime/CaCO₃).

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