Comparative Study on the Amino Acid Profile of Eucheuma Cottonni and Gracilaria Sp. Seaweed

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Abstract. Seaweed is a macro algae that lives in the sea having no roots, stems and leaves. Generally, seaweed contains carbohydrates (sugars or vegetable-gum), less fat, vitamins A, B1, B2, B6, B12, and C, beta-carotene and minerals, such as potassium, calcium, phosphorus, sodium, iron, iodine, protein, and amino acids. This study aimed to find out the amount of amino acids contained in Eucheumacottonii and Gracilariasp seaweeds and see if there are differences in amino acid levels in this samples taken from Silampayang Parigi Moutong District. Type of samples taken were Eucheumacottonii and Gracilariasp seaweed of approximately 5 meters from the beach. The analysis of amino acids used High Performance Liquid Chromatography (HPLC) and Ultra Performance Liquid Chromatography (UPLC).The test results showed that the Eucheumacottonii seaweed obtained 17 types of amino acids with the highest levels was from serine by 6.99 mg/g, while the lowest is tryptophan of 0.59 mg/g. Mean while,Gracilariasp obtained 16 amino acids types with the highest level was from glutamic acid of 2.46 mg/g and the lowest was from methionine of 0.39 mg/g. Both Eucheumacottonii and Gracilariasp seaweeds have significant differences of amino acids levels with P value <0.05.

Keywords : Seaweed, Eucheumacottonii, Gracilariasp

1. Introduction

Seaweed is a low-level plants that cannot be distinguished between the roots, stems and leaves. All parts of the plant are called thallus. Overall, these plants have similar morphologies, although they are actually different . Seaweed is a commodity that is widely recognized by the world. The growth of worldwide demand increases in line with in the use of seaweed for various purposes, among others in the field of industry, food, textile, paper, paint cosmetics and pharmaceuticals (drugs). In Indonesia, the use of seaweed for the industry starts

from gelatin industrial and alginates carrageenan industrial (Ministry of Commerce, 2013). In the pharmaceutical industry, gelatin is also used as raw material for wrapping drug capsules and vitamins, as well as a mixture of laxatives and toothpaste.

According to the Ministry of Maritime Affairs and Fisheries [15], Palu City, every year, seaweed production increases rapidly.,iIn 2015, the total production of Eucheumacottonii and Gracilariasp wet seaweeds in Central Sulawesi was 1,015,231.2 tons consisting of Eucheumacottonii by 894 835,2 tons and Gracilariasp by 120.395 tons. Generally, seaweed contains carbohydrates (sugars or vegetable-gum), less fat, vitamins A, B1, B2, B6, B12,C, beta-carotene and minerals, such as potassium, calcium, phosphorus, sodium, iron, iodine, protein and amino acids.

The content of amino acids, vitamins and minerals of seaweed reaches 10-20 times more than the other plants [1]. Generally, the amino acids contained in Eucheuma are acid aspartate, acid glutamate, serine, glycine, alanine, cysteine, proline, tyrosine, histidine, arginine, threonine, valine, methionine, isoleucine, leucine, fenilanin and lysine [2].

In study conducted by Famelia R [3], protein and amino acids can also be used as a dietary supplement to improve physical performance,in which the amino acid supplementation consumed immediately after endurance strength exercise and is particularly useful to increase muscle mass which is marked bythe increased diameter and the number of muscle fiber myofibril, in addition to improve the muscle endurance, although not significantly. The effect is also seen in the improvement of liver and kidney tissue structure. Amino acid supplements also affect some blood parameters significantly, namely an increase in the quality of erythrocytes and blood hemoglobin levels.

Based on the description above in mind, it is known that in any kind of seaweed contains various types and levels of amino acids. It is caused by several environmental factors that can affect the growth of seaweed such as temperature, light, sanitation, water movement (current) and the pH of the water[2] . Until now has not found the publication of a comparative study of the amino acid profile of seaweed EucheumaCottonii and Gracilariasp in the village of the Silampayang Villages ParigiMoutong district.

2. Materials and Method

2.1 Materials

The materials used in this study were 1 kg of each Eucheu macottonii and Gracilariasp seaweed taken from Silampayang Village, Kasimbar Sub-District, ParigiMoutong District, CentralSualwesi. Other materials used were hydrochloric acid (HCl) 6N, hydrochloric acid (HCl) 0.1N, hydrochloric acid (HCl (p)), aquabidest, AABA solution, AccQ-Fluorine borate, reagent borate A, sodium hydroxide (NaOH), 0.2 M sodium citrate buffer solution, 0.20 µm filter, standard amino acid solution, L-tryptophan standard solution and filter paper.

2.2 Sampling

Types of samples taken were seaweed Eucheumacottonii and Gracilariasp seaweeds from SilampayangVillage, Parigi Moutong District. Sampling was done in the morning, by taking directly in captivity within approximately 5 meters from the beach. Age of the sample used was 1.5 - 3 days (45-50 days). Sample handling aiming to maintain the quality of the sample removal was done by placing seaweed in a container that contains sea water habitat.

2.3 Amino Acid Analysis Using UPCL Tools

a. Sample solution

The sample was weighed as much as 0.1 grams and added 5 ml of 6N HCl then divortex thereafter, hydrolyzed for 22 hours at a temperature of 1100C and chill. After that, it was moved to a 50 ml flask and added by aquabidest to mark boundaries and then filtered with a filter of 0.20 lm. Subsequently, pipetteof 500 mL was filtrated and addedby 40 mL of aquabidest AABA solution and add approximately 460 mL. Then, a solution of 10 mL pipette and added AccQ-Flour 70 mL Borate in the vortex. Furthermore, added more flour reagent A divortex as much as 20 mL and then allowed to be leftfor 1 minute. Furthermore, incubated for 10 min at 55^{oC} and then injectedon UPLC system (SIG, 2018).

b. Standard Solution/Raw Solution

Pipette40 µl standard mix amino acids then added by 40 µl of internal AABA standard continued by an addition of 920 µl of aquabidest and then homogenized. Take 10 µl of standard, add 70µl of AccQ-Flour Borate thanvortex. Next,added another 20 µl of reagent flour A after being vortexed and left for 1 minute then incubated for 10 minutes at 55°C and injected into the UPLC system [14].

2.4 Tryptophan Analysis Using an HPCL Tool

a. Preparation of sample solutions

The sample was weighed approximately of 0.1 gram and added to the tube with a lid and added 10 ml of 4.2 M NaOH solution and homogenized it in the vortex. Then, the sample was hydrolized in the oven at 110°C for 22 hours. Furthermore, cooled the hydrolysis sample at room temperature, and transferredthe hydrolysis into 50 ml beaker. Next, rinse the tube with 1 ml of 0.2 M sodium citrate buffer solution, pH 4.25. Then, hold the rinse into a beaker. The rinsing stage was carried out in three repetitions. After that, neutralized the solution with 3.5 ml HCl (p) then shaked it. The pH of the solution with HCl or NaOH

was adjusted until it reached pH 4.25 approximately 0.05. Next,the sample solution was movedinto a 50 ml volumetric flask and dilutedwith the aquabidest until the mark was homogenized. Then,the sample solution was moved into a centrifuge tube, then centrifuge it at 14000 rpm for three minutes. After that strain the supernatant was filtered with filter paper and the filterate was filtered again with RC 0.45 µm membrane filter and lastinjected into the HPLC system [14] .

b. Standard solution

Pipette of infusion fluid sample (pipetting adapted to the content of L-Tryptophan in the sample) then put it into a 100 ml volumetric flask then dissolved and diluted it to the boundary mark. Furthermore, filteredwith a 0.45 µm RC filter membrane and then injected into the HPLC system[4]

2.5 Data Analysis

The content of amino acids in the material can be calculated by the formula below:

$$Amino\ Acid\ Levels\left(\frac{mg}{kg}\right) = \frac{Rasioanalit\ sampelx\left(C.standar\frac{pmol}{1000000000}\right)xBMxfpx\ 100}{Rasio\ analit\ standar\ x\ bsample\ weights\ (g)}$$

Amino Acid levels (mg/g) = *Amino Acid levels* (mg/kg)/1000

Note:Ratio = Area analyte / total area AABA width

Fp = volume 1 (µl) / pipetting (µl) x volume 2 (µl)

Tryptophan Levels (mg/kg) = (area-intercept)/slope x final volume (ml) x fp

Weight (g)

3. Results and Discussion

In this seaweed sample testing, the results obtained were thatthere were18 kinds of amino acids contained in the seaweed. Amino acids weredivided into two types, which were essential and non-essentialamino acids. The results can be seen in Figures 1 and 2 and in Table 1.

a. Essential Amino Acids of *Eucheuma cottonni* and *Gracilaria sp*

Essential amino acids found in both types of seaweed can be seen on the Figure 1.

b. Non-essential Amino Acids Eucheuma cottonni and Gracilaria sp

Non-essential amino acid found in both types of seaweed can be seen on the Figure 2.

c. Statistical test results

The statistical test used in this study were unpaired T test, whichwas done because this research aimedto compare amino acid profiles of Eucheuma cottonii and Gracilaria sp. It was said that there were significant differences between the two samples if the P value below 0.05. If the value of P> 0.05, this means that there was no difference and if the value of p <0.05, this means that there was a significant difference between the two types of seaweed. Statistical test results can be seen in the table 1.

Amino acid is an organic component containing amino and carboxyl. The composition of the amino acid content can determine the quality of the protein. Proteins which contain all the essential amino acids in the amount the body needshas a high quality. If there is a deficiency of one or more essential amino acids,it means that this protein haslow quality [4]. Generally, seaweed contains carbohydrates (sugars or vegetable-gum), less fat, vitamins such as A, B1, B2, B6, B12, C, beta-carotene and minerals, such as potassium, calcium, phosphorus, sodium, iron, iodine, protein and amino acids [5].

In Indonesia, seaweed was used for the industry starting from gelatin, carrageenan and alginates. The main benefit of gelatin is as a stabilizer, emulsifier, thickener, purification and gel maker. Gelatin is most widely used as a hydrocolloid especially in the food, pharmaceutical and cosmetics [6]. In the field of pharmaceutical, gelatin can also be used as raw material for wrapping drug capsules and vitamins as well as a mixture of laxatives and toothpaste . The use of seaweed as carrageenan in the manufacture of products as a gelling agent or stabilizer, suspending and tekstus forming emulsions. Carrageenan is also used in the cosmetics industry, textile, paint, medicine and fodder. The alginate is also used in the pharmaceutical field as an emulsifier, stabilizer, and a suspending agent in the manufacture of tablets, capsules, patches, filter, as a laxative, while in the cosmetics industry alginate serves as an emulsifier in the manufacture of creams, lotions, shampoos and hair dye .Protein and amino acids are very beneficial as a dietary supplement to improve the performance of physical [3], stimulate the brain function, increase the level of muscle energy, help lower excessive blood sugar levels, maintain the health of the heart, soothe the tensed nerves, prevent buildup fat in the liver and arteries mainly supplying blood to the brain, etc [7] .

In this study,samples used were Eucheumacottonni and Gracilariasp seaweed taken from ParigiMoutong obtained approximately 5 meters from the beach and sampling was conducted in the morning. According to Anggadiredja et al [2[the age of seaweed harvested and well used is at the range of 1.5-2 months or 45-50 days after planting. When the harvest doneless than suchage, it will produce low-quality seaweed for agar or carrageenan content and low strength of the resulting gel.

This research was conducted with the analysis of the number of amino acids using High Performance Liquid Chomatography tool (HPLC) and Ultra Performance Liquid

Chomatography (UPLC), wherein the tool has advantages and disadvantages of each. Advantages of the HPLC method is the capability to resolution and higher speed, the HPLC column can be reused without repackaging, better control parameters affecting the separation efficiency and drawbacks, namely the size of the particles used ranged 3-10µ and injection volume 5µ . As for the UPLC method has the advantage of having a very high ability in terms of resolution, speed and sensitivity analysis [8]. UPLC also usesfine particles ofless than 2µ tosave time and reduce solvent consumption. However,the shortcomings of this method areeasily clogged if filtration column if eluent is not good and the sample must be thoroughly filtered. Therefore, UPLC method is more sensitive than the HPLC method [9]

In the analysis of the amino acid arginine, kind of serine, glycine, aspartate, glutamate, alanine, proline, cystine, tyrosine, histidine, threonine, lysine, methionine, valine, isoleucine, leucine and phenylalanine used UPLC instruments. Columns wereused in instrument UPLC is ACCQ-Tag Ultra C18, where ACCQ-Tag Ultra C18 (Oktadensil silica) was a stationary phase that is most widely used because it is able to separate compounds with a polar low, medium or high [10] and detectors used are PDA (Photodiode Array) detectors with 260 nm wavelength.This can be interpreted that the detector emits waves at 260 nm wavelength and captures fluorescence emissions emitted by the sample.

As for the analysis of compounds, it used Tryptophan HPLC instrument.Tryptophan included in the category of non-polar aromatic amino acid which is unstable so HPLC instrument was used. Columns used in HPLC instrument was Lichospher 25 cm x 4.0 mm, 5 mL wasstationary phase often used with reversed phase properties of C-18 and is suitable for the separation of neutral compounds, acids and damp weak and detectors used wasPDA (photodiode Array) with a wavelength of 280 nm.This may mean that the detectors emit at a

wavelength of 280 nm and emission capture fluorescence emitted by the sample. The advantages of PDA detectors (Photodiode Array), is that it iscapable of providing a collection of commatograms at different wavelengths at a single process [10].

Generally, the amino acid usually dissolved in water and insoluble in organic solvents nonpolar namely ether, acetone, and chloroform [11] because based on itschemical properties, amino acid is a weak base, so that prior to the analysis, hydrolysis was first performed aiming to break down polysaccharides into monosaccharides to produce free amino acids [4]. For the use of an internal standard solution or AABA (a-amino-N-butyric acid), it aimed as a volumetric error correction factor during sample preparation and corrects the loss of amino acid residues during the hydrolysis process which will be detected by reduced internal standards, so that the use of internal standard solutions can increase precision. Furthermore, the amino acids werefirst derivatized to form derivatives that can fluoresce, change the molecular structure or polarity of the analyte so that it will produce a better chromatogram peaks [11].

From the testing that has been done on the type of Eucheumacottoniiseaweed, it obtained 17 types of amino acids with the highest levels was fromserine by6.99 mg/g and the lowest was from tryptophan by 0.59 mg/g (Figure 1). For Gracilariaspseaweed sample, it gained as much as 16 amino acids with the highest levels of the amino acid was a glutamic acid by2.46 mg/g whilethe lowes was methionine by0.39 mg/g. From the results obtained, there are two types of amino acids which cannot be detected because they have cysteine LOD (Limit of detection) values of 48.42 mg/kg and histidine of 40.86 mg/kg due to their very small sensitivity that cannot be detected by HPLC or UPLC devices.

After the amino acid levels in each seaweed was obtained, statistical tests was then proceed. This test was performed in order to prove and obtain scientific data that there was a significant difference between the levels of Eucheumacottonii and Gracilariaspseaweed which can be seen in Figure1. Statistical tests conducted in this study was the unpaired t-test which found that there was significant differences between two types of seaweed if the P value < 0.05, this means that there are significant differences between the types of Eucheumacottonii and Gracilariaspseaweeds. As [13] has studied, Eucheumadenticilatum contains 19 types of amino acids with the highest levels was fromaspartate and asparagine by 2,791 nmol and the lowest was fromglycine by 0.105 nmol.

From the study results, it can be described that every type of seaweed growth, they have variousamino acid content. It is caused by several factors such as the environment that could affect the growth of seaweed and temperature factors, sanitation, light, water movement (currents), the pH of the water [12].

4. Conclusion

Based on the results of research and discussion, it can be concluded that:Eucheumacottoniisample obtained its highest levels of amino acids from serine by6.99 mg/g, while the lowest was tryptophan by 0.59 mg/g.Meanwhile,Gracilariasphashighest levels of amino acids fromglutamic acid by2.46 mg/g, while the lowest was methionine by0.39 mg/g.The results of the analysis of amino acid content of Eucheumacottoniiseaweed obtained that there were17 types of amino acids and Gracilariaspobtained 16 types of amino acids. T-test showed that there weresignificant differences between the two types of seaweed.

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