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Biochemical study of amino acid profile of *Kappaphycus alvarezii* and *Gracilaria salicornia* seaweeds from Gerupuk Waters, West Nusa Tenggara (NTB)

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Abstract

This study aimed to analyze the amino acid profile of *Kappaphycus alvarezii* and *Gracilaria salicornia* seaweeds from Gerupuk Waters, West Nusa Tenggara (NTB). Samples were collected in January using a transect method, then dried and ground to powder to determine the amino acid content. Amino acid analysis was performed using high-performance liquid chromatography (HPLC). An amino acid standard curve was used to calculate amino acid content of each sample. The results of the analysis showed that there were 15 amino acids present in *K. alvarezii* seaweed, consisting of seven essential amino acids and eight non-essential amino acids. Meanwhile, *G. salicornia* seaweed contained 13 amino acids, consisting of seven essential amino acids and six non-essential amino acids. In *K. alvarezii*, the amino acid with the highest level was glutamate (13.73%), while in *G. salicornia*, the amino acid with the highest level was serine (13.02%).The results of the nutritional evaluation indicate that lysine is a limiting amino acid in *K. alvarezii* seaweed and methionine is a limiting amino acid in *G. salicornia* seaweed.

Keywords: amino acid, *Gracilaria salicornia*, Gerupuk Waters, *Kappaphycus alvarezii*, seaweed

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INTRODUCTION

Seaweed is an important marine biological resource with high nutritional value, and thus it becomes one of the most promising plants of the future. With its unique biochemical structure and composition, seaweed is widely used in various industries, such as those engaged in the foods, energy, medicine and cosmetic sectors, as well as utilized for biotechnology (Komalavalli and Lalitha 2015, Norziah and Ching 2000, Ommee and Payap 2012). Most seaweed contains essential amino acids which are almost identical to the essential amino acids found in eggs (Norziah and Ching 2000). In addition, most seaweed contains quite a lot of aspartic acid, glutamic acid, and arginine in the composition of total amino acids (Rajasulochana *et al*. 2010). The varied nutritional composition of seaweed is affected by the species, habitat, and environmental conditions (Zawawi *et al*. 2014).

Kappaphycus alvarezii and *Gracilaria salicornia* are two species of seaweed that are in the class of Rhodophyceae, largely found in Gerupuk Waters, West Nusa Tenggara (NTB). *K. alvarezii* is a species of seaweed with a high economic value that has been widely cultivated as a source of carrageenan (Madhavarani and Ramanibai 2014). Meanwhile, *G. salicornia* has the potential to produce jelly which, due to its high chlorophyll content, has potential as antioxidant properties (Vijayavel and Martinez 2010, Vimala and Poonghuzhali 2015).

The results of Xiren and Aminah's (2017) study suggest that *K. alvarezii* originating from Langkawi and Sabah Waters, Malaysia contains 19 amino acids, with aspartic acid, glutamic acid, alanine, and leucine being the dominant amino acids. On another side, the information on the amino acid content of *Gracilaria sp.* genus suggests that the aspartic acid and glutamic acid of this species of seaweed are present in a dominant number (Fateminasab 2014, Kumar and Kaladharan 2007, Norziah and Ching 2000). This research provides limited information on the sea surrounding Malaysia. In fact, *K. alvarezii* and *G. salicornia* are widely spread in several marine areas in Southeast Asia, including Indonesia. Biochemical composition of seaweed is reported to be generally affected by geographical locations and conditions of the surrounding environment

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Table 1. Amino acid composition of *K. alvarezii* and *G. salicornia* seaweeds in Gerupuk Waters, NTB

No.	Amino acids	*K. alvarezii*		*G. salicornia*	
		mg/g	*(% b/b)*	*mg/g*	*(% b/b)*
	Essential amino acids				
1	Histidine	16.01 ± 0.36	1.01	22.92 ± 0.67	7.92
2	Threonine	24.90 ± 0.57	9.9	22.51 ± 0.63	7.51
3	Methionine	16.86 ± 0.67	1.86	16.11 ± 0.67	1.11
4	Valine	24.94 ± 059	9.94	22 ± 0.59	7
5	Phenylalanine	21.05 ± 0.15	6.05	17.94 ± 0.25	2.94
6	Isoleucine	21.37 ± 0.30	6.37	19.85 ± 0.42	4.85
7	Leucine	23.43 ± 0.04	8.43	21.60 ± 0.56	6.6
	Total EAA	148.55 ± 3.62	43.56	142.93 ± 2.57	37.93
	% of total AA	33.64	47.58	49.58	46.49
	Non-essential amino acids				
8	Aspartic acid	33.26 ± 0.09	12.26	ND	ND
9	Glutamic acid	116.73 ± 0.16	13.73	27.93 ± 0.11	11.93
10	Serine	26.82 ± 0.27	4.82	29.02 ± 0.12	13.02
11	Glycine	29.71 ± 0.31	7.71	21.85 ± 0.58	5.85
12	Arginine	24.04 ± 0.26	2.04	24.04 ± 0.30	2.34
13	Alanine	29.33 ± 0.17	7.33	25.11 ± 0.85	9.11
14	Tyrosine	18.06 ± 0.10	0.06	17.40 ± 0.21	1.4
15	Lysine	15.05 ± 0.01	0.05	ND	ND
	Total non-EAA	293 ± 32.94	48	145.53 ± 11.77	43.65
	% of total AA	66.36	52.42	50.42	53.51
	Total AA	441.55	91.56	288.28	81.58
	EAA/non-EAA	0.51	0.91	0.98	0.87
	EAA/total AA	0.34	0.48	0.50	0.46

*AA: amino acid; EAA: essential amino acid; Non-EAA: non-essential amino acid; ND: not determined

(Boyaci et al. 2014, Davooabadi and Aghajani 2013, Rohani 2012).

There is an abundance of *K. alvarezii* and *G. salicornia* in Gerupuk, NTB, but the utilization of this seaweed is limited due to limited knowledge of its nutritional value and limited human resources. Therefore, this research was intended to analyze the amino acid profile of *K. alvarezii* and *G. salicornia* seaweeds from Gerupuk Waters, West Nusa Tenggara (NTB), in order to provide information on the nutritional value of both species.

MATERIALS AND METHODS

Sampling Methods

Seaweed samples used in this research were *K. alvarezii*, obtained from Gerupuk Waters, NTB, at the ordinates of 8º55'3,46"S Lat. and 116º20'3,49" E Lon. and *G. Salicornia*, collected from the same waters at the ordinates of 8º55'1,4" S Lat. and 116º19'57,22 E Lon. Sampling of both seaweeds was conducted during the month of January. The collected sample was then identified in the Indonesian Science Center (LIPI), North Jakarta.

Samples of both seaweed species were collected in a fresh condition. Samples were placed inside plastic polybags, stored on ice, and placed in a cool box. The seaweed samples were immediately taken to the laboratory. In the laboratory, the seaweed samples were washed with clean water to remove dirt. After washing, samples were wiped with tissue paper to remove any excess water. The seaweed samples were then dried in an oven at 37 °C until reaching a constant weight. The dried samples were mashed using a blender and sieved to obtain the sample powder which was then stored in a refrigerator (Lima-Filho *et al*. 2002).

Analysis of Amino Acids

The amino acid analysis was performed using high-performance liquid chromatography (HPLC) schimadzu LC 10 A (colomn lichrospher 100 Rp-18 (sum). The eluent used was 50 mM Natrium asetat and sodium dibasic phospat : THF : Methanol (96 : 2 : 2), pH 6.8.

One gram each sample was put into the reaction tube, 4 ml of HCl 6 N was then added and the mixture refluxed for 24 hours at 110 °C. After that, the samples were cooled and neutralized with 6 N NaOH to pH 7, then filtered using 0.2 µm Whatman filter paper. As much as 10 µL of each sample was taken and 300 µL of OPA was added. The samples were then left for 1 minute for perfect derivation. After that, 20 µL of the sample was injected into the HPLC column for separation of the different amino acids. The standard amino acid solution used in HPLC analysis consisted of 20 types of standard amino acid. HPLC analysis performed twice for each sample. The amount of each amino acid contained in both seaweed samples was determined using a standard amino acid curve. Meanwhile, the amino acid scores were determined using the following formula:

$$\text{Amino Acid Score of the Sample} = \frac{\text{Essential Amino Acid Content of the Sample}}{\text{FAO / WHO Standard Amino Acid Pattern}}$$

Data Analysis

The amino acid data were analyzed descriptively and presented in the form of narration and table.

RESULTS

The results of the amino acid analysis of *K. alvarezii* and *G. salicornia* seaweeds in Gerupuk Waters, NTB, can be seen in **Table 1** and **Fig. 1**. Based on the results of the amino acid analysis using HPLC (**Table 1**), there were 15 amino acids in *K. alvarezii* seaweed, consisting

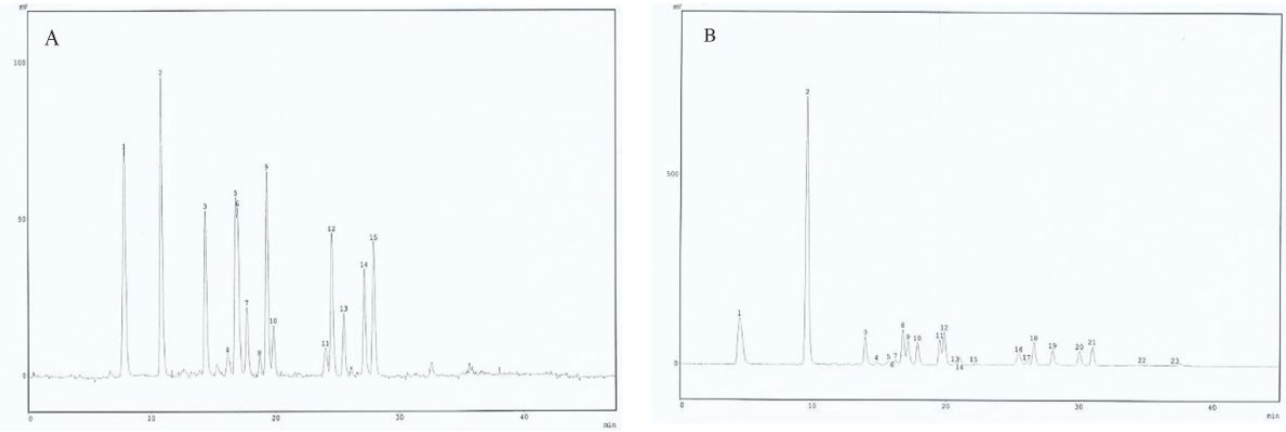

Fig. 1. Chromatograms of amino acids in seaweeds, (A) *G. Salicornia* and (B) *K. Alvarenzii*

Table 2. Essential amino acids (mg/g protein) in *K. alvarezii* and *G. salicornia* compared to the amino acid reference pattern recommended by FAO (1991)

Essential amino acids	***K. alvarezii***	***G. salicornia***	***FAO/WHO (1991)***
Isoleucine	22.75	17.32	28
Leucine	12.77	10	66
Lysine	0.08	ND*	58
Phenylalanine + Tyrosine	9.70	6.89	63
Methionine	7.44	4.44	25
Threonine	29.11	22.09	34
Valine	28.4	20.00	35

*ND, not determined

of seven essential amino acids and eight non-essential amino acids. Meanwhile, in *G. salicornia* seaweed, 13 amino acids were identified, consisting of seven essential amino acids and six non-essential amino acids. Furthermore, the percentages of the total essential amino acids contained in *K. alvarezii* and *G. salicornia* seaweeds were 47.58% and 46.49% of the total amino acids. Meanwhile, the percentages of the total non-essential animo acids contained in *K. alvarezii* and *G. salicornia* seaweeds were 52.42% and 53.51%, respectively.

After the amino acid composition of *K. alvarezii* and *G. salicornia* (**Table 1**) were determined, the next step was to evaluate the nutritional value by comparing the essential amino acid content of the samples with the amino acid reference pattern recommended by Food Agriculture Organization of United Nations (FAO) in 1991. This is particularly helpful in initial estimation of the nutrient content of seaweed protein (Kumar and Kaladharan 2007, Siddique et al. 2013, Xiren and Aminah 2017).

Amino acid score data presented in **Table 2** show that lysine is a limiting amino acid in *K. alvarezii* seaweed and methionine is a limiting amino acid in *G. salicornia* seaweed due to its lowest chemical value. Meanwhile, threonine is the amino acid with the highest chemical score in both *K. alvarezii* and *G. salicornia* seaweeds.

DISCUSSION

The samples of *K. alvarezii* and *G. salicornia* seaweed had the same essential amino acid profile, which included histidine, leucine, threonine, valine, methionine, isoleucine, and phenylalanine. However, both seaweed samples differed in their non-essential amino acid profile; aspartate and lysine were present only in *K. alvarezii*, but did not exist in *G. salicornia*. According to previous studies on the amino acid profile of *K. alvarezii* seaweed originating from Brazil (Gressler *et al*. 2010) and Malaysia (Matanjun *et al*. 2009), 16 amino acids were identified, consisting of nine essential amino acids and seven non-essential amino acids. Gressler *et al*. (2010) stated that the profile of amino acids can determine the nutritional quality of food, especially in the evaluation of new protein sources. Amino acid content is linearly dependent on seaweed protein content in which low protein content causes low amino acid content. Therefore, the factors affecting amino acid content are similar to those affecting protein content, involving species, environment, and harvested period (Hardjani *et al*. 2017).

The result of essential amino acid percentages in *K. alvarezii* and *G. salicornia* in **Table 1** were consistent with findings of Dawczynski *et al*. (2007), namely that the essential amino acid content of some types of seaweed was 30% of the total amino acids. Several previous studies have reported that the total essential amino acids of *Caulerpa lentillifera* and *Ulva Reticulata* accounted for nearly 40% of the total amino acids (Ratana-arporn and Chirapart, 2006), those of *Kappaphycus alvarezii* dan *Hypnea musciformis* reached 45–49% (Dawczynski *et al*. 2007, Kumar and Kaladharan 2007), and those of *Gelidium pusillum* reach 52.08% (Siddique *et al*. 2013).

The results in the **Table 1** also showed that glutamic acid (13.73%) and aspartic acid (12.26%) contained in *K. alvarezii* seaweed were the amino acids that occurred at the highest level. On the other hand, the amino acids in *G. salicornia* seaweed that occurred at the highest level were glutamic acid (11.93%) and serine (13.02%). Several previous studies have also shown that aspartic acid and glutamic acid content of red seaweed ranged from 15% to 33% (Gressler *et al*. 2009, Matanjun *et al*. 2009, Ortiz *et al*. 2009). The content of aspartic acid and glutamic acid in red and green seaweeds were significantly higher than that of brown seaweed. The high content of aspartic acid and glutamic acid contributes to providing a distinctive flavor in seaweed; its savory flavor (*umami* flavor), for example, leads to its use as food seasoning to stimulate appetite in elderly people. Meanwhile, serine can provide a sweet taste that results in the complex flavor of seaweed (Yaich *et al*. 2011, Helaly 2013). Furthermore, Holds and Kran (2011) reported that red algae contain many glutamic acids. The taste of *nori* (dried or edible seaweed often used in Japanese cookery) is the result of a large number of different amino acids, including alanine, glutamic acid, and glycine.

The high percentage of glutamic acid, aspartic acid, and serine in *K. alvarezii* and *G. salicornia* seaweeds can be used as the consideration in the utilization of other potentials of both seaweeds, especially in the health sector. Khanifar *et al*. (2011) mentioned that glutamic acid is a precursor of glutamine and GABA. Aspartic acid helps to protect the liver and is involved in the metabolism of DNA and RNA. Moreover, it plays a role in the immune system by increasing the production of immunoglobulins and the formation of antibodies. Meanwhile, serine is essential in maintaining blood sugar levels, plays a role in the central nervous system including the development of the myelin sheath, and is necessary for muscle growth and maintenance.

The nutritional evaluation in **Table 2** indicate that lysine is a limiting amino acid in *K. alvarezii* seaweed and methionine is a limiting amino acid in *G. salicornia* seaweed. Meanwhile, threonine is the highest amino acids in both *K. alvarezii* and *G. salicornia* seaweeds. This result is proportional with the result of the study by Kumar and Kaladharan (2017). They suggest that leucine and lysine are limiting amino acids in *K. alvarezii*, while threonine and tryptophane had the highest chemical scoret. In contrast, lysine and methionine are limiting amino acids in *Gracilaria sp*, while threonine and tryptophane had the highest score. Furthermore, the study of Xiren and Aminah (2017) showed that lysine is a limiting amino acid in *K. alvarezii* seaweed species from both Langkawi and Sabah Waters in Malaysia.

In general, it can be seen that the content of *K. alvarezii* and *G. salicornia* seaweeds is rich in aromatic amino acids (i.e. threonine) and has limited sulfur amino acids (i.e. lysine). This suggests that *K. alvarezii* and *G. salicornia* seaweeds can be a source of complementary protein for humans and animals.

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