International Food Research Journal 24(3): 1255-1260 (June 2017)
Journal homepage: *http://www.ifrj.upm.edu.my*

Proximate composition and total amino acid composition of *Kappaphycus alvarezii* found in the waters of Langkawi and Sabah, Malaysia

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Article history

Received: 23 May 2016
Received in revised form: 2 July 2016
Accepted: 15 July 2016

Keywords

Kappaphycus alvarezii
Proximate composition
Amino acid content

Abstract

Proximate composition and amino acid content of red seaweeds, *Kappaphycus alvarezii*, from Langkawi and Sabah in Malaysia were determined in order to evaluate their potential nutritional value. The crude fibre content of seaweed from Sabah (at 8.95%) was found to be significantly higher than that of Langkawi (at 7.86%) ($P<0.05$). The two seaweeds from Langkawi and Sabah respectively contained lipids (1.06, 0.97% dry weight), ash (16.305, 17.18% fresh sample), proteins (6.24, 6.89% dry weight) and moisture (80.87, 81.86% fresh sample) ($P>0.05$). Total 17 amino acids were found in both seaweeds, aspartic acid, gulamic acid, leucine are the major constituent and followed by. This study showed that *Kappaphycus alvarezii* from both habitats contained different amount of some of the essential amino acid and proximate composition it can be used as ingredients to improving nutritive value in human diets.

Introduction

Seaweeds have been commonly used to human consumption in many parts of the world, In Malaysia, seaweeds are traditionally used as food and medicine apart from being an important hydrocolloids source (Phang, 2006) because they contain essential minerals of food nutrients for human nutrition (Ruperez, 2002; Gressler *et al*., 2010). They are identified as valuable sources of protein, elements (high levels of K and Cl), fiber and essential amino acids (Ommee and Payap, 2012). The fibre content of seaweed varieties is higher than those found in most fruits and vegetables. The protein in seaweeds contains all essential amino acids (EAA) included histidine, arginine, threonine, tyrosine, tyrosine, valine, methionine, lysine, leucine and phenyl alanine. And the EAA contents of some species are comparable to those of soy and egg proteins. In addition, many seaweed species contain high concentration of arginine, aspartic acid and glutamic acid (Rajasulochana *et al*., 2010).

The red *Kappaphycus alvarezii* seaweed falls under the class of Rhodophyceae. It is economically important specie which has been extensively cultivated in more than 20 countries for a source of carrageenan (Madhavarani and Ramanibai, 2014). *Kappaphycus alvarezii* is found in Sabah and Langkawi.

Sabah coastline surrounded by coral reefs, rocky shores, sandy area, mudflats and mangroves with high humidity and rich of organic matter tends to seaweeds growth and inhabits a variety of this substratum along the coastline (Zawawi *et al*., 2014). Langkawi coastline surrounded by coral reefs, rocky shores, mudflats, mangroves and lowland forests. The seaweed flora of Langkawi is quite distinct from that of Sabah. It may have element widespread to the Andaman Sea flora (Phang *et al*., 2005).

The nutritional composition of seaweeds vary depends on their species, maturity, environmental growth conditions such as sea water and sun light (Ito and Hori, 1989; Ortiz *et al*., 2006; Zawawi et al., 2014). Changes in their ecological conditions have an influence on the synthesis of nutrients (Lobban *et al.*, 1985; Ommee and Payap, 2012). The biochemical composition of *Kappaphycus alvarezii* from Langkawi is poorly known and utilization of seaweeds from Langkawi is limited to people living in the coastal areas only.

The purpose of this study was to determine the proximate composition and amino acid content of seaweeds from the two different places. In order to provide more intensive nutrient information, the samples were collected from both Langkawi and Sabah for analysis.

Materials and Methods

Sampling was carried out during one growing season, in July, from the Langkawi Island in the state of Kedah Peninsular Malaysia, and from the coastal area of Sabah, East Malaysia. Samples were thoroughly rinsed and soaked in water for 117 minutes (Siah *et al.*, 2014), and then soaked in 5%

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lemon juice overnight to eliminate a fishy odour (Xiren and Aminah, 2014).

Moisture content analysis

Moisture content of seaweeds was determined according to the method described by AOAC (2000) with slight modifications. Weight of aluminium dish was recorded as M1. About 3 g soaked seaweed samples were weighed into an aluminium dish and recorded as M2. Aluminium dish containing sample was then placed in an oven at 105°C for four hours. It was then cooled in a desiccator and weighed as M3. Percentage of moisture content was calculated by using the following formula:

$$\text{Percentage of moisture content (\%)} = \frac{M_3 - M_1}{M_2} \times 100$$

Ash content analysis

Ash content of seaweeds was determined according to the method described by AOAC (2000) with slight modifications. Soaked seaweed Samples (3g) was placed in a crucible and then heated in a fume hood until fumes were no longer produced. The sample was placed in a cooled muffle furnace and heated overnight at 450 – 500°C.

Total ash (g) = (weight of crucible + ash) – (weight of crucible)

$$\text{Percentage of ash content (\%)} = \frac{\text{Weight of ash}}{\text{Weigh of sample}} \times 100$$

Crude protein analysis

Crude protein content of seaweeds was determined according to the method described by AOAC (2000) with slight modifications as recommended by Kjeltec 2300 (Foss Analytical, Denmark). Briefly, a 4 gram pre-dried sample was weighed into digestion tubes. Two Kjeltabs Cu 3.5 (catalyst salts) was added into each tube. About 12 ml of concentrated sulphuric acid (H_2SO_4) was carefully added into the tube and then shaken gently. Digestion procedure was performed using pre-heated (420°C) digestion block of InKjel 625M (Behr, Germany) for 60 minutes until clear blue/green solution was obtained. Digested samples were cooled for 10-20 minutes. Distillation was then performed using distillation unit of Kjeltec 2300 (Foss, Denmark) and the percentage of protein was calculated by multiplying the percent of nitrogen found with a factor of 6.25.

Percentage of nitrogen (%):

$$\frac{0.1 \times (\text{volume of sample titration} - \text{volume of blank titration})}{\text{Weight of sample (mg)}} \times 14 \times 100$$

Percentage of protein content (%) = Percentage of nitrogen (%) x 6.25

Fat content analysis

Fats were extracted from the seaweed powder following the method described by AOAC (2000). The 2g of pre-dried seaweed sample was weighted into a pre-dried extraction thimble, a pre-dried boiling flask was weighed and 200 ml hexane as solvent for 2 to 2.5 hours in the boiling flask. The flask was further dried in an oven at 105°C for 1 hour. It was then cooled in a desiccator and weighed.

Percentage of Fat Content (%):

$$= \frac{[(\text{Weight of flask + fat}) - (\text{Weight of flask})]}{\text{Weight of sample (mg)}} \times 100$$

Crude fibre content analysis

Crude fibre was determined by sequential extraction of pre-dried seaweed samples (0.1g) with 1.25% H_2SO_4 (200 ml) and 1.25% NaOH (200ml) using the fibre-bag as a container. For drying and ashing, the crucible with sample was dried in an oven for 5 hours at 105°C and cool in desiccators and this is weighed as M1 and ashed in the muffle furnace (Carbolite, United Kingdom) at 525°C overnight and cooled in desiccators and weighed as M2. The weight of crucible with sample after drying and ashing was recorded and the crude fibre content was calculated (AOAC, 2000).

$$\text{Percentage of fiber content (\%)} = \frac{M1 - M2}{\text{Weigh of sample}} \times 100$$

Determination of total amino acids composition

Analysis of the amino acids was determined through a process of acid hydrolysis according to waters AccQ.Tag Aminno acid Analysis procedure (Cohen and Michaud, 1993). Approximately 0.3 g of powdered sample was weighed into a glass-stoppered test tube and hydrolyzed with 5 mL of 6 NHCl at 110°C for 24 h. Samples were cooled to room temperature before it was filtered through a filter paper (Sartorius Grade 292) into a 100 mL volumetric flask. An internal standard (400 µL) of 50 µmoL mL−1α-Aminobutyric Acid (AABA) in 0.1 M HCl was added and made up to 100 mL with distilled water. The aliquot was filtered through 0.20 mm polytetrafluoro ethylene micro filter. As for derivatization, 10 µL of filtered hydrolysated samples or standards were transferred into a 1.5 mL glass vial and 70 µL of borate buffer solution was added to it and mixed well. Then, a 20 µL of AccQ fluor reagent (3 mgmL−1in acetonitrile) was added to the mixture

and thoroughly mixed through a vortex for several seconds (Maizura *et al.,* 2013). 10µLof samples and standards were injected into a HPLC (Waters 2475, Waters Co., Milford, MA, USA) with the flow rate set at 1 mL min^{-1}. Analysis of the amino acids was performed with AccQ Tag column (3.9×150 mm, particle size 4 µm). The mobile phase A was Eluent A (200 mL AccQ Tag to 2 L of Milli-Q water) and mobile phase B, was Eluent B (60% acetonitrile). The linear gradient condition was set as follows: 100% A and 0% B at start, 98% A and 2% B at 0.5 min, 91% A and 9% B at 15 min, 87% A and 13% B at 19 min, 65% A and 35% B at 33 min, 65% A and 35% B at 35 min, 0% A and 100% B at 36 min, 0% A and 100% B at 39 min, 100% A and 0% B at 40 min and 100% A and 0% B at 50 min. Detection was carried out by a fluorescence detector (λ excitation at 250 nm and λ emission at 395 nm).

Statistical analysis

All determinations were performed at least in triplicate. Statistical comparisons of the results were performed by one-way ANOVA using SPSS ver.19.The Duncan was used to identify significant differences at $P<0.05$ between the mean values of the two species (Bryman and Cramer, 2012).

Results and Discussion

Proximate composition

The proximate composition of *Kappaphycus alvarezii* seaweeds from two different localities is as shown in Table 1. The protein contents found in both seaweeds ranged from 6.2 -6.8% of their dry weight (WW).However, significant ($P<0.05$) differences were found between the two seaweeds. This result concurred with a previous report described by Fisal *et al.* (2012) that seaweeds from Sabah had protein contents within a wide range of between 5.2-13.2% (DW). In this study the protein contents of both seaweed samples were higher than that of Sargassum Polycystum (5.4% DW) (Matanjun *et al.*, 2009), and that Langkawi's *Kappaphycus alvarezii* had protein content similar to that of *G.domingensis* (6.2% DW) (Gressler *et al*., 2010). These seaweeds appeared to be an interesting potential source of food proteins as they had protein contents higher than that found in several local vegetables such as peas, lettuce, red spinach and broccoli, corresponding to that reported by Norziah and Ching (2000). However, the protein contents of the two seaweeds were lower than those of other seaweed species such as Porphyratenera (47% DW) and Palmaria Palmata (35% DW) (Fleurence, 1999). These levels varied depending on water quality in the seas surrounding the islands (Zawawi *et al*., 2014).

Table 1. Proximate composition of *Kappaphycus alvarezii* from Langkawi and Sabah

	Kappaphycus alvarezii (Langkawi)	*Kappaphycus alvarezii* (Sabah)
Moisture (% fresh sample)	86.8± 0.7[a]	84.8 ± 0.6[a]
Ash (% fresh sample)	16.3 ± 0.05[a]	17.1 ± 0.8[a]
Protein (% dry weight)	6.2 ± 0.4[b]	6.8 ± 0.9[a]
Fiber (% dry weight)	7.8 ± 0.6[b]	8.9 ± 0.5[a]
Fat (% dry weight)	1.0 ± 0.6[a]	0.9 ± 0.7[a]

Different letters indicate significant differences ($P<0.05$). Values are expressed as mean (n=2).

The lipid contents varied from 0.9% WW in *Kappaphycus alvarezii* from Sabah to 1% WW to that found in Langkawi. These content levels were consistent with the previous reports (0.8-1.2% DW) (Abirami and Kowsalya, 2011). Two seaweeds which contained lipid higher than that of other species were *Gracilaria verrucosa* (0.27% DW) and *Sargassum polycystum* (0.71% DW) (Fisal *et al.*, 2012). However, the differences in lipid contents of the same species can be due to their geographical origin and water quality in the seas surrounding the islands. (Marinho-Soriano *et al*., 2006; Zawawi *et al*., 2014).

The ash contents of the two analyses samples were 16.3% fresh sample in *Kappaphycus alvarezii* from Langkawi and 17.1% fresh sample in *Kappaphycus alvarezii* from Sabah. The amounts of ash obtained in the present study were in agreement with the previous studies (Fisal *et al*., 2012). The ash contents in most marine seaweeds were usually much higher than those found in vegetables (0.3-4.8% DW). The difference in ash contents of the seaweeds depend on their species, physiological factors, environmental changes, methods of mineralization and type of processing adopted (Norziah and Ching, 2000).

In this study, the amounts of crude fibres of the two selected seaweeds ranged from 7.8-8.9% WW. However, significant ($P<0.05$) differences were found between the two seaweeds. The contents of total crude fibres in seaweeds were higher than those in vegetables such as soya beans (5.5% WW) and peas (2.7% DW) (Norziah and Ching, 2000). They have several positive physiological effects on humans in preventing constipation, colon cancer, cardiovascular disease and obesity. Therefore, both *Kappaphycus alvarezii* seaweeds can be used as ingredients for the production of high-fibre foods in industry (Benjama

Table 2. Amino acid composition (mg/100 mg) of *Kappaphycus alvarezii* from Langkawi and Sabah

Amino acid	*Kappaphycus alvarezii* (Langkawi)	*Kappaphycus alvarezii* (Sabah)
Hydroxyproline	ND	ND
Aspartic acid	0.44[b]	0.80[a]
Serine	0.26[b]	0.38[a]
Glutamic acid	0.45[b]	0.79[a]
Glycine	0.23[b]	0.35[a]
Histidine*	0.04	ND
Arginine*	0.16[b]	0.28[a]
Threonine*	0.19[b]	0.32[a]
Alanine	0.32[b]	0.49[a]
Proline	0.39[a]	0.28[b]
Tyrosine*	0.09[b]	0.13[a]
Valine*	0.24[b]	0.43[a]
Methionine *	0.07[b]	0.13[a]
Lysine*	0.14[b]	0.26[a]
Isoleucine*	0.21[b]	0.36[a]
Leucine*	0.32[b]	0.56[a]
Phenyl alanine*	0.24[b]	0.37[a]
Total AA	3.79	5.93
*EAA	1.70	2.84
Non-EAA	2.09	3.09
EAA/Non-EAA	0.81	0.92
EAA/Total AA	0.45	0.48

Values are expressed as mean (n=2). Different letters indicate significant differences (P<0.05).
* EAA, Essential amino acid.
Non-EAA, Non-essential amino acid.
ND, not determined.

and Masniyom, 2011).

Total amino acid composition

The amino acid contents of the two *Kappaphycus alvarezii* seaweeds are illustrated in Table 2. Their essential amino acids (EAA) included histidine, arginine, threonine, tyrosine, tyrosine, valine, methionine, lysine, leucine and phenyl alanine. However, the analytical method used could not determine Hydroxyproline. The level of different essential amino acids ranged from 1.70 to 2.84 mg/100mg DW. According to Mabeau and Fleurence (1993), the high levels of aspartic and glutamic acids were responsible for the special flavour and taste of these seaweeds. Both seaweeds were rich in asparatic acid, glutamic acid and Leucine. The two seaweeds contained large amount of glutamic acid and aspartic acid which are responsible for the seaweed taste (Vinoj and Kaladharan, 2007).

The means of total amino acid contents in Kappaphycus alvarezii from Langkawi and Sabah were 3.79 mg/100mg DW and 5.93 mg/100mg DW, respectively. The protein contents of Kappaphycus alvarezii from Langkawi and Sabah were 6.2 and 6.8% DW, respectively. The ratios of EAA to total amino acids were 0.45 in Kappa from Langkawi, and 0.48 in those from Sabah respectively, indicated a good ratio of EAA to non-EAA in both seaweeds.

Amino acids in these seaweeds constituted mainly aspartic acid, glutamic acid, proline, alanine and leucine, but were lower in histidine. Both seaweed samples exhibited similar amino acid patterns in which aspartic and glutamic acids together constituted a large part of the amino acid fraction. Moreover, the seaweeds were generally rich in glycine and alanine but poor in histidine. Similar results were reported in other studies (Vinoj and Kaladharan, 2007). These findings suggested that the two *Kappaphycus alvarezii* seaweeds can be used as alternative nutrient sources of protein and amino acid for human and animal consumption (Ommee and Payap, 2012).

Table 3. Essential amino acid of *Kappaphycus alvarezii* from Langkawi and Sabah in comparison with FAO/WHO/UNU reference value (%)

Essential Amino Acid	*Kappaphycus alvarezii* (Langkawi)	*Kappaphycus alvarezii* (Sabah)	FAO/WHO (1991) Ref. Value
Lysine	2.9	3.7	5.8
Threonine	3.8	4.6	3.4
Valine	5.1	6.2	3.5
Leucine	6.6	8.0	6.6
Isoleucine	4.4	5.2	2.8
Phenylalanine	6.8	7.1	6.3
Tyrosine	0.1	0.1	-
Arginine	3.3	4.1	-
Histidine	0.7	-	-

Furthermore, the levels of all their essential amino acids were comparable to those of the FAO/WHO (1991) requirement pattern. From the table (see Table 3), leucine (6.6-8.0%), valine (5.1-6.2%), Threonine (3.8-4.6%) and isoleucine (4.4-5.2%) score higher than their respective reference standards. The values for phenylalanine (6.8-7.1%) are comparable to their respective reference values. The Lysine contents of *Kappaphycus alvarezii* from Langkawi and Sabah are below the reference values. However, the levels of other essential amino acids in this study were above the FAO/WHO (1991) requirement (see Table 3). With respect to the FAO/WHO (1991) requirement pattern, *Kappaphycus alvarezii* from Langkawi and Sabah seemed to be able to contribute adequate levels of total EAA for human.

Conclusion

This study showed that some of the amino acids, protein and fiber content of *Kappaphycus alvarezii* from Sabah were significantly higher ($p \leq 0.05$) than those grown in Langkawi. A total of 19 amino acids

were found in the both seaweeds. Among all the amino acids present, asparatic, glutamic, alanine and leucine were found to be the major components. In the case of ash and crude fiber contents in these two localized seaweeds were higher than those found in several vegetables grown locally.

Acknowledgement

This research was supported by the National University of Malaysia under project Numbers STGL-004-2007 and STGL-016-2012.

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