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Amino acid content in seaweeds from the Magellan Straits (Chile)

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ABSTRACT

Total proteins, amino acid (AA) profile and AA score (AAS) were determined in 73 red, green and brown seaweeds collected in the sub-Antarctic ecoregion of Magallanes. Significant differences were found between the genera and seaweed colour for protein contents, essential AA (EAA), non-essential AA (NEAA) and ratio EAA/NEAA. A serving of brown seaweed would contribute to a lower protein intake than a portion of red or green seaweed. However, AAS and the EAA index (EAAI) showed that brown seaweeds had a better protein quality than red and green seaweeds. Sulfur AA were the limiting AA in red and green seaweeds while leucine was the limiting AA in brown seaweed. A high concentration of lysine was found, which is often the limiting amino acid in animal feeds. Seaweeds might be important sources of proteins with high level of EAA, however the protein and AA content varies depending on the seaweed colour and genus.

1. Introduction

Red and brown seaweeds have been an important element of the diet in East Asian countries such as Japan, Korea and China, since ancient times. In western countries seaweeds are utilised mainly as a source of polysaccharides (alginates, carrageenans and agar) for food and pharmaceutical purposes (Mabeau and Fleurence, 1993; Jiménez-Escrig and Sánchez-Muniz, 2000; Cao et al., 2016). However, in these countries, there is an increasing interest in using marine algae as food, due to its beneficial and health-promoting nutrients. Among these compounds, there are polyunsaturated fatty acids, essential amino acids, fibre, minerals and trace elements, vitamins, phenolics and carotenoids, among others (Bocanegra et al., 2009; Brown et al., 2014). Including marine algae in the human diet has impacts on growth, body weight, mineral bioavailability, lipid metabolism, blood pressure and antioxidant properties (Bocanegra et al., 2009). Thus, the analysis of the chemical composition in seaweeds is essential in the search for additional healthy food sources in human and animal nutrition.

This study sought to examine and compare for the first time the total protein content, amino acid profile and amino acid score in several genera of seaweeds collected from the Straits of Magellan (Chile) in order to find out the differences between seaweed genus and colour.

2. Material and methods

2.1. Sampling and preparation

Seventy-three samples of seaweeds were collected monthly between January and June 2007, in the sub-Antarctic ecoregion of Magallanes, Southern Chile, located in the south-western part of the South American continent (48°36′ to 56°00′ S; 66°25′ to 75°40′ W). The zone of sampling included the coastal marine protected area and Francisco Coloane park, the middle of the Straits of Magellan and the Cape Horn Biosphere Reserve. These samples included nine genera of the Rhodophyta division (red algae, Ceramium, $n=1$; Heterosiphonia, $n=1$; Iridaea, $n=2$; Gigartina (popularly known as Luga), $n=3$; Mazzaella, $n=6$; Nothogenia, $n=4$; Polysiphonia, $n=1$; Porphyra, $n=18$; Sarcothalia, $n=2$), six genera of the Chlorophyta division (green algae, Ballia, $n=1$, Cladophora, $n=3$, Codium, $n=2$, Entemorpha, $n=9$, Monostroma, $n=1$, Ulva, $n=7$) and three genera of the Ochrophyta division (brown algae, Adenocystis, $n=7$, Lessonia, $n=2$, Macrocystis, $n=3$).

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Seaweed samples were identified based on examination of morphological and anatomical characteristics by the Centre for Aquaculture and Subantarctic Marine Resources Laboratory at the Department of Natural Sciences and Resources, University of Magallanes, Chile. The genera were chosen for their potential as a food source and their protein and nutritional richness. Intertidal benthic macroalgae were randomly and manually collected at low tide from the surface of beach rocks, whereas subtidal macroalgae were collected by scuba divers. Samples were washed with seawater from the sampling site and stored in plastic bags and were transported in coolers with ice to the laboratory refrigerators at 4 °C prior to analysis. In the laboratory, the samples were washed with Milli-Q deionized water (Millipore, Bedford, MA) to remove epiphytes, salt and foreign matter; they were then dried at room temperature (20 °C) for five days. Finally, the samples were milled to a particle size of less than 1.0 mm and kept in polypropylene tubes at 4 °C.

2.2. Analytical methods

2.2.1. Determination of amino acids (AA)

The total amino acids were determined according to the procedure described by Bosh et al. (2006) with slight modifications. The dry sample (30 mg) was weighed into a glass tube, and 2 mL of 6 M HCl was added. Hydrolysis was carried out at 110 °C for 24 h. Each hydrolyzed extract was passed through a wet filter paper; and then 10 mL of internal standard and 2.5 mM L-2-amino-*n*-butyric acid (Sigma-Aldrich Chemical Co., St. Louis, MO) were added. This solution was diluted with Milli-Q water to 250 mL. An aliquot was filtered with 0.45-μm filter GHP (Waters Corporation, Milford, MA). The amino acids were subjected to HPLC analysis after derivatization with 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate (AQC), using AccQ·Fluor Reagent Kit (Waters Corporation). Then, 10 μL of filtered hydrolysed sample or standard (amino acid hydrolysed standard; Waters), 70 μL of 200 mM borate buffer and 20 μL of reconstituted AccQ·Fluor reagent were added into the vial followed by mixing with a vortex for several seconds. Finally, the vial was heated in a heating block at 55 °C for 10 min. Ten microlitres of sample or standard solution were injected into the HPLC system with a fluorescence detector (Waters 474 coupled to separation module Waters 2690). The separation of the AQC derivatives was carried out using a 3.9 × 150 mm AccQ·Tag column, at 37 °C with gradient elution, and the flow rate was 1.0 mL/min. Three eluents were used: eluent **A** was an acetate-phosphate buffer (Waters ACC·Tag), eluents **B** and **C** were acetonitrile and Milli-Q water, respectively. The gradient program was as follows: 0.00 min, 100% **A**; 0.50 min, 99% **A** and 1% **B**; 18.00 min, 95% **A** and 5% **B**; 19.00 min, 91% **A** and 9% **B**; 29.50 min, 83% **A** and 17% **B**; 34.00 min, 60.0% **B** and 40% **C**; 44.00–55.00 min, 100% **A**. The amino acid-AQC derivatives were detected by their fluorescence, upon excitation at 250 nm and emission at 395 nm. The HPLC peaks were identified by comparing their retention times with data obtained from standards (amino acid hydrolysed standard; Waters). Good resolution and separation of the sixteen AA analysed in the real samples of green, red and brown seaweeds was achieved using the conditions of elution described in the experimental section. The analysis time for the determination of all the AA was around of 50 min; validation of the method was described in Rodríguez Galdón et al. (2010). All these analyses were performed in triplicate, and the results expressed as g of AA per 100 g of dry weight (DW).

2.2.2. Amino acid chemical score (AAS)

This chemical parameter is commonly used to measure the protein nutritional quality (FAO/WHO, 1991):

AAS (%) = [mg of AA per g of test protein/mg of AA per g of reference protein] × 100

The reference protein used was the FAO/WHO (1991) and FAO/WHO/UNU (2007) amino acid pattern. The chemical score of the protein is the lowest value of the chemical scores of all the essential AA.

Table 1
Concentrations of proteins and groups of amino acids (expressed as g/100 g DW) for seaweed genera, grouped according to their colour.

	genera	EAA	NEAA	ratio EAA/NEAA	sum AA	crude protein (Kjeldahl)
overall		6.54	11.6	0.56	15.5	19.9
brown	*Adenocystis*	5.07	8.92	0.57	12.0	12.6
	Lessonia	3.60	6.77	0.53	8.87	12.5
	Macrocystis	5.86	9.36	0.62	13.0	17.3
red	*Ceramium*	9.32	15.4	0.61	21.2	28.0
	Heterosiphonia	10.1	16.4	0.62	22.7	32.5
	Iridaea	8.71	13.3	0.66	18.8	24.1
	Gigartina	4.39	7.09	0.61	9.83	9.67
	Mazzaella	4.86	9.89	0.49	12.5	15.3
	Nothogenia	7.04	12.4	0.57	16.7	23.3
	Polysiphonia	8.65	12.7	0.68	18.4	30.4
	Porphyra	7.09	13.0	0.54	17.2	22.0
	Sarcothalia	4.19	7.03	0.60	9.64	13.5
green	*Ballia*	7.42	13.9	0.54	18.2	22.0
	Cladophora	8.03	13.8	0.58	18.6	24.5
	Codium	5.49	9.32	0.59	12.7	14.8
	Enteromorpha	6.58	12.0	0.55	15.8	19.9
	Monostroma	6.56	12.6	0.52	16.3	19.6
	Ulva	8.08	14.4	0.56	19.2	26.4
	red	6.63^{b}	11.8^{b}	0.56	15.5^{b}	20.4^{b}
	brown	7.17^{b}	12.8^{b}	0.56	17.1^{b}	22.5^{b}
	green	5.02^{a}	8.67^{a}	0.58	11.7^{a}	14.0^{a}
p (sig)[*]		0.004	0.000	0.689	0.001	0.007

EAA: Essential amino acids; NEAA: Non-Essential amino acids; Sum AA: Sum of all the amino acid residues after hydrolysis less water mass.
[*] Results in the same column with the different superscript were significantly different).