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Edible Azorean macroalgae as source of rich nutrients with impact on human health

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ABSTRACT

Fucus spiralis, *Porphyra* sp. and *Osmundea pinnatifida* are macroalgae consumed as food in some of the Azorean Islands, but little information is available on their nutritional value. This paper presents information on the nutritional aspects in terms of proteins (9.71–24.82%), lipids (5.23–8.88%), soluble carbohydrates (17.59–25.37%), ash (22.31–38.55%), minerals (0.96–1.82 for Na/K ratios) and fat soluble vitamins A (1.20–1.41%) and E (4.86–51.14%) contents on dry weight basis. Fatty acid (SFA, MUFA, PUFA and *n*6/*n*3 and h/H ratios) and amino acid profiles, and antioxidant properties were also reported. All the three species contained measurable quantities of 9 of 10 essential amino acids (41.62–63.50% of the total amino acids). The regular consumption of these algae allied with the low level of seawater pollution around Azores Islands will improve human health conditions and will have a protective effect on the most common degenerative diseases.

1. Introduction

Marine algae, commonly referred to as "seaweeds" for the commercially available macroalgae, have for centuries (documented since 600 BC) been consumed whole among the East Asian populations of China, Korea, Japan and other countries. In the meantime in Western countries they have been utilized as sources of phycocolloids, as a gelling agent (e.g. alginates, carrageenans and agar), or meal for animal nutrition and for various applications in the food industry. Macroalgal communities are available almost throughout the year and due to the marine ecosystems in which they grow, they provide nutrition and an accommodating environment for other marine living organisms. Because of these properties, macroalgae are some of the most important organisms maintaining the ecosystem's stability (Wilson, 2002).

The Archipelago of Azores has a long tradition in the use of marine macroalgae as fertilizers in local agriculture and/or horticulture and also as food supplement (e.g., *Fucus spiralis*, *Porphyra* sp., *Laurencia* and *Osmundea*) or for chemicals extraction (e.g., *Pterocladiella capillacea* and *Gelidium microdon*) (Neto, Tittley, & Raposeiro, 2006). The consumption of seaweeds is widespread and accepted as a common practice in some of the Azorean Islands. Being isolated in the middle of the Atlantic Ocean (37°40′N and 25°31′W) and taking into account the low pollution levels of seawater (Neto et al., 2009), the Azores Islands have become a very promising location as a natural resource for marine organisms which may produce new ingredients of so-called functional or health-promoting foods that have a medicine-like effect in treating or preventing certain diseases. The consumption of macroalgae can increase the intake of proteins, fibre, minerals, vitamins, essential amino acids and polyunsaturated fatty acids that lower the occurrence of some chronic diseases (diabetes, obesity, heart diseases, cancers, etc.), which are accordingly associated with low fibre diets of western countries (Southgate, 1990). As is well established, the variability in the chemical composition of macroalgae, aside from the species, may be the result of its developmental stage,

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geographical location, habitat, season and nutrient content of the growth medium, among others environmental factors (e.g., Fleurence, 1999; Khotimchenko, Vaskovsky, & Titlyanova, 2002). It is also known that different sampling methodologies and drying method could also affect the biochemical composition and consequently the nutritional value of algae (Chan, Cheung, & Ang, 1997).

The biochemical composition of the edible Azorean macroalgae (EAM) has received limited attention in recent years (Patarra, Leite, Pereira, Baptista, & Neto, 2013; Patarra, Paiva, Neto, Lima, & Baptista, 2011). As a result, the objective of the present investigation was to provide a more detailed information on the biochemical and nutritional composition of the three most consumed EAM (*Osmundea pinnatifida*, *F. spiralis* and *Porphyra* sp.) in order to determine if these algae may be of nutritional value for human consumption and have some impact on human health, which in turn can provide an incentive for the biodiversity conservation and for the maintenance of a clean environment while also having a potential effect on the regional economy.

2. Material and methods

2.1. Chemicals and reagents

Dichloromethane, chloroform, methanol and hexane, HPLC grade were purchased from Fluka Chemika (Steinheim, Switzerland). Ethanol, acetone, sodium chloride, anhydrous sodium sulphate, potassium hydroxide, phenol, triethylamine (TEA), ascorbic acid, Kjeldahl catalyst and sulphuric acid were obtained from E. Merck (Darmstadt, Germany). Deionized water used for samples preparation was obtained from a Millipore water purification system (Millipore, Bedford, MA, USA). Derivatisation reagent (14% boron trifluoride in methanol) and certified preformulated multi-standard cation kit were obtained from Alltech Associates (Deerfield, IL, USA). Fatty acids methyl esters standards "FAME Mix C4-C24 (18919 Supelco)" and "PUFA No. 1 Marine Source (47033 Supelco)", DPPH (2,2-diphenyl-1-picrylhydrazyl) solution, butylated hydroxyltoluene (BHT), HPLC grade sodium acetate trihydrate ($CH_3COONa{\cdot}3H_2O$), disodium hydrogen phosphate (Na_2HPO_4) and sodium hydroxide were purchased from Sigma–Aldrich (St. Louis, MO, USA). Amino acid standard mixture, sequanal grade 6 N HCl and phenyl isothiocyanate (PITC), were from Pierce Chemicals (Rockford, IL, USA). Acetonitrile (HPLC grade) was from Fisher Scientific (Fair Lawn, NJ, USA). Purified water (Millipore, Bedford, MA, USA) with at least 18 MΩ resistivity was used for the preparation of all reagents, eluents, and buffers. All were filtered through 0.2 μm membranes.

2.2. Macroalgal collection

Rhodophyta *O. pinnatifida* (Hudson) Stackhouse and Ochrophyta (Phaeophyceae) *F. spiralis* (Linnaeus) samples were collected in January (2013). Rhodophyta *Porphyra* sp. (C. Agardh) were collected in January (2007), from the littoral zone (namely, ETAR da Pranchinha) of São Miguel Island, in the Azores Archipelago. Voucher specimens were deposited in the Herbarium AZB – Ruy Telles Palhinha of the Department of Biology at the University of Azores.

2.3. Sample preparation

Within 24 h of collection, a sample of each species was first washed in seawater to remove epiphytes, sand and other foreign material and finally the algae were carefully rinsed with distilled water to remove salts and then partially dried with a paper towel. The samples were then dried and stored in an air-tight container in a freezer (−80 °C) until further analysis. Prior to the analytical procedures, the algae samples were defrosted and dried at 65 °C during 48 h (avoiding overheating that could lead to oxidation) and then were cut into small pieces, grounded into a fine powder using a dry grinder or by pestle and mortar and finally re-dried at 40 °C and stored in dark under N_2 in a desiccators at a refrigerated temperature.

2.4. Nutrient analysis

This study focuses on a range of general nutritional components from EAM. For all analyses (with the exception of moisture content) triplicate algal samples were again dried in an oven prior to use.

2.4.1. Dry weight and moisture content determination

The dry weight (DW) of fresh matter was estimated after drying the EAM samples at 70 °C for 18 h in a hot air oven until a constant weight was obtained. The moisture content of the fresh material was calculated by subtracting the dry sample weight from the spun wet weight.

2.4.2. Determination of crude proteins

The organic nitrogen content was quantified using the modified Kjeldahl procedure (AOAC, 1990) in a Velp Scientifica UDK 132 apparatus. Each of the EAM samples (1 g of homogenized dry powder algal material) was digested with sulphuric acid 96%, then distilled with acid boric solution (2%) and titrated with 0.1 M HCl. Estimation of the crude protein content was calculated multiplying the organic nitrogen by a conversion factor of 6.25.

2.4.3. Extraction of crude lipids, total lipid determination and fatty acids profile

Crude lipid content was determined gravimetrically after soxhlet extraction (AOAC, 2000) with dichloromethane:methanol 2:1 (v/v) during 4 h of reflux, in order to obtain high-yield, following the Folch, Lees, and Solam-Stanley (1957) method. Fatty acid (FA) profile was performed by gas chromatography (GC), after transmethylation using 0.5 N KOH methanol solution and derivatisation with 14% boron trifluoride in methanol according to the modified protocol of Leite, Lima, and Baptista (2007). Fatty acids methyl esters (FAME) were analysed on a Bruker model 450 GC equipped with split/splitless injector and a flame ionization detector, using a wall-coated open tubular (WCOT) fused silica CPWAX58 (FFAP)-CB column from Varian (25 m × 0.25 mm i.d., 0.20 μm film thickness). The temperature was held at 150 °C for 2 min, programmed at a rate of 4 °C/min to 250 °C and then held at this temperature for 10 min. Helium was the carrier gas at a flow rate of 1.3 mL/min. The injector and detector temperatures were held constants at 260 °C and 280 °C, respectively. FAME identification were carried out by comparison of their retention times with those of the FAME standard and for the more difficult assignments were also confirmed by GC/MS experiments carried out with a Varian 3800 GC (Palo Alto, CA, USA) interfaced with a 4000 Varian MS, using the same analytical conditions. The mass spectrometric conditions were: electron impact ionization at 70 eV, source temperature 220 °C, 100 μA trap current, and the sweep time was 1.5 s/decade scan, with a mass range of 50–450 *m*/*z*. FAME peaks were quantified by area normalization using the workstation software from Bruker. The repeatability of the GC analysis of FA were evaluated in measurements of the retention time by repeated injections ($n = 5$). The recovery was calculated based on the difference between the total concentration determined in the spiked samples and the concentration observed in the non-spiked samples.

2.4.4. Determination of ash

The total inorganic material (ash) in insoluble indigestible fractions (1 g) was determined gravimetrically after incineration of

dried algal material at 550 °C for 2–3 h using an electric muffle furnace (AOAC, 1995), cooled in a desiccators and reweighted. The ash content was determined using the equation: % Ash = weight of ash/weight of sample × 100.

2.4.5. Determination of soluble carbohydrates content

Algae carbohydrate content has been reported by several authors as the weight difference using protein, lipid, fibre, moisture and ash content data. In this study, the total soluble carbohydrate content of EAM was extracted from samples with 2.5 N HCl and the concentrations determined by the phenol–sulphuric acid colourimetric method described by Dubois, Gilles, Hamilton, Rebers, and Smith (1956). Percent soluble carbohydrate was calculated based on absorptions at 490 nm in a Shimadzu 160-A UV/VIS spectrophotometer model 1800. The results were calculated from a multiple level glucose calibration curve at five different concentrations, constructed from peak-area *versus* glucose concentration. The linear regression analysis provided the equation $y = 0.0787\ x - 0.1001$, with the correlation coefficient (R^2) of 0.9989.

2.4.6. Mineral analysis

The EAM samples for mineral determination were analysed by ion chromatography. Samples (25 mg of algal material in 1 mL of deionised water) were frozen at −80 °C for 12 h, defrosted and homogenized with a potter (2600 rpm) during 10 min, centrifuged at 3500 rpm during 8 min and 100 μL of supernatant was added to 900 μL of deionized water and 20 μL was injected in HPLC.

Sample were analysed by an Alltech Associates (Deerfield, IL, USA) HPLC with a free-metal pump model 426, a rheodyne manual peek injection valve and coupled to an Alltech conductivity detector model 650. Universal cation exchange column (100 × 4.6 mm i.d., 7 μm particle size), was used with 3 mM methanosulfonic acid as mobile phase at a flow rate of 0.8 mL/min. Calibration curves were obtained from the pre-formulated cation mixture (Li 0.2 ppm, Na 1.3 ppm, NH_4 5 ppm, K 2.5 ppm, Mg 2.0 ppm and Ca 2.0 ppm) and the corresponding equations provided by linear regression analysis were used for the quantification the EAM mineral content.

2.4.7. Amino acids analysis (composition)

For amino acid determination, protein was precipitated with trichloroacetic acid and the protein pellet was recovered by centrifugation following the Barbarino and Lourenço (2005) methodology. Triplicate subsamples of 600 μg of protein pellet, were placed in 4 mL screw-cap vials and 100 μL of 6 N hydrochloride acid containing 1% phenol were then added. The vials were closed under nitrogen, placed in a heating block at 110 °C for 24 h. The tubes were cooled and their content were used for the derivatisation step. The derivatisation procedure was a modification of the method of Sánchez-Machado, López-Cervantes, López-Hernández, Paseiro-Losada, and Simal-Lozano (2003). Amino acid standard solution or sample hydrolysate (50 μL) was placed in a tube and dried in a vacuum oven for 20 min at 42 °C. Methanol:water:TEA (2:2:1, v/v/v; 50 μL) was then added to the residue and the resulting solution was vacuum-dried for 20 min at room temperature (RT). Methanol:water:TEA:PITC (7:1:1:1, v/v/v/v; 50 μL) was then added, and the tubes were mixed (vortex) during 15 s, then left for 20 min at RT. The resulting solution was vacuum-dried during 100 min at RT and 100 μL of 5 mM Na_2HPO_4 (containing 5% acetonitrile) was added and vortex mixing for 15 s before injecting (20 μL) in HPLC.

Amino acids profiles were evaluated on an Agilent Technologies (PaloAlto, CA, USA) HPLC system series 1200 on a HP Aminoquant (200 × 2.1 mm) reverse-phase column using the following solvent system: phase (A) 0.14 M ammonium acetate with 0.05% TEA (pH 6.4) and phase (B) acetonitrile:water (60:40, v/v), using a linear gradient elution: $t = 0$ min – 5% B, $t = 13$ min – 5% B, $t = 20$ min – 40% B, $t = 22$ min – 45% B, $t = 25$ min – 50% B, $t = 26$ min – 80% B, $t = 27$ min – 100 B, $t = 31$ min – 5% B at a flow rate of 0.35 mL/min under UV detection at 254 nm. In each sample, the amino acids content were measured in triplicate, and the values were expressed as mg/g of protein.

2.4.8. Antioxidant properties

The free radical scavenging activity (FRSA) of the macroalgae species were determined in a methanol solution of DPPH, used as a stable radical, according to the method described by Moyneux (2004). This methodology measures the hydrogen atom or electron donor capacity of an extract to the stable radical DPPH formed in solution. In other words, it measures the capacity of the extract to scavenge free radicals in solution. An aliquot of 2.0 mL of the methanol solution of macroalgae species or BHT at 2.0 mg/mL concentration was added to a test tube, with 1 mL of DPPH methanol solution (4.5 mg/100 mL in methanol, w/v). Methanol was used as a blank to zero the absorbance (Abs), BHT was used as reference sample and a mixture without macroalgae extract or BHT was used as the control (c). The Abs was measured at 517 nm over a period of 30 min of reaction time using a Shimadzu 160-A UV/VIS spectrophotometer. The FRSA of the samples (s) were calculated as a percentage of DPPH decolouration using the following equation: $\text{FRSA}\ (\%) = (1 - \text{Abs}_s/\text{Abs}_c) \times 100$. Results are expressed as mean values ± SD of three different Abs measurements of two extracts per macroalgae species.

2.4.9. Fat soluble vitamins content

The extraction and quantification of fat soluble vitamins was performed by the method of Sánchez-Machado, López-Hernández, and Paseiro-Losada (2002) with some modifications. A sample (500 mg) was weighed and placed in a tube with 50 mg of ascorbic acid and 5 mL of KOH solution (0.5 M in methanol) was added, and immediately vortex mixed for 20 s. The tube were placed in a heating block at 80 °C for 30 min (removing them every 5 min and vortex again 5 s). After cooling in iced water 1 mL of distilled water and 6 mL of hexane was added, and the mixture was rapidly vortex mixed for 1 min, then centrifuged for 8 min at 2500 rpm. A 2 mL volume of the upper phase was transferred to another test tube and dried under nitrogen. The residue was redissolved in 500 μL of methanol:acetonitrile 30:70 (v/v). A 50 mL aliquot was injected into the HPLC column.

2.5. Statistical analysis

All determinations were performed at least in triplicate, the data were expressed as means ± standard deviations (SD). One-way analysis of variance test (ANOVA) was carried out to assess for any significant differences between the means. Differences between means at the 5% ($P < 0.05$) level were considered significant.

3. Results and discussion

The biochemical composition and nutritional value of the three most consumed EAM (*O. pinnatifida*, *F. spiralis* and *Porphyra* sp.) were evaluated in this study. The nutrient levels were shown in comparison to standards and common marketplace foods or with intake of reference nutrients or also with selected nutrients from terrestrial foodstuffs. Some perspectives on the potential uses of these algae for the development of new food supplements or food additives for human or animal consumption that augment a nutritional balanced diet were also discussed.

3.1. Proteins

The EAM content of crude protein (Table 1) ranged from the lowest value of 9.71 ± 0.03% to the highest value of 24.82 ± 0.05% of DW for *F. spiralis* and *Porphyra* sp., respectively, and were within the ranges of brown algae (3–15% of DW), and red and green algae (10–47% of DW) (Darcy-Vrillon, 1993; Fleurence, 1999). These values were consistent with earlier studies on Azorean marine algae (Patarra et al., 2011) and also for *Enteromorpha* spp. (9.45%) reported by Aguilera-Morales, Casas-Valdez, Carrillo-Dominguez, Gonzalez-Acosta, and Perez-Gil (2005), and for *Undaria pinnatifida* (18.00%), *Porphyra* sp. (24.11%) and *Palmaria* sp. (13.87%) reported by Sanchez-Machado, Lopez-Hernandez, Paseiro-Losada, and Lopez-Cervantes (2004). The EAM show higher protein content than in some grains like oats (13.4%), wheat (13.8%), corn (9.4%), rice (7.1%), soybean (13.0%) and other cereals (Morales de Leon, Babinsky, Bourges, & Camacho, 2000). Variations in the protein content of EAM can be attributed to different species, environmental factors or a combination of both (Fleurence, 1999).

3.2. Lipids

The highest content of EAM crude lipids (Table 1) was present in *Porphyra* sp. (8.88 ± 0.05% of DW) and *O. pinnatifida* (7.53 ± 0.07% of DW) whereas the lowest content was found in *F. spiralis* (5.23 ± 0.03% of DW). However, Patarra et al. (2013) registered lower content of lipids for the same species. Indeed, different sampling methodologies could affect the lipid composition (Chan et al., 1997). Similar algae lipids results were reported by McDermid and Stuercke (2003) for the edible Hawaiian algae *Ulva fasciata* (5.10 ± 0.2%), *Codium reediae* (6.30 ± 0.1%), *Porphyra vietnamensis* (4.40 ± 0.2%) and *Dictyota sandvicensis* (20.20 ± 0.1%), and also by Chakraborty and Santra (2008) for *Lola capillaris* (4.05 ± 0.24%) and *Enteromorpha intestinalis* (7.13 ± 1.33%) from Sunderban, India. Furthermore, according to Nelson, Phleger, and Nichols (2002), the content of algae lipids is higher in the winter and spring and is lower in the summer, and it also varies according to the geographical location, climate and environmental conditions (like temperature, salinity and nutrient content of the growth medium).

3.3. Ash

The ash obtained in EAM (Table 1) ranged from 22.31 ± 0.38% to 38.55 ± 0.68% of DW for *F. spiralis* and *O. pinnatifida*, respectively, and were within the ranges previously reported (e.g., Rupérez, 2002). These values are similar to the *Sargassum sinicola* (37.2%), reported and recommended for human consumption by Manzano and Rosales (1989).

3.4. Soluble carbohydrates

The total soluble carbohydrates content of EAM (Table 1) show the highest value of 25.37 ± 0.29% of DW in *Porphyra* sp. while the lowest values of 17.61 ± 0.39% and 17.59 ± 0.27% of DW were recorded in *O. pinnatifida* and *F. spiralis*, respectively. Similar results were also reported by Chakraborty and Santra (2008) for *Gelidiella acerosa* (14.34 ± 1.74%), *Dictyota ceylinica* (18.52 ± 0.71%), *Polysiphonia mollis* (25.81 ± 1.34%) and *Catenella repens* (28.96 ± 7.96%). However, Ortiz et al., 2009 registered higher content of carbohydrates (ranged from 58.4% to 70.9% of DW). According to Rosemberg and Ramus (1982) the algae carbohydrate synthesis are related to periods of maximum growth and increased photosynthetic activity. On the other hand, these periods were influenced by increased values of temperature, salinity and sunlight intensity. The carbohydrate values for the EAM are above the contents reported by several authors for vegetables and fruits and they only resemble the carbohydrate content in dried fruit (McCance, Widdowson, & Holland, 1993).

3.5. Minerals

The ion chromatography (cation exchange column) referred in methods provide an efficient separation of all EAM minerals. The HPLC repeatability of the calcium (higher retention time and consequently with higher diffusion) determination ($n = 5$) was 1.97% indicating a high degree of repeatability. The accuracy of the calcium was also evaluated by the determination of the recovery in a sample of a known level of calcium as referred in methods. Results with the relative standard deviation (RSD) were better than 2.35% and the mean recovery ranged from 97.7% to 99.1% indicating a high degree of the method accuracy for the determination of calcium (and for the other minerals presenting lower retention times and consequently lower diffusion during the HPLC separation) under the analytical conditions used.

Mineral content of marine algae is high relative than that of the most common vegetables (Ortega-Calvo, Mazuelos, Hermosín, & Sáiz-Jiménez, 1993). Macroalgae have the capacity to accumulate minerals according to environmental conditions (e.g., Mabeau & Fleurence, 1993). Significant differences in the amount of Na, K, Mg, Cs and Ca of EAM are shown in Table 2. The results are very similar to the reported in literature (MacArtain, Gill, Brooks, Campbell, & Rowland, 2007). The *F. spiralis* contains 12 times more potassium than cheddar cheese, 15 more magnesium than whole milk, and 20 times more calcium than bananas. *Porphyra* sp. contains 32 times more potassium than cheddar cheese, 36 times more magnesium than whole milk, and 21 times more calcium than bananas. *O. pinnatifida* contain 19 times more potassium than cheddar cheese, 38 times more magnesium than whole milk, and 46 times more calcium than sirloin steak. The Na/K ratios were 1.46, 0.96 and 1.82 for *F. spiralis*, *Porphyra* sp. and *O. pinnatifida*, respectively. Rupérez (2002) reported low Na/K ratios, below 1.5, for the brown seaweed *Fucus vesiculosus* (1.27) and for the red seaweeds *Porphyra tenera* (1.04) and *Chondrus crispus* (1.34). In contrast, sausages and olives have Na/K ratios of 4.89 and 45.63, respectively (Ortega-Calvo et al., 1993). It is well known that intakes of high Na/K ratios have been related to the higher incidence of hypertension. Marine algae can therefore help balance high Na/K ratio diets.

3.6. Amino acids

The reverse-phase HPLC separation of the derivatised amino acids, referred in methods, provide an efficient separation of all

Table 1
Composition (moisture, protein, lipid, ash, and soluble carbohydrates) of the edible Azorean macroalgae (EAM).[a]

EAM	Humidity (% of FW)	Protein (% of DW)	Lipid (% of DW)	Ash (% of DW)	Carbohydrates (% of DW)
Fucus spiralis	87.7	9.71 ± 0.03	5.23 ± 0.03	22.31 ± 0.38	17.59 ± 0.27
Porphyra sp.	87.1	24.82 ± 0.05	8.88 ± 0.05	28.16 ± 0.52	25.37 ± 0.29
Osmundea pinnatifida	90.6	20.79 ± 0.12	7.53 ± 0.07	38.55 ± 0.68	17.61 ± 0.39

[a] Values are mean ± SD ($n = 3$). FW, fresh weight. DW, dry weight.

Table 2
Mineral content of edible Azorean macroalgae (mg/100 g dry weight) compared to some whole foods.[a]

Macroalgae/Foods	Na	K	Mg	Cs	Ca	Na/K
Fucus spiralis	1429 ± 4.20	975.9 ± 3.5	163.2 ± 0.7	109.9 ± 0.3	118.1 ± 0.4	1.46
Porphyra sp.	2382.6 ± 7.8	2481.1 ± 10.8	396.4 ± 2.9	97.3 ± 0.2	124.5 ± 0.5	0.96
Osmundea pinnatifida	2669.2 ± 9.3	1464.2 ± 4.5	418.6 ± 2.1	421.6 ± 2.5	411.5 ± 2.7	1.82
Brown rice[b]	28.0	1160.0	520.0	–	110.0	0.024
Whole milk[b]	55.0	140.0	11.0	–	115.0	0.39
Cheddar cheese[b]	670.0	77.0	25.0	–	720.0	8.70
Sirloin steak[b]	49.0	260.0	16.0	–	9.0	0.19
Bananas[b]	1.0	400.0	340.0	–	6.0	0.003
Peanuts[b]	2.0	670.0	210.0	–	60.0	0.003

[a] Values are mean ± SD (n = 3).
[b] Values for whole foods from McCance et al. (1993) in mg/100 g weight.

amino acids in 30 min. The repeatability of the retention times were evaluated in repetitive injections (n = 5) and ranged from 1.87% to 2.35%, indicating a high degree of repeatability. The accuracy of the three different amino acids (serine, methionine and lysine – short, medium and long retention times) were evaluated by the determination of the recoveries calculated based on the difference between the total concentration determined in the spiked samples and the concentration observed in the non-spiked samples. The results show the RSD better than 3.45% and the mean recovery ranged from 96.5% to 99.5%, indicating a high degree of the method accuracy under the analytical condition used.

Table 3 shows the profiles of the EAM amino acids which contain all of the essential amino acids (EAA) in different proportions, except for tryptophan, which was destroyed during acid hydrolysis. The EAA percentage of the total amino acids were 41.62%, 56.73% and 63.50% for *O. pinnatifida*, *Porphyra* sp. and *F. spiralis*, respectively. These values were higher than showed by Sánchez-Machado et al. (2003) that reported 39.5% of EAA for *Porphyra* sp., 37.0% for *Palmaria* sp. and 38.5% for *U. pinnatifida*. In comparison with some food this results showing that *O. pinnatifida* have similar EAA content than casein (43.6%) and *Porphyra* sp. and *F. spiralis* present higher EAA content than the casein, leguminous plants (45.4%) and ovalbumin (52.4%). The highest EAA was leucine (18.45 ± 0.27 mg/g of protein) found in *Porphyra* sp. and the lowest amino acid was histidine (0.91 ± 0.03 mg/g of protein) found in *O. pinnatifida*. The most limiting EAA were histidine, valine, arginine, methionine and lysine in *O. pinnatifida* as compared with that in *Porphyra* sp. and *F. spiralis*. The highest amount of the EAA valine, methionine, isoleucine, lysine, methionine, phenylalanine and arginine were found in *F. spiralis* as compared with that in *O. pinnatifida* and *Porphyra* sp. The comparison of the EAM amino acid profile with the protein standards (casein, ovalbumin and leguminous plants) show that the concentration of glutamic acid (21.1%) in casein, and arginine (13–14%) in ovalbumin are higher than those found for all the EAM. The high levels of aspartic and glutamic acids in EAM are responsible for the special flavour and taste (Mabeau, 1991). All three EAM samples had similar non-essential amino acid profiles, showing however higher values in the Ochrophyta (*F. spiralis*) as compared with the Rhodophyta (*Porphyra* sp. and *O. pinnatifida*). The levels of EAA of all the three EAM were comparable to those of the FAO/WHO (1991) requirement pattern.

3.7. Fatty acids (FA)

Table 4 shows the FA profiles of the EAM samples, and the different FA groups, in percentage of the total FAME. The SFA, palmitic acid (C16:0) was the most abundant FA in the EAM with exception of *F. spiralis* for which the most abundant FA was oleic acid (C18:1, *n*9). The total MUFA of EAM ranged from the lowest value of 17.31 ± 0.92% followed by 20.64 ± 1.09% to the highest value of 27.10 ± 1.44% of the total FAME, for *Porphyra* sp., *O. pinnatifida* and *F. spiralis*, respectively. Oleic acid (C18:1, *n*9) was the predominant FA within this group showing the values of 8.65 ± 0.67%, 14.19 ± 1.13% and 21.04 ± 1.67% of the total FAME for *Porphyra*

Table 3
Amino acid profiles of edible Azorean macroalgae (mg of amino acids per g of protein) and some tradition foods (%).[a]

Amino Acid (AA)	*Osmundea pinnatifida*	*Porphyra* sp.	*Fucus spiralis*	Casein standard[b]	Ovalbumin[c]	Leguminous plant[c]
Aspartic acid	13.69 ± 0.20	13.73 ± 0.25	5.62 ± 0.09	6.4 ± 0.47	4.7–5.4	6.2
Glutamic acid	12.17 ± 0.18	10.30 ± 0.15	12.12 ± 0.10	21.1 ± 1.78	6.4–8.7	9.9
Serine	2.72 ± 0.07	5.55 ± 0.13	11.47 ± 0.12	4.8 ± 0.31	–	6.8
Glycine	2.56 ± 0.06	7.19 ± 0.07	7.45 ± 0.05	1.6 ± 0.16	–	3.4
Histidine[d]	0.91 ± 0.03	2.33 ± 0.06	3.20 ± 0.03	4.0 ± 0.29	4.1	1.2–1.4
Arginine[d]	3.78 ± 0.02	5.93 ± 0.05	11.72 ± 0.10	3.7 ± 0.27	13–14	11.7
Threonine[d]	5.17 ± 0.26	11.21 ± 0.10	10.85 ± 0.09	3.7 ± 0.28	3.0	4.0
Alanine	0.79 ± 0.01	3.17 ± 0.02	4.06 ± 0.05	2.6 ± 0.21	–	6.7
Proline	15.88 ± 0.15	6.12 ± 0.04	6.90 ± 0.07	11.3 ± 1.03	–	2.8
Tyrosine	2.09 ± 0.07	5.27 ± 0.06	7.78 ± 0.08	4.1 ± 0.29	2.3–2.6	1.8
Valine[d]	2.22 ± 0.03	7.15 ± 0.04	11.14 ± 0.12	6.0 ± 0.43	5.4	4.5
Methionine[d]	1.95 ± 0.06	2.05 ± 0.05	6.36 ± 0.06	0.5 ± 0.04	3.1	3.8–4.0
Leucine[d]	16.54 ± 0.08	18.45 ± 0.27	15.46 ± 0.19	8.8 ± 0.61	4.8	7.3
Isoleucine[d]	nd	8.20 ± 0.11	15.31 ± 0.20	4.7 ± 0.42	6.2	3.6
Phenylalanine[d]	2.20 ± 0.09	3.73 ± 0.02	9.82 ± 0.08	4.8 ± 0.39	4.1	2.4
Lysine[d]	2.77 ± 0.03	8.26 ± 0.12	12.54 ± 0.12	7.4 ± 0.51	7.7	6.4–6.5
Triptofan[d]	nd	nd	nd	–	–	–
EAA (%)	41.62	56.73	63.50	43.6	52.4	45.4

[a] Values are mean ± SD (n = 3). nd, not detected. EAA, essential amino acids.
[b] Referred in Barbarino and Lourenço (2005).
[c] Referred in Fleurence (1999).
[d] EAA for humans.

Table 4
Gas chromatography determination of fatty acid composition (% of total FAME) of edible Azorean macroalgae (EAM).[a]

Fatty acids (FAME)	TR of FAME (min)	EAM		
		Fucus spiralis	*Porphyra* sp.	*Osmundea pinnatifida*
Lauric, C12:0	3.556	tc	tc	tc
Tridecanoic, C13:0	4.525	11.73 ± 0.93	3.27 ± 0.26	8.35 ± 0.66
Myristic, C14:0	5.75	1.29 ± 0.10	1.56 ± 0.12	0.71 ± 0.06
Myristoleic, C14:1 *c*9 (*n*5)	6.271	0.60 ± 0.05	0.74 ± 0.06	0.51 ± 0.05
Pentadecanoic, C15:0	7.207	0	0	0
Pentadecenoic, C15:1 *c*10 (*n*5)	7.811	0	0	0
Palmitic, C16:0	8.903	18.77 ± 1.49	47.41 ± 3.51	45.93 ± 3.41
Palmitoleic, C16:1 *c*9 (*n*7)	9.309	1.10 ± 0.08	2.75 ± 0.22	1.99 ± 0.16
Heptadecanoic, C17:0	10.623	tc	tc	tc
Heptadecenoic, C17:1 *c*10 (*n*7)	11.087	1.44 ± 0.12	tc	tc
Stearic, C18:0	12.487	0.77 ± 0.06	2.08 ± 0.16	1.15 ± 0.09
Oleic, C18:1 *c*9 (*n*9)	12.845	21.04 ± 1.67	8.65 ± 0.67	14.19 ± 1.13
Cis-7-Octadecenoic, C18:1 *c*7 (*n*11)	12.939	tc	5.17 ± 0.41	3.95 ± 0.31
Linolelaidic, C18:2 *t*9,12 (*n*6)	13.666	6.36 ± 0.51	4.24 ± 0.38	1.03 ± 0.08
Linoleic (LA), C18:2 *c*9,12 (*n*6)	13.759	tc	tc	tc
Arachidic, C20:0	14.233	0.47 ± 0.04	tc	tc
γ-Linolenic (GLA), C18:3 *c*6,9,12 (*n*6)	14.868	9.35 ± 0.74	8.74 ± 0.69	0.32 ± 0.03
Eicosenoic, C20:1 *c*11 (*n*9)	16.196	tc	0	tc
α-Linolenic (ALA), C18:3 *c*9,12,15 (*n*3)	16.516	tc	5.37 ± 0.43	tc
Heneicosanoic, C21:0	17.381	0.56 ± 0.05	tc	tc
Eicosadienoic, C20:2 *c*11,14 (*n*6)	17.871	0.34 ± 0.03	tc	0.23 ± 0.02
Behenic, C22:0	18.004	tc	tc	tc
Dihomo-γ-linolenic (DHGLA), C20:3 *c*8,11,14 (*n*6)	18.289	14.30 ± 1.13	3.39 ± 0.27	4.65 ± 0.37
Erucic, C22:1 *c*13 (*n*9)	18.577	tc	tc	tc
Eicosatrienoic, C20:3 *c*11,14,17 (*n*3)	19.484	11.69 ± 0.93	3.19 ± 0.25	16.35 ± 1.29
Arachidonic (AA), C20:4 *c*5,8,11,14 (*n*6)	19.812	0.43 ± 0.03	1.34 ± 0.11	tc
Tricosanoic, C23:0	20.143	0	0	0.64 ± 0.05
Docosadienoic, C22:2 *c*13,16 (*n*6)	20.991	0.52 ± 0.04	1.74 ± 0.14	tc
Lignoceric, C24:0	21.526	0	tc	0
Eicosapentaenoic (EPA), C20:5 *c*5,8,11,14,17 (*n*3)	23.263	1.05 ± 0.08	tc	0
Nervonic, C24:1 *c*15 (*n*9)	23.579	2.92 ± 0.23	0	0
Docosahexaenoic (DHA), C22:6 *c*4,7,10,13,16,19 (*n*3)	35.159	1.29 ± 0.11	1.19 ± 0.09	0.68 ± 0.05
Fatty acids groups				
Saturated FA (SFA)		33.59 ± 1.79	54.32 ± 2.53	56.78 ± 2.64
Monounsaturated FA (MUFA)		27.10 ± 1.44	17.31 ± 0.92	20.64 ± 1.09
Polyunsaturated FA (PUFA)		38.97 ± 2.08	24.96 ± 1.33	22.23 ± 1.18
C18 PUFA		15.71 ± 0.84	18.35 ± 0.98	1.35 ± 0.07
C20 PUFA		27.81 ± 1.48	7.92 ± 0.42	21.23 ± 0.13
t FA (TFA)		6.36 ± 0.34	4.24 ± 0.23	1.03 ± 0.06
*n*3		14.03 ± 0.75	9.75 ± 0.52	17.03 ± 0.91
*n*6		24.94 ± 1.33	15.21 ± 0.81	5.20 ± 0.28
*n*9		23.93 ± 1.24	8.65 ± 0.46	14.19 ± 0.76
*n*6/*n*3		1.78	1.56	0.31
h/H		3.29	0.86	0.92

[a] Values are mean ± SD (*n* = 3). tc, trace. 0, compound not detected in sample. RT, retention time. FAME, fatty acids methyl esters. *c*, cis. *t*, trans. *n*, omega. *n*6/*n*3, omega 6 to omega 3 PUFA ratio. h/H, hypocholesterolemic (MUFA + PUFA) to hypercholesterolemic (C14:0 + C16:0) FA ratio.

sp., *O. pinnatifida* and *F. spiralis*, respectively. The total PUFA ranged from the lowest value of 22.23 ± 1.18% followed by 24.96 ± 1.33% to the highest value of 38.97 ± 2.08% of the total FAME for *O. pinnatifida*, *Porphyra* sp. and *F. spiralis*, respectively. The EPA was found only in *F. spiralis* (1.05 ± 0.08% of total FAME) and DHA were found in the three EAM samples with the values of 0.68 ± 0.05%, 1.19 ± 0.09% and 1.29 ± 0.11% of the total FAME for *O. pinnatifida*, *Porphyra* sp. and *F. spiralis*, respectively. GLA and eicosatrienoic acid (C20:3, *n*3) were present in all species, the latter being the most abundant PUFA in *F. spiralis* and *O. pinnatifida*. These results are in general in agreement with the ones published by Patarra et al. (2013). The SFA content ranged from the 33.59 ± 1.79% followed by 54.32 ± 2.53% to 56.78 ± 2.64% of the total FAME for *F. spiralis*, *Porphyra* sp. and *O. pinnatifida*, respectively. The ratio of the series *n*6 FA and *n*3 FA ranged from the 0.31 followed by 1.56 to the highest value of 1.78 for *O. pinnatifida*, *Porphyra* sp. and *F. spiralis*, respectively. These values were similar to the reported by Sanchez-Machado et al. (2004) that presented *n*6/*n*3 ratio of 1.21 for *Porphyra* spp., 0.13 for *Palmaria* spp. and 0.49 for *U. pinnatifida*. The ratio of h/H ranged from 0.86 followed by 0.92 to the highest value of 3.29 for *Porphyra* sp., *O. pinnatifida* and *F. spiralis*, respectively. The determination of the ratio h/H is important because the hypocholesterolemic FA (h) reduce the low density lipoproteins (LDL-Cholin), also known as bad cholesterol, and the hypercholesterolemic FA (H) increase it (Leite et al., 2007). Clinical studies described by several authors (e.g. Ginsberg et al., 1990)

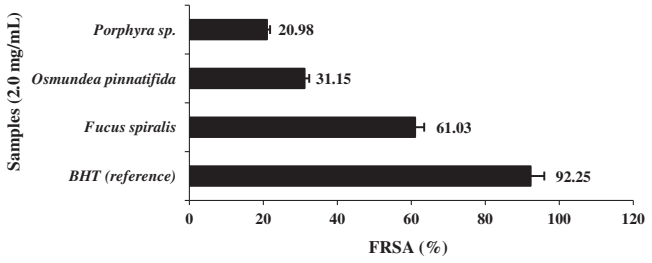

Fig. 1. Comparison of free radical scavenging activity (FRSA) of methanolic extract from edible Azorean macroalgae (EAM) with standard butylated hydroxytoluene (BHT) at 2.0 mg/mL, after 30 min reaction.

Table 5
Fat soluble vitamins content of edible Azorean macroalgae (mg/100 g dry weight) compared to some whole foods.[a]

	A	D2	D3	α-Toc	δ-Toc	γ-Toc	K1	K3
Fucus spiralis	1.41 ± 0.01	0.21 ± 0.02	0.83 ± 0.02	51.14 ± 0.27	tc	tc	tc	tc
Porphyra sp.	1.27 ± 0.04	0.17 ± 0.02	1.05 ± 0.01	27.34 ± 0.16	1.01 ± 0.01	0.18 ± 0.01	tc	tc
Osmundea pinnatifida	1.20 ± 0.02	tc	nd	4.86 ± 0.02	tc	14.19 ± 0.10	0.92 ± 0.02	1.64 ± 0.01
Oats	–	–	–	1.49[b]	–	–	–	–
Olive oil	–	–	–	8–14[c]	–	–	–	–
Cod liver oil	28[c]	–	0.23[c]	8–14[c]	–	–	–	–
Eggs	–	–	0.03[c]	–	–	–	–	–
Spinach	–	–	0.3–0.8[c]	–	–	–	–	–

[a] Values are mean ± SD (n = 3). Toc, tocoferol. tc, trace. nd, not detected.
[b] Referred in Roeck-Holtzhauer et al. (1991).
[c] Referred in Panfili et al. (2003).

have shown that diets rich in MUFA and PUFA and low in SFA reduce the total cholesterol and LDL-Cholin plasma. The EAM exhibited an interesting ratio h/H, suggesting they have a high nutritional value from the FA point of view.

3.8. Antioxidants

The antioxidant activity of the EAM compounds is mainly due to their redox properties, which can play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides. The extracts react with the DPPH in solution that converts it to 1,1-diphenyl-2-picrylhydrazine, due to its hydrogen-donating ability at a very rapid rate. Fig. 1 shows the FRSA results of the EAM samples that were analysed in triplicate. The greatest anti-free radical activity after 30 min reaction was observed for *F. spiralis* (61.03 ± 4.29%) followed by *O. pinnatifida* (31.15 ± 2.42%) and *Porphyra* sp. (20.98 ± 1.62%). These results are very similar to the reported by Paiva, Patarra, Neto, Lima, and Baptista (2012), and particularly the *F. spiralis* presents a higher FRSA as compared with the known and well recognized antioxidant green tea from *Camellia sinensis* (53.5%) (Rego, Paiva, Lima, & Baptista, 2011). The SD of FRSA was lower than 5.21% that reveals good repeatability. The FRSA results of all EAM samples show slightly increased values with increasing reaction time. The knowledge of the kinetics of the DPPH consumption is also important because free radicals in the organism are short-lived species, what implies that the impact of a substance as an antioxidant depends on its fast reactivity towards free radicals. Above 90% can be considered as a full absorption inhibition of DPPH, because after completing the reaction the final solution always possesses some yellowish colour and therefore its absorption inhibition compared to colourless methanol solution cannot reach 100%. The BHT, used in the same conditions, as a positive control, presents a FRSA of 92.25 ± 5.21%. The results of antioxidant properties show that EAM revealing to be a valuable species that could be explored from biotechnology and commercial perspectives.

3.9. Fat soluble vitamins

The reverse-phase HPLC separation of the fat soluble vitamins, referred in methods, provide an efficient separation of all vitamins. The repeatability of the retention times were evaluated in repetitive injections (n = 5) and ranged from 1.37% to 2.35%, indicating a high degree of repeatability. The accuracy of the three different vitamins (vitamin A, vitamin D3 and vitamin K1 – short, medium and long retention times) were evaluated by the determination of the recoveries calculated based on the difference between the total concentration determined in the spiked samples and the concentration observed in the non-spiked samples. The results show the RSD better than 3.15% and the mean recovery ranged from 97.5% to 99.2%, indicating a high degree of the method accuracy under the analytical condition used.

The content of fat-soluble vitamins in EAM, shown in Table 5, reveals a vitamin A content of 1.41, 1.27 and 1.20 mg/100 g of DW for *F. spiralis*, *Porphyra* sp. and *O. pinnatifida*, respectively. The vitamin A content was found in all three EAM species at moderate amount, i.e., about 12 times lower than the vitamin A-rich vegetable such as carrot (16.9 mg/100 g). The EAM vitamin E content presents 51.14, 27.34 and 4.86 mg/100 g of DW for *F. spiralis*, *Porphyra* sp. and *O. pinnatifida*, respectively, in contrast with Ito and Hori (1989) that point out that sublittoral brown seaweed, like *F. spiralis*, are rather poor in vitamin E. Similar vitamin E content were reported by Ortiz et al. (2009) that reported values of 8.65 and 45.35 mg/100 g of DW for *Gracilaria chilenis* and *Codium fragile*, respectively. It is well known that vitamin E plays an important role in health helping to inhibit LDL oxidation and prostaglandin and tromboxan formation. *F. spiralis* shows the highest α-tocopherol content that is 34 times higher than in oats (1.49 mg/100 g) and 4 times higher than in olive oil (12 mg/100 g) (Panfili, Fratianni, & Irano, 2003; Roeck-Holtzhauer, Quere, & Claire, 1991). According to Sánchez-Machado et al. (2002) the Phaeophyta algae contain more α-tocopherol than Rhodophyta that is also shown in our results.

4. Conclusions

The habitat of marine algae varies from species to species and many of them are exposed to direct sunlight in different aqueous environments. As a result they produce many compounds at different levels that will have some effect on human health. The EAM samples evaluated in this study were shown to be a rich sources of proteins, vitamins, polyunsaturated fatty acids, minerals, high ash content (that can contribute with important microelements to human and animal nutrition), and also contain acceptable amounts of 9 out of 10 EAA as compared to terrestrial foodstuffs. The antioxidant properties, FA profiles (MUFA and PUFA), the n3/n6 and h/H ratios and also the non-animal nature of the EAM nutrients lends them for use in many food supplements and/or pharmaceutical products that will augment a nutritionally balanced diet. As a result, the regular consumption of EAM allied with the low level of seawater pollution around the Azores Islands will improve human health conditions and will have a protective effect on the most common degenerative diseases. In addition, the EAM with high protein levels associated with other important nutrients may also be used in the production of foods for fish farmed by aquaculture. According to Mustafa, Wakamatsu, Takeda, Umino, and Nakagawa (1995) a *Porphyra* meal used as a food additive in fish feed appeared to improve body weight gain and increase triglyceride and protein deposit in muscles as well as improve the resistance of fish to stress or diseases.

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