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Health-promoting ingredients from four selected Azorean macroalgae

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ABSTRACT

This study presents, for the first time, the nutritional and health promoting aspects of four selected Azorean macroalgae (*Ulva compressa*, *Ulva rigida*, *Gelidium microdon* and *Pterocladiella capillacea*) in terms of total lipids, fatty acids (FA) profile, *n6*/*n3* and hypocholesterolemic (h)/hypercholesterolemic (H) FA ratios, minerals, total essential amino acids (AA), anti-ageing and anti-phenylketonuria AA content, coenzyme Q_{10}, α-tocopherol, total phenolics, antioxidant properties and energy value, on a dry weight basis. The results revealed low lipid content (1.02–4.32%) but significant PUFA content (29.57–69.22% of total FA), suitable FA ratios (0.48–9.49 for *n6*/*n3* and 1.26–4.22 for h/H), balanced macromineral ratios (0.27–1.91 for Na/K and 0.15–1.07 for Ca/Mg), appreciable amount of essential AA (45.27–58.13% of total AA), high amount of anti-ageing AA, low Phe content, coenzyme Q_{10} (1.25–8.27 μg/g), α-tocopherol (2.61–9.14 mg/100 g), high total phenolic content (27.70–55.07 mg of gallic acid equivalents/g extract), significant free radical scavenging activity (29.32–47.73%) and valuable energy content (6.80–9.80 kJ/g). A regular consumption of these algae either directly or through food supplements may improve human health or may have a protective effect on some diseases and ageing process. They can also be used for producing pharmaceuticals and cosmeceuticals with potential economic value.

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1. Introduction

In Asian countries, marine macroalgae (seaweeds) have been consumed as sea vegetables for centuries (documented since 600 BCE) while in the Western countries they are traditionally used as sources of phycocolloids (e.g. agar, carrageenans and alginates) or meal for animal nutrition. Nowadays, edible seaweeds are increasingly consumed in Europe, especially in France, the first European country to regulate the use of *Ulva* species as foods and condiments for humans. In Spain, seaweeds are considered novel foods (Bocanegra, Bastida, Benedí, Ródenas, & Sánchez-Muniz, 2009; Lordan, Ross, & Stanton, 2011; Smith, 2004). In fact, seaweeds are increasingly being recognized as an excellent source of health-promoting compounds, including proteins with high nutritional value (Fleurence, 1999), bioactive peptides (Harnedy & FitzGerald, 2013), dietary fiber with good balance of both insoluble and soluble fibers (Ortiz et al., 2006), polysaccharides, polyunsaturated fatty acids, minerals, vitamins and polyphenols, that may have protective effects against cancer, allergy, diabetes, oxidative stress, inflammation, thrombosis, obesity, lipidemia, hypertension and other degenerative disorders. However, besides the numerous research reports on their therapeutic properties, seaweeds remain a relatively underutilized sustainable resource for use as a natural complementary and alternative therapy to commercial synthetic drugs against certain chronic diseases (Mohamed, Hashim, & Rahman, 2012). Taking into account the current increasing demand for seaweed food products, several reports have appeared on the nutritional composition of edible seaweeds from some coastal areas. However, limited published data is available on the amount of important nutrients and their potential use. It is well known that among the various compounds found in seaweeds, antioxidants (such as carotenoids, tocopherols and polyphenols) have been attracted the attention of scientific community (Mohamed et al., 2012). However, the available information on coenzyme Q_{10} content is scarce (Klein, Walter, Lange, & Buchholz, 2012) despite its strong antioxidant properties. Concerning the macroalgal mineral composition, the dietary intake ratios of Na/K and Ca/Mg are also rarely highlighted, as well as the h/H fatty acids ratios, despite its importance on human health. Macroalgal specific amino acids, such as phenylalanine content, with impact on the diet of phenylketonuria patients, and the anti-ageing amino acids content are also poorly explored in previous reports. Rarely mentioned is also the macroalgal energy value (Renaud & Luong-Van, 2006), despite its importance in human and animal nutrition.

The Azores Islands (Portugal), due to their isolation in the middle of the Atlantic Ocean, are a very promising location for marine natural resources that may produce unique bioactive compounds. Indeed, like

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most flora, the chemical composition of seaweeds, and consequently their nutritional and medicinal value, depends on many factors, including species and its developmental stage, geographical location, habitat, season, climate and environmental conditions, among others (e.g. Fleurence, 1999). Seaweeds are abundant and structuring organisms on the coastal areas of the Azores Islands, known as environmentally healthy habitats (Neto et al., 2009). Some species have a markedly seasonal pattern whereas others are available throughout the year (Neto, Tittley, & Raposeiro, 2006). Traditionally, the Azorean population have gathered seaweeds to use as fertilizers in local agriculture and/or horticulture, and also to eat (e.g. *Fucus spiralis*, *Porphyra*, *Laurencia*, *Osmundea* and *Ulva*) or for agar production (e.g. *Gelidium microdon* and *Pterocladiella capillacea*) (Neto et al., 2006). However, little information is available on their nutritional and/or pharmacological value. To the best of our knowledge, there is only one full report on the nutritional composition of *F. spiralis*, *Porphyra* sp. and *O. pinnatifida* (Paiva, Lima, Patarra, Neto, & Baptista, 2014) and two detailed pharmacological studies: one dealing with the isolation of secondary metabolites from *U. rigida* and *G. microdon* and the evaluation of their antitumor and antimicrobial activities (Silva et al., 2013), and the other reporting the isolation and characterization of angiotensin I-converting enzyme (ACE) inhibitory peptides from *U. rigida* protein hydrolysate (Paiva, Lima, Neto, & Baptista, 2016).

As a result, the aim of the present study was to investigate, for the first time, the nutritional aspects, the health promoting ingredients content and the antioxidant activity of the Azorean locally abundant *Ulva compressa*, *Ulva rigida*, *G. microdon* and *P. capillacea*, in order to evaluate their use as functional foods and/or for producing health-care products for the pharmaceutical, medical, cosmetic and food industries.

2. Material and methods

2.1. Chemicals and reagents

Acetonitrile (ACN), dichloromethane, methanol and hexane, HPLC grade, were purchased from Fluka Chemika (Steinheim, Switzerland). Ethanol, acetone, sodium chloride, sodium sulphate anhydrous, potassium hydroxide, phenol, triethylamine (TEA), ascorbic acid, boric acid, Kjeldahl catalyst, sulphuric acid, standard glucose, ammonium acetate, hydrochloric acid and trichloroacetic acid (TCA) were obtained from E. Merck (Darmstadt, Germany). Derivatization reagent (14% boron trifluoride in methanol) and certified preformulated multi-standard cation kit were purchased 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)”, coenzyme Q_{10}, α-tocopherol, 2,2-diphenyl-1-picrylhydrazyl (DPPH), butylated hydroxyltoluene (BHT), gallic acid, Folin-Ciocalteu reagent (2 N), sodium carbonate anhydrous, sodium acetate trihydrate ($CH_3COONa.3H_2O$), disodium hydrogen phosphate (Na_2HPO_4), sodium hydroxide, ammonium sulphate and methanosulfonic acid were obtained from Sigma-Aldrich (St. Louis, MO, USA). Amino acid (AA) standard mixture, sequanal grade 6 N HCl and phenyl isothiocyanate (PITC) were purchased from Pierce Chemicals (Rockford, IL, USA). All other reagents used in this study were reagent grade chemicals. Deionised water was obtained from a Milli-Q water purification and filtration system with an 18 MΩ.cm resistivity (Millipore, Bedford, MA, USA). All reagents, eluents and buffers were filtered through 0.2 μm membranes.

2.2. Macroalgae collection

All samples used in this study were collected from the littoral of São Miguel Island (37°40′ N and 25°31′ W), and voucher specimens were prepared and deposited in the Herbarium AZB – Ruy Telles Palhinha of the Department of Biology at the University of Azores. The Rhodophyta *Gelidium microdon* Kϋtzing (SMG-13-03) and *Pterocladiella capillacea* (S.G. Gmelin) Santelices & Hommersand (SMG-13-05) were collected in January 2013, and the Chlorophyta *Ulva compressa* Linnaeus (SMG-13-15) and *Ulva rigida* C. Agardh (SMG-13-12) in April 2013.

2.3. Macroalgae samples preparation

Within 24 h of collection the algal samples were first washed in seawater to remove encrusting material and epiphytes, carefully rinsed with distilled water to remove salts, partially dried with a paper towel, and then air-dried and stored in an air-tight container in a freezer (−80 °C) for not >6 months until further analysis. Prior to the analytical procedures, the samples were defrosted and dried at 40–45 °C during 48 h (avoiding overheating that could lead to oxidation). Dried triplicate samples were grounded into a fine powder of 0.5 mm particle size, redried at 40 °C and stored in dark under N_2 in desiccators at a refrigerated temperature of 4–5 °C. The moisture content of the fresh algal samples *U. compressa*, *U. rigida*, *G. microdon* and *P. capillacea* were 90.00 ± 0.91%, 84.50 ± 0.70%, 83.20 ± 0.88% and 86.00 ± 0.65%, respectively, calculated by subtracting the weight of dry sample from the wet weight.

2.4. Nutrient analysis

2.4.1. Total lipid determination and fatty acids (FA) profile

The crude lipid content was determined gravimetrically after soxhlet extraction (AOAC, 2000) during 4 h of reflux with chloroform:methanol 2:1 (v/v) following the Folch, Lees, and Solam-Stanley (1957) methodology. To characterize the lipid profile, a cold extraction with chloroform:methanol (2:1, v/v) in the absence of light to minimize lipids oxidation was adopted (Folch et al., 1957). For FA profile determination, the sample was transmethylated using 0.5 N potassium hydroxide methanol solution and derivatized with 14% boron trifluoride in methanol according to the modified protocol of Leite, Lima, and Baptista (2007). Fatty acids methyl esters (FAME) were analysed by GC and GC/MS on a fused silica CP-Wax 58 (FFAP) CB column (25 m × 0.25 mm i.d., 0.20 μm film thickness) from Varian (Palo Alto, CA, USA), using the analytical conditions previously described by Paiva et al. (2014).

2.4.2. Mineral determination

For mineral determination the macroalgae samples (dry powder) were prepared as previously described by Paiva et al. (2014), digested with hydrochloric acid followed by SCAN 1000 to neutralize the acid (Alltech Associates protocol – Deerfield, IL, USA), and then analysed by ion chromatography on an universal cation exchange free-metal column (10 cm × 4.6 mm i.d., 7 μm particle size) from Alltech Associates (Deerfield, IL, USA), using the conditions described by the referred authors. 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 of the algae mineral content.

2.4.3. Amino acids (AA) determination

The protein was precipitated with TCA and the protein pellet was recovered by centrifugation following the Barbarino and Lourenço (2005) methodology. The protein pellet was hydrolyzed using 6 N HCl and derivatized with PITC, as previously described (Paiva et al., 2014; Sánchez-Machado, López-Cervantes, López-Hernández, Paseiro-Losada, & Simal-Lozano, 2003). The AA profile was performed by HPLC analysis on a HP Aminoquant reverse-phase column (20 cm × 2.1 mm i.d., 5 μm particle size), using the analytical conditions described by Paiva et al. (2014), and the AA content was expressed as mg/g of protein.

2.4.4. Coenzyme Q_{10} determination

For coenzyme Q_{10} determination the macroalgae samples were prepared according to Rujiralai, Raekasin, Cheewasedtham, and Cheewasedtham (2014), and then analysed by HPLC on a Zorbax Eclipse

XBD-C18 reverse phase analytical column (25 cm × 4.6 mm i.d., 5 μm particle size) (Agilent Technologies, Santa Clara, CA, USA) using an isocratic elution of MeOH:Ethanol (50:50, v/v) at a constant flow-rate of 0.8 mL/min and UV detection at 275 nm. Peak identification was assigned by retention time, chromatographic pattern, spiked of the authentic standard to the sample and also confirmed by superimposing the UV spectra of the peak with the corresponding coenzyme Q_{10} standard spectrum. The detector signal was recorded and processed (peak area determination) by Agilent Technologies ChemStation software, using an external standard methodology.

2.4.5. Vitamin E (α-tocopherol) determination

For vitamin E determination the macroalgae samples were prepared as previously described by Paiva et al. (2014), and then analysed by HPLC on a Zorbax Eclipse XBD-C18 reverse phase analytical column (25 cm × 4.6 mm i.d., 5 μm particle size) (Agilent Technologies, Santa Clara, CA, USA) using a linear gradient of phase (A) MeOH:H_2O (90:10, v/v) and phase (B) ACN (t = 0–3 min – 10% B, t = 12 min – 80% B and t = 17 min – 99% B) at a constant flow-rate of 0.6 mL/min and UV detection at 280 nm. The quantification method as referred in Section 2.4.4.

2.4.6. Total phenolic content (TPC) determination

The finely powdered dry samples were extracted with methanol, in the ratio of 1:10 (g/mL), at room temperature using a magnetic stirring bar for 2 h. The residue was re-extracted twice with methanol in the same conditions and extracts were combined and concentrated to dryness at 40 °C using a rotary evaporator under reduced pressure. TPC in macroalgae methanolic extracts was determined by using Folin–Ciocalteu colorimetric methodology according to Waterhouse (2002) with modifications, as previously described (Rainha et al., 2013). The TPC values were expressed as mg of gallic acid equivalents (GAE) per g of dry weight (DW) extract, after the reducing sugars interferences correction as stated by Waterhouse (2002).

2.4.7. Energy value determination

The energy content of the selected Azorean macroalgal biomass was determined following the methodology of Renaud and Luong-Van (2006) (a modification of the Brett and Groves (1979) method), multiplying the values obtained for protein (15.66–23.40% of DW), soluble carbohydrate (14.45–19.76% of DW) and lipid (1.02–4.32% of DW) by 23.86, 17.16 and 36.42 kJ/g, respectively. The protein and soluble carbohydrate contents of the macroalgae samples were determined as described by Paiva et al. (2014), and the lipid content as described in Section 2.4.1.

2.5. Biological activity assay

2.5.1. Free radical scavenging activity (FRSA) determination

The FRSA of the macroalgae samples was determined according to the method of Molyneux (2004) with slight modifications. An aliquot of 2.0 mL of the macroalgae sample solution 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). BHT was used as reference sample and a mixture without macroalgae sample or BHT was used as the control. The absorbance (Abs) was measured at 517 nm after 30 min in the dark. The FRSA of the macroalgae samples were calculated as a percentage of DPPH decolouration using the following equation: $\%FRSA = (1 - Abs_{sample} / Abs_{control}) \times 100$.

2.6. Statistical analysis

All determinations were performed at least in triplicate and the results 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.

The Pearson correlation analysis was performed between antioxidant activity and total phenolic contents.

3. Results and discussion

3.1. Nutrient analysis

3.1.1. Crude lipid content and fatty acids (FA) profile

The lipid content of the studied algae (see Table 1) showed a narrow range (1.02–4.32% of DW), that is within the range (0.30–7.20%) referred for several marine seaweed (Yuan, 2008). Although the lipid content of the studied macroalgae is relatively low, they contain a relative higher level of PUFA as compared to land vegetables (Darcy-Vrillon, 1993). The Table 1 shows the FA profiles and the different FA groups, in percentage of the total FAME (tFAME). The total SFA content was higher in *U. rigida* (46.79% of tFAME) followed by *P. capillacea* and *U. compressa* (39.09 and 37.57% of tFAME, respectively), and lower in *G. microdon* (23.27% of tFAME), being the palmitic acid (C16:0) the most abundant FA in the first three species. This acid was also reported to be the most abundant FA in Tunisian *U. rigida* (Frikha et al., 2011) and in other macroalgae (e.g. Sánchez-Machado, López-Cervantes, López-Hernández, & Paseiro-Losada, 2004). Concerning the total unsaturated FA (the dominant FA group in all studied algae), the MUFA content was higher in *U. compressa* (32.85% of tFAME) followed by *U. rigida* and *P. capillacea* (22.11 and 17.87% of tFAME, respectively), and lower in *G. microdon* (7.50% of tFAME). Within this group, *cis*-7-octadecenoic acid (C18:1*n*11) was the predominant FA for *U. compressa* (19.42% of tFAME) and *G. microdon* (4.19% of tFAME), while the oleic acid (C18:1*n*9), reported to decrease the level of LDL-cholesterol, was the major MUFA in *U. rigida* (15.99% of tFAME) and *P. capillacea* (9.15% of tFAME). Frikha et al. (2011) also reported that oleic acid was the major MUFA for Tunisian *U. rigida*. The PUFA content was higher in *G. microdon* (69.22% of tFAME) followed by *P. capillacea* (44.43% of tFAME), and lower in *U. rigida* and *U. compressa* (31.69 and 29.57% of tFAME, respectively). Among this group, γ-linolenic acid (C18:3*n*6) and arachidonic acid (C20:4*n*6), an eicosanoid precursor, were only found in the two *Ulva* species, being the γ-linolenic acid the most abundant PUFA in these algae (ca 17.0% of tFAME, for each algae). Dihomo-γ-linolenic acid (C20:3*n*6) and eicosatrienoic acid (C20:3*n*3) were the predominant PUFA in *G. microdon* (35.61 and 23.86% of tFAME, respectively) and *P. capillacea* (11.55 and 28.26% of tFAME, respectively). The eicosapentaenoic acid (EPA, C20:5*n*3), another eicosanoid precursor, was found only in traces and docosahexaenoic acid (DHA, C22:6*n*3) was found in all studied algae. Additionally, α-linolenic acid (ALA, C18:3*n*3) was only found in *G. microdon* (2.99% of tFAME). This amount is twice-higher than the linoleic acid (LA, C18:2*n*6) value, considered suitable for normal metabolism (optimal intake of LA relative to ALA) (Simopoulos, 1999).

The dietary *n*6/*n*3 FA ratio affects the regulation of metabolic functions and the development of metabolic syndrome, including insulin sensitivity, inflammation, lipid profiles and adiposity (Burghardt et al., 2010). The *n*6/*n*3 FA ratio values of 0.48, 1.46, 8.43 and 9.49 found for *P. capillacea*, *G. microdon*, *U. rigida* and *U. compressa*, respectively, are within the currently recommended values by WHO (Ortiz et al., 2006). Sánchez-Machado et al. (2004) also reported very low *n*6/*n*3 ratios for red macroalgae (0.13 to 1.21). Similar results were also reported earlier by Patarra, Leite, Pereira, Baptista, and Neto (2013) for *P. capillacea* (0.88). A healthy h/H FA ratio was also found for all species, namely 1.26, 1.90, 2.09 and 4.22 for *U. rigida*, *U. compressa*, *P. capillacea* and *G. microdon*, respectively. This h/H FA ratio is of fundamental importance because the hypocholesterolemic FA (h) reduces the low density lipoproteins (LDL) cholesterol, also known as bad cholesterol, whereas the hypercholesterolemic FA (H) increases it.

Table 1
Total lipid content (% dry weight) and gas chromatography determination of fatty acid composition (% of total FAME) of the studied macroalgae[a].

Total lipid and fatty acids contents	TR of FAME (min)	Macroalgae species			
		Ulva compressa	*Ulva rigida*	*Gelidium microdon*	*Pterocladiella capillacea*
Total lipids	–	1.67 ± 0.16	1.02 ± 0.09	2.44 ± 0.09	4.32 ± 0.33
Fatty acid	–	–	–	–	–
Lauric, C12:0	3.556	tc	tc	tc	tc
Tridecanoic, C13:0	4.525	0.83 ± 0.07	2.02 ± 0.06	1.81 ± 0.14	5.27 ± 0.49
Myristic, C14:0	5.75	1.72 ± 0.12	tc	0.86 ± 0.10	tc
Myristoleic, C14:1 *c*9 (*n*5)	6.271	tc	tc	tc	tc
Pentadecanoic, C15:0	7.207	tc	0	tc	tc
Pentadecenoic, C15:1 *c*10 (*n*5)	7.811	tc	0	tc	tc
Palmitic, C16:0	8.903	31.21 ± 1.98	42.76 ± 2.98	17.33 ± 1.50	29.79 ± 1.67
Palmitoleic, C16:1 *c*9 (*n*7)	9.309	1.24 ± 0.09	2.25 ± 0.21	1.66 ± 0.12	1.66 ± 0.14
Heptadecanoic, C17:0	10.623	2.41 ± 0.19	0	tc	tc
Heptadecenoic, C17:1 *c*10 (*n*7)	11.087	8.13 ± 0.56	0	tc	tc
Stearic, C18:0	12.487	0.62 ± 0.08	0	1.32 ± 0.09	2.52 ± 0.16
Oleic, C18:1 *c*9 (*n*9)	12.845	3.11 ± 0.12	15.99 ± 1.31	1.65 ± 0.33	9.15 ± 0.42
Cis-7-Octadecenoic, C18:1 *c*7 (*n*11)	12.939	19.42 ± 1.53	0	4.19 ± 0.40	3.04 ± 0.21
Linolelaidic, C18:2 *t*9,12 (*n*6)	13.666	7.35 ± 0.61	4.89 ± 0.41	1.34 ± 0.11	1.47 ± 0.11
Linoleic (LA), C18:2 *c*9,12 (*n*6)	13.759	tc	1.56 ± 0.10	1.45 ± 0.13	1.39 ± 0.09
Arachidic, C20:0	14.233	0.78 ± 0.10	0	tc	tc
γ-Linolenic (GLA), C18:3 *c*6,9,12 (*n*6)	14.868	16.76 ± 1.56	17.11 ± 1.03	tc	tc
Eicosenoic, C20:1 *c*11 (*n*9)	16.196	0.95 ± 0.05	2.7 ± 0.24	tc	4.02 ± 0.31
α-Linolenic (ALA), C18:3 *c*9,12,15 (*n*3)	16.516	tc	tc	2.99 ± 0.33	tc
Heneicosanoic, C21:0	17.381	tc	2.01 ± 0.19	1.95	1.51 ± 1.29
Eicosadienoic, C20:2 *c*11,14 (*n*6)	17.871	tc	tc	tc	tc
Behenic, C22:0	18.004	tc	0	tc	tc
Dihomo-γ-linolenic (DHGLA), C20:3 *c*8,11,14 (*n*6)	18.289	1.94 ± 0.30	tc	35.61 ± 2.56	11.55 ± 0.86
Erucic, C22:1 *c*13 (*n*9)	18.577	tc	1.17 ± 0.08	tc	tc
Eicosatrienoic, C20:3 *c*11,14,17 (*n*3)	19.484	2.16 ± 0.43	2.11 ± 0.27	23.86 ± 1.51	28.26 ± 1.42
Arachidonic (AA), C20:4 *c*5,8,11,14 (*n*6)	19.812	0.70 ± 0.04	2.76 ± 0.30	tc	tc
Tricosanoic, C23:0	20.143	tc	tc	tc	0
Docosadienoic, C22:2 *c*13,16 (*n*6)	20.991	tc	2.01 ± 0.21	2.63 ± 0.22	0
Lignoceric, C24:0	21.526	tc	0	tc	0
Eicosapentaenoic (EPA), C20:5 *c*5,8,11,14,17 (*n*3)	23.263	tc	tc	tc	0
Nervonic, C24:1 *c*15 (*n*9)	23.579	tc	tc	tc	0
Docosahexaenoic (DHA), C22:6 *c*4,7,10,13,16,19 (*n*3)	35.159	0.66 ± 0.03	1.25 ± 0.07	1.34 ± 0.09	1.76 ± 0.12
Total saturated fatty acids (SFA)	–	37.57 ± 2.19	46.79 ± 2.18	23.27 ± 1.41	39.09 ± 2.44
Total monounsaturated fatty acids (MUFA)	–	32.85 ± 1.84	22.11 ± 1.17	7.5 ± 0.81	17.87 ± 1.10
Total polyunsaturated fatty acids (PUFA)	–	29.57 ± 1.52	31.69 ± 1.42	69.22 ± 3.30	44.43 ± 2.17
Total C18 PUFA	–	24.11 ± 1.12	23.56 ± 0.99	5.78 ± 0.78	2.86 ± 0.11
Total C20 PUFA		4.80 ± 0.48	4.87 ± 0.49	59.47 ± 3.02	39.81 ± 2.22
Total trans fatty acids (TFA)	–	7.35 ± 0.63	4.89 ± 0.26	1.34 ± 0.20	1.47 ± 0.09
Total *n*3 fatty acids	–	2.82 ± 0.35	3.36 ± 0.18	28.19 ± 1.72	30.02 ± 2.01
Total *n*6 fatty acids	–	26.75 ± 1.55	28.33 ± 1.39	41.03 ± 2.54	14.41 ± 0.94
Total *n*9 fatty acids	–	4.06 ± 1.21	19.86 ± 1.05	1.65 ± 0.66	13.17 ± 0.73
Ratio *n*6/*n*3	–	9.49	8.43	1.46	0.48
Ratio h/H	–	1.90	1.26	4.22	2.09

[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.

The obtained results for *P. capillacea* and *G. microdon* indicated that they may be used in diets to improve a balanced *n*6/*n*3 FA ratio in order to prevent inflammatory, cardiovascular and nervous system disorders (Ortiz et al., 2006). *Ulva* species are more indicated for diets directed to reduce the LDL-cholesterol plasma (Taboada, Millán, & Míguez, 2010).

3.1.2. Mineral composition

In general the mineral content of marine algae is high, relatively to the most common vegetables (Ortega-Calvo, Mazuelos, Hermosín, & Saiz-Jimenez, 1993), due to the diversity of minerals in marine habitats (MacArtain, Gill, Brooks, Campbell, & Rowland, 2007). Significant differences in the amount of the macrominerals Na, K, Mg and Ca of the studied algae are shown in Table 2. The *U. rigida* and *U. compressa* show a superior content of Mg and Ca as compared with the other studied algae, and with some common foods. Similar findings were reported by Frikha et al. (2011) for Tunisian *U. rigida*. It is known that Ca helps to produce the heartbeat, and Mg regulates it. In other words, Ca and Mg require each other for their functions in heart muscle and must be provided to our body in balanced amounts (Rosanoff, 2010; Seelig & Heggtveit, 1974). The results of our study show that the red algae *G. microdon* and *P. capillacea* presented a well-balanced Ca/Mg ratio (approx. 1:1) as compared to some common foods. Our study also revealed a significant content of K, particularly for *P. capillacea* and *G. microdon*. In addition, the Na/K ratio of all studied algae shows a low value (<2), specially the *P. capillacea* (0.27), *G. microdon* (0.35) and *U. rigida* (0.70). Rupérez (2002) also reported low Na/K ratios, below 1.5, for the red macroalgae *Porphyra tenera* (1.04) and *Chondrus crispus* (1.34). It is well known that food intake of high Na/K ratio have been related to higher incidence of hypertension (Ortega-Calvo et al., 1993). Consuming *P. capillacea*, *G. microdon* and *U. rigida* as food supplement may, therefore, exert hypotensive effects.

3.1.3. Selected amino acids (AA) with specific functional properties

3.1.3.1. The algae with anti-ageing AA. Resulting from methionine and lysine (two essential AA), carnitine helps to boost mitochondrial function (Liu, Killilea, & Ames, 2002), and plays an important role during the mitochondrial decline contributing to anti-ageing activity within the body (Cao et al., 2011). Arginine, another essential AA during the first months of a baby life, is involved in many metabolic processes and is important in the treatment of heart diseases and high blood pressure. Arginine also

Table 2
Mineral content in the studied macroalgae (mg/100 g dry weight)[a] compared to some common whole foods, and Na/K and Ca/Mg ratios.

Macroalgae and foods	Minerals				Mineral ratios	
	Na	K	Ca	Mg	Na/K	Ca/Mg
Ulva compressa	1322.49 ± 3.17	693.32 ± 2.27	242.57 ± 1.15	1594.12 ± 3.11	1.91	0.15
Ulva rigida	576.08 ± 2.34	817.46 ± 3.81	324.93 ± 1.94	1775.13 ± 4.74	0.70	0.18
Gelidium microdon	433.08 ± 2.60	1238.98 ± 3.00	74.73 ± 0.15	127.01 ± 0.39	0.35	0.59
Pterocladiella capillacea	635.62 ± 3.26	2369.52 ± 10.45	174.00 ± 1.45	162.72 ± 1.25	0.27	1.07
Brown rice[b]	28.0	1160.0	110.0	520.0	0.02	0.21
Whole milk[b]	55.0	140.0	115.0	11.0	0.39	10.45
Cheddar cheese[b]	670.0	77.0	720.0	25.0	8.70	28.80
Sirloin steak[b]	49.0	260.0	9.0	16.0	0.19	0.56
Bananas[b]	1.0	400.0	6.0	34.0	0.003	0.02
Peanuts[b]	2.0	670.0	60.0	210.0	0.003	0.29

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

improves the circulation and strengthens the immune system (Gad, 2010), having, according to this author, a better anti-ageing benefits than any other specific nutraceutical or pharmaceutical product. Table 3 shows the selected AA of the studied algae and their potential impact on the ageing process. *U. compressa* presents the highest amount of methionine followed by *P. capillacea* and *G. microdon* (6.55, 4.21 and 4.17 mg/g protein, respectively). A similar result was observed for the amount of lysine (11.34, 5.34 and 5.32 mg/g protein, respectively). *U. compressa* presents also the highest amount of arginine followed by *G. microdon* and *P. capillacea* (14.11, 8.48 and 5.46 mg/g protein, respectively). Thus, supplements made of these three species may have an anti-ageing benefit.

3.1.3.2. Selected algae for patients with phenylketonuria disorder. It is well known that phenylketonuria (PKU) is a disorder that occurs when "phenylalanine hydroxylase" (PAH) enzyme is either not working properly or missing. Phenylalanine (Phe) is found in almost every food, except pure fat and sugar. The function of the PAH is to change the Phe into other substances. When a child with PKU eats food containing Phe, it builds up in the blood and causes problems such as intellectual disabilities, and treatment is necessary to prevent mental retardation (Sheard, 2000). Results on *U. rigida* (Table 3) reveal a very low Phe content (1.78 mg/g protein) and a reasonable content of total essential AA (48.38%) indicating that *U. rigida* can be an excellent food supplement for PKU patients.

3.1.4. Coenzyme Q_{10} content

Coenzyme Q_{10} (CoQ_{10}) is a lipid-soluble compound found in virtually all cells of human body (Battino et al., 1990) and is also supplied to the organism by various foods. CoQ_{10}, an antioxidant, has many positive effects: strengthens the heart muscle and the immune system, raises work capacity, prolongs life and acts as an essential electron carrier in the mitochondrial respiratory chain. With age, the amount of CoQ_{10} in our cells decreases and with most people over 35–40 there is a deficit. CoQ_{10} is now widely used as an important nutritional supplement and cosmetic additive (e.g. Ernster & Dallner, 1995; Jeya, Moon, Lee, Kim, & Lee, 2010). The assessed CoQ_{10} content of the studied algae (Table 4) shows values of 8.27, 1.59, 1.42 and 1.25 μg/g of DW for *P. capillacea*, *U. rigida*, *U. compressa* and *G. microdon*, respectively, comparable or superior to those of common foods (Mattila & Kumpulainen, 2001), such as some vegetables, fruits, dairy products and eggs. Thus, these algae can be used as complementary dietary sources of CoQ_{10} with beneficial health effects. *P. capillacea*, due to the superior CoQ_{10} content, may also be used in the development of pharmaceutical and/or cosmeceutical products.

3.1.5. Vitamin E (α-tocopherol) content

It is well known that vitamin E, which is exclusively obtained from the diet, is a powerful lipophilic antioxidant, like the referred CoQ_{10} compound (Ernster & Dallner, 1995), that help to inhibit LDL oxidation, and prostaglandin and tromboxan formation. According to some authors, brown algae contain α-, β- and γ-tocopherols, while green and red algae contains only α-tocopherol (Bocanegra et al., 2009). The content of α-tocopherol in the studied algae (Table 4) reveals values of 9.14, 4.22, 3.25 and 2.61 mg/100 g of DW for *U. compressa*, *G. microdon*, *U. rigida* and *P. capillacea*, respectively. These values are comparable or superior to those reported for some common foods (Sheppard, Weihrauch, & Pennington, 1992) and the level in *U. rigida* is also higher than the one reported for the same species from other location (1.97 mg/100 g of DW) (Taboada et al., 2010). All species may supplement diets improving the vitamin E intake.

3.1.6. Total phenolic content (TPC)

It is well known that natural antioxidants, like phenolic compounds, play an important role against various diseases (atherosclerosis, cancer, chronic inflammation, cardiovascular disorders, hypertension) and ageing process, directly related to oxidative stress (Oroian & Escriche, 2015). According to some studies (Heo, Cha, Lee, Cho, & Jeon, 2005;

Table 3
Selected amino acids content of the studied macroalgae (mg amino acid/g protein)[a] and content of essential amino acids (on a dry weight basis).

Amino acid (AA)	Macroalgae species			
	Ulva compressa	*Ulva rigida*	*Gelidium microdon*	*Pterocladiella capillacea*
Arginine[b]	14.11 ± 0.07	2.99 ± 0.02	8.48 ± 0.09	5.46 ± 0.06
Lysine[b]	11.34 ± 0.09	2.38 ± 0.04	5.32 ± 0.08	5.34 ± 0.06
Methionine[b]	6.55 ± 0.13	1.43 ± 0.04	4.17 ± 0.06	4.21 ± 0.09
Phenylalanine[b]	8.45 ± 0.06	1.78 ± 0.03	4.42 ± 0.05	5.24 ± 0.06
Total AA[c]	228.08	59.67	90.78	129.53
Total EAA[c]	110.77	28.87	52.77	58.64
EAA (%)	48.57	48.38	58.13	45.27

[a] Values are mean ± SD (n = 3).
[b] Essential amino acid (EAA).
[c] Unpublished results.

Table 4
Coenzyme Q_{10} and vitamin E (α-tocopherol) contents in the studied macroalgae (dry weight)[a] compared to some common whole foods.

Macroalgae and Foods	Lipophilic antioxidants	
	Coenzyme Q_{10} (μg/g)	α-Tocopherol (mg/100 g)
Ulva compressa	1.42 ± 0.09	9.14 ± 0.27
Ulva rigida	1.59 ± 0.08	3.25 ± 0.20
Gelidium microdon	1.25 ± 0.04	4.22 ± 0.15
Pterocladiella capillacea	8.27 ± 0.11	2.61 ± 0.11
Olive oil	–	11.9[b]
Fish	8.5–15.9[c]	7.5–22.0[b]
Egg	1.2[c]	0.99[b]
Apple	1.3[c]	0.4[b]
Cauliflower	2.7[c]	–
Potato	0.5[c]	0.05[b]
Tomato	0.9[c]	–
Carrot	1.7[c]	0.4[b]

[a] Values are mean ± SD (n = 3).
[b] Values for whole foods from Sheppard et al. (1992) in mg/100 g product.
[c] Values for whole foods from Mattila and Kumpulainen (2001) in μg/g fresh weight.

Heo, Cha, Lee, & Jeon, 2006) the TPC is lower in green than in brown and red algae methanolic extracts. Within this context, the present study on the TPC of methanolic extracts (see Table 5) revealed higher value in *G. microdon* (55.07 mg GAE/g extract) followed by *U. compressa*, *U. rigida* and *P. capillacea* (31.10, 29.90 and 27.70 mg GAE/g extract, respectively). Frikha et al. (2011) presented an inferior value for Tunisian *U. rigida* (10.77 mg GAE/g extract). Heo et al. (2006) presented similar results for Korean *P. capillacea* (23.97 mg GAE/g extract), but a lower value for another species of *Gelidium* (*G. amansii*, 24.06 mg GAE/g extract). Thus, supplements or cosmetics based on *G. microdon* may act as natural antioxidants.

3.1.7. Calculated energy value

As shown in Fig. 1, the Rhodophyta species *P. capillacea* and *G. microdon* presented significantly higher nutritive value ($P < 0.05$), in terms of higher calculated energy values (9.8 ± 0.29 and 9.5 ± 0.29 kJ/g, respectively) as compared with the Chlorophyta species *U. rigida* and *U. compressa* (7.1 ± 0.22 and 6.8 ± 0.21 kJ/g, respectively). Renaud and Luong-Van (2006) reported similar calculated energy values for some Chlorophyta that ranged from 2.1 to 8.8 kJ/g collected in the summer and from 2.2 to 5.8 kJ/g for the winter season and for Rhodophyta ranged from 5.2 to 8.9 kJ/g for the summer and from 5.4 to 10.3 kJ/g collected in the winter season.

3.2. Biological activity assay

3.2.1. DPPH free radical scavenging activity (FRSA)

Results on the in vitro evaluation of algae antioxidant activity through the ability of their crude methanolic extracts to scavenge DPPH radicals are presented in Table 5. The greatest activity after 30 min of reaction time was observed for *G. microdon* (47.73%) followed by *U. compressa* (40.21%), whereas the lowest activity was found in *U. rigida* (29.32%) and *P. capillacea* (26.14%). The BHT, used in the same

Table 5
Free radical scavenging activity (FRSA) after 30 min reaction and total phenolic content of the studied macroalgae methanolic dry extracts[a].

Macroalgae and BHT	FRSA (%)	Total phenolic content (mg GAE/g dry extract)
Ulva compressa	40.21 ± 2.84	31.10 ± 1.25
Ulva rigida	29.32 ± 0.02	29.90 ± 1.20
Gelidium microdon	47.73 ± 3.01	55.07 ± 2.13
Pterocladiella capillacea	26.14 ± 1.90	27.70 ± 1.12
BHT	91.22 ± 4.88	–

[a] Values are mean ± SD (n = 3). BHT (butylated hydroxyltoluene) was used as positive control. GAE, gallic acid equivalents.

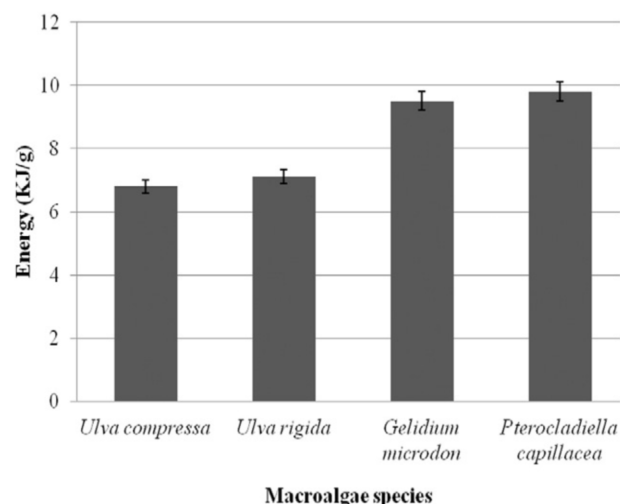

Fig. 1. Calculated energy value of the studied macroalgae (dry weight). Values are mean ± SD (n = 3).

conditions, as a positive control, presents a FRSA of 91.22%. Similar results were obtained by Frikha et al. (2011) for Tunisian *U. rigida* (23.0%) and by Heo et al. (2006) for Korean *P. capillacea* (24.14%) and *Gelidium amansii* (33.63%), using all a similar methodology. However, it is very difficult to compare data of antioxidant activities in macroalgae species because each author used different extraction protocol methods and units (for a review see Tierney, Croft, & Hayes, 2010). Concerning the relationship between FRSA and TPC in algae extracts, several researchers reported a positive correlation (e.g. Heo et al., 2006), that is in agreement with our study ($r = 0.850$, $P < 0.01$).

4. Conclusions

The algae species evaluated in this study clearly represent a rich source of beneficial nutrients and bioactive compounds (e.g. MUFA, PUFA, EAA, macrominerals, phenolics and lipophilic antioxidants), and energy content. This justifies its direct use as a nutritionally balanced functional diet. In addition, the low total lipids content, the balanced *n*3/*n*6 and h/H FA ratios (higher than one) and Na/K and Ca/Mg mineral ratios, may have a potential impact in hypertension and cardiovascular-disorder patients. Furthermore, the significant FRSA activity, allied with pristine seawaters in the Azores region, lends them for use as an excellent low-fat healthy promoting ingredients and/or pharmaceutical and cosmeceutical products. Additionally, the studied algae also showed a higher content of anti-ageing AA and also *U. rigida* revealed to be an excellent source of EAA and low Phe content for patients with PKU disorder.

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References

AOAC (2000). *Official methods of analysis* (17th ed.). Washington: Association of Official Analytical Chemists.
Barbarino, E., & Lourenço, S. (2005). An evaluation of methods for extraction and quantification of protein from marine macro- and microalgae. *Journal of Applied Phycology*, *17*, 447–460.
Battino, M., Ferri, E., Gorini, A., Villa, R. F., Rodriquez Huertas, J. F., Fiorella, P., ... Marchetti, M. (1990). Natural distribution and occurrence of coenzyme Q homologues. *Membrane Biochemistry*, *9*, 179–190.

Bocanegra, A., Bastida, S., Benedí, J., Ródenas, S., & Sánchez-Muniz, F. J. (2009). Characteristics and nutritional and cardiovascular-health properties of seaweeds. *Journal of Medicinal Food*, *12*, 236–258.

Brett, J. R., & Groves, T. D. (1979). Physiological energetics. In W. S., & D. J. (Eds.), *Fish physiology. VIII.* (pp. 279–351). London: Academic Press.

Burghardt, P. R., Kemmerer, E. S., Buck, B. J., Osetek, A. J., Yan, C., Koch, L. G., ... Evans, S. J. (2010). Dietary n-3:n-6 fatty acid ratios differentially influence hormonal signature in a rodent model of metabolic syndrome relative to healthy controls. *Nutrition and Metabolism*, *7*, 53. http://dx.doi.org/10.1186/1743-7075-7-53.

Cao, Y., Qu, H. J., Li, P., Wang, C. B., Wang, L. X., & Han, Z. W. (2011). Single dose administration of L-carnitine improves antioxidant activities in healthy subjects. *Tohoku Journal of Experimental Medicine*, *224*, 209–213.

Darcy-Vrillon, B. (1993). Nutritional aspects of the developing use of marine macroalgae for the human food industry. *International Journal of Food Science and Nutrition*, *44*, 23–35.

Ernster, L., & Dallner, G. (1995). Biochemical, physiological and medical aspects of ubiquinone function. *Biochimica et Biophysica Acta*, *1271*, 195–204.

Fleurence, J. (1999). Seaweed proteins: Biochemical nutritional aspects and potential uses. *Trends in Food Science & Technology*, *10*, 25–28.

Folch, J., Lees, M., & Solam-Stanley, G. H. (1957). A simple method for the isolation and purification of total lipid from animal tissue. *Journal of Biological Chemistry*, *226*, 497–509.

Frikha, F., Kammoun, M., Hammami, N., Mchirgui, R. A., Belbahri, L., Gargouri, Y., ... Ben-Rebah, F. (2011). Chemical composition and some biological activities of marine algae collected in Tunisia. *Ciencias Marinas*, *37*, 113–124.

Gad, M. Z. (2010). Anti-aging effects of L-arginine. *Journal of Advanced Research*, *1*, 169–177.

Harnedy, P. A., & FitzGerald, R. J. (2013). Bioactive proteins and peptides from macroalgae, fish, shellfish and marine processing waste. In S. -K. (Ed.), *Marine proteins and peptides: Biological activities and applications* (pp. 5–39). U.K.: John Wiley & Sons, Ltd.

Heo, S. -J., Cha, S. -H., Lee, K. -W., Cho, S. K., & Jeon, Y. -J. (2005). Antioxidant activities of chlorophyta and phaeophyta from Jeju Island. *Algae*, *20*, 251–260.

Heo, S. -J., Cha, S. -H., Lee, K. -W., & Jeon, Y. -J. (2006). Antioxidant activities of red algae from Jeju Island. *Algae*, *21*, 149–156.

Jeya, M., Moon, H. -J., Lee, J. -L., Kim, I. -W., & Lee, J. -K. (2010). Current state of coenzyme Q_{10} production and its applications. *Applied Microbiology and Biotechnology*, *85*, 1653–1663.

Klein, B. C., Walter, C., Lange, H. A., & Buchholz, R. (2012). Microalgae as natural sources for antioxidative compounds. *Journal of Applied Phycology*, *24*, 1133–1139.

Leite, J., Lima, E., & Baptista, J. (2007). Azorean bovine milk conjugated linoleic acid. Effect of green pasture diet, storage and processing temperature. *Le Lait*, *87*, 167–179.

Liu, J., Killilea, D. W., & Ames, B. N. (2002). Age-associated mitochondrial oxidative decay: improvement of carnitine acetyltransferase substrate-binding affinity and activity in brain by feeding old rats acetyl-L-arnitine and/or R-α-lipoic acid. *Proceedings of the National Academy of Sciences*, *99*, 1876–1881.

Lordan, S., Ross, R. P., & Stanton, C. (2011). Marine bioactives as functional food ingredients: Potential to reduce the incidence of chronic diseases. *Marine Drugs*, *9*, 1056–1100.

MacArtain, P., Gill, C. I. R., Brooks, M., Campbell, R., & Rowland, I. R. (2007). Nutritional value of edible seaweeds. *Nutrition Reviews*, *1*, 535–543.

Mattila, P., & Kumpulainen, J. (2001). Coenzymes Q_9 and Q_{10}: Contents in foods and dietary intake. *Journal of Food Composition and Analysis*, *14*, 409–417.

McCance, R. A., Widdowson, E. M., & Holland, B. (1993). *Composition of foods* (6th ed.). Cambridge: Royal Society of Chemistry.

Mohamed, S., Hashim, S. N., & Rahman, H. A. (2012). Seaweeds: A sustainable functional food for complementary and alternative therapy. *Trends in Food Science & Technology*, *23*, 83–96.

Molyneux, P. (2004). The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. *Songklanakarin Journal of Science and Technology*, *26*, 211–219.

Neto, A. I., Tittley, I., & Raposeiro, P. M. (2006). *Flora Marinha do Litoral dos Açores. Rocky shore marine flora of the Azores.* Horta, Portugal: Secretaria Regional do Ambiente e do Mar.

Neto, A. I., Brotas, V., Azevedo, J. M. N., Patarra, R. F., Álvaro, N. M. V., Gameiro, C., ... Nogueira, E. M. (2009). *Qualidade de águas costeiras do Grupo Oriental do arquipélago dos Açores e proposta de monitorização.* Ponta Delgada, Portugal: Departamento de Biologia, Universidade dos Açores.

Oroian, M., & Escriche, I. (2015). Antioxidants: Characterization, natural sources, extraction and analysis – A review. *Food Research International*, *74*, 10–36.

Ortega-Calvo, J. J., Mazuelos, C., Hermosín, B., & Saiz-Jimenez, C. (1993). Chemical composition of *Spirulina* and eukaryotic algae food-products marketed in Spain. *Journal of Applied Phycology*, *5*, 425–435.

Ortiz, J., Romero, N., Robert, P., Araya, J., Lopez-Hernández, J., Bozzo, C., ... Rios, A. (2006). Dietary fiber, amino acid, fatty acid and tocopherol contents of the edible seaweeds *Ulva lactuca* and *Durvillaea Antarctica*. *Food Chemistry*, *99*, 98–104.

Paiva, L., Lima, E., Patarra, R. F., Neto, A. I., & Baptista, J. (2014). Edible Azorean macroalgae as source of rich nutrients with impact on human health. *Food Chemistry*, *164*, 128–135.

Paiva, L., Lima, E., Neto, A. I., & Baptista, J. (2016). Isolation and characterization of angiotensin I-converting enzyme (ACE) inhibitory peptides from *Ulva rigida* C. Agardh protein hydrolysate. *Journal of Functional Foods* in press.

Patarra, R. F., Leite, J., Pereira, R., Baptista, J., & Neto, A. I. (2013). Fatty acids composition of selected macrophytes. *Natural Product Research*, *27*, 665–669.

Rainha, N., Koci, K., Coelho, A. V., Lima, E., Baptista, J., & Fernandes-Ferreira, M. (2013). HPLC-UV-ESI-MS analysis of phenolic compounds and antioxidant properties in *Hypericum undulatum* shoot cultures and wild-growing plants. *Phytochemistry*, *86*, 83–89.

Renaud, S. M., & Luong-Van, J. T. (2006). Seasonal variation in the chemical composition of tropical Australian marine macroalgae. *Journal of Applied Phycology*, *18*, 381–387.

Rosanoff, A. (2010). Rising Ca:Mg intake ratio from food in USA adults: A concern? *Magnesium Research*, *23*, S181–S193.

Rujiralai, T., Raekasin, N., Cheewasedtham, W., & Cheewasedtham, C. (2014). Development of an effective extraction process for coenzyme Q_{10} from *Artemia*. *Chemical Papers*, *68*, 1041–1048.

Rupérez, P. (2002). Mineral content of edible marine seaweeds. *Food Chemistry*, *79*, 23–26.

Sánchez-Machado, D. I., López-Cervantes, J., López-Hernández, J., Paseiro-Losada, P., & Simal-Lozano, J. (2003). High-performance liquid chromatographic analysis of amino acids in edible seaweeds after derivatization with phenyl isothiocyanate. *Chromatographia*, *58*, 159–163.

Sánchez-Machado, D. I., López-Cervantes, J., López-Hernández, J., & Paseiro-Losada (2004). Fatty acids, total lipid, protein and ash contents of processed edible seaweeds. *Food Chemistry*, *85*, 439–444.

Seelig, M. S., & Heggtveit, H. A. (1974). Magnesium interrelationships in ischemic heart disease: A review. *American Journal of Clinical Nutrition*, *27*, 59–79.

Sheard, N. F. (2000). Importance of diet in maternal phenylketonuria. *Nutrition Reviews*, *58*, 236–239.

Sheppard, A. J., Weihrauch, J. L., & Pennington, J. A. T. (1992). Analysis and distribution of vitamin E in vegetable oils and foods. In L., & J. (Eds.), *Vitamin E in health and disease* (pp. 9–31). New York: Marcel Dekker.

Silva, M., Vieira, L. M. M., Almeida, A. P., Silva, A. M. S., Seca, A. M. L., Barreto, M. C., ... Kijjoa, A. (2013). Chemical study and biological activity evaluation of two Azorean macroalgae: *Ulva rigida* and *Gelidium microdon*. *Oceanography*, *1*. http://dx.doi.org/10.4172/ocn.1000102.

Simopoulos, A. P. (1999). Essential fatty acids in health and chronic disease. *The American Journal of Clinical Nutrition*, *70*, 560S–569S.

Smith, A. J. (2004). Medicinal and pharmaceutical uses of seaweed natural products: A review. *Journal of Applied Phycology*, *16*, 245–262.

Taboada, C., Millán, R., & Míguez, I. (2010). Composition, nutritional aspects and effect on serum parameters of marine algae *Ulva rigida*. *Journal of the Science of Food and Agriculture*, *90*, 445–449.

Tierney, M. S., Croft, A. K., & Hayes, M. (2010). A review of antihypertensive and antioxidant activities in macroalgae. *Botanica Marina*, *53*, 387–408.

Waterhouse, A. L. (2002). Determination of total phenolics. In R. E. (Ed.), *Current protocols in food analytical chemistry (units I.1.1.1–I1.1.8)*. New York: John Wiley & Sons.

Yuan, Y. V. (2008). Marine algal constituens. In C., & F. (Eds.), *Marine nutraceuticals and functional foods* (pp. 259–292). Boca Raton: CRC Press.