Food Chemistry 183 (2015) 197–207

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Chemical composition of red, brown and green macroalgae from Buarcos bay in Central West Coast of Portugal

Dina Rodrigues [a,*], Ana C. Freitas [a,b], Leonel Pereira [c], Teresa A.P. Rocha-Santos [a,b], Marta W. Vasconcelos [d], Mariana Roriz [d], Luís M. Rodríguez-Alcalá [d], Ana M.P. Gomes [d], Armando C. Duarte [a]

[a] CESAM – Centre for Environmental and Marine Studies & Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal
[b] ISEIT/Viseu, Instituto Piaget, Estrada do Alto do Gaio, Galifonge, 3515-776 Lordosa, Viseu, Portugal
[c] MARE – Marine and Environmental Sciences Centre/IMAR Faculdade de Ciências e Tecnologia, Universidade de Coimbra, 3004-517 Coimbra, Portugal
[d] CBQF – Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Universidade Católica Portuguesa/Porto, Rua Dr. António Bernardino Almeida, 4200-072 Porto, Portugal

ARTICLE INFO

Article history:
Received 24 March 2014
Received in revised form 9 March 2015
Accepted 18 March 2015
Available online 21 March 2015

Keywords:
Chemical composition
Elements
Fatty acids
FTIR-ATR characterization
Seaweeds

ABSTRACT

Six representative edible seaweeds from the Central West Portuguese Coast, including the less studied *Osmundea pinnatifida*, were harvested from Buarcos bay, Portugal and their chemical characterization determined. Protein content, total sugar and fat contents ranged between 14.4% and 23.8%, 32.4% and 49.3% and 0.6–3.6%. Highest total phenolic content was observed in *Codium tomentosum* followed by *Sargassum muticum* and *O. pinnatifida*. Fatty acid (FA) composition covered the branched chain C13ai to C22:5 n3 with variable content in n6 and n3 FA; low n6:n3 ratios were observed in *O. pinnatifida*, *Grateloupia turuturu* and *C. tomentosum*. Some seaweed species may be seen as good sources of Ca, K, Mg and Fe, corroborating their good nutritional value. According to FTIR-ATR spectra, *G. turuturu* was associated with carrageenan seaweed producers whereas *Gracilaria gracilis* and *O. pinnatifida* were mostly agar producers. In the brown algae, *S. muticum* and *Saccorhiza polyschides*, alginates and fucoidans were the main polysaccharides found.

1. Introduction

Algae, in particular, edible seaweeds, are a very interesting natural source of compounds with biological activity that could be used as functional ingredients (Plaza, Cifuentes, & Ibánez, 2008). Considering their great taxonomic diversity, research on identification of biologically active compounds is encouraging in such a vast untapped resource (Lordan, Ross, & Stanton, 2011). Some algae live in complex habitats and are often subject to extreme conditions. Their metabolism can be influenced by parameters such as water temperature, salinity, light and nutrients, being forced to quickly adapt to continuously new environmental conditions and in order to survive they produce a wide variety of biologically active secondary metabolites (Plaza et al., 2008). Seaweeds can in fact provide several compounds at different quantities and for that, research on identification of bioactive compounds from algae can be seen as an almost unlimited field (Lordan et al., 2011).

Seaweeds are known to be of low calorie content, rich in polysaccharides, minerals, vitamins, proteins, steroids and dietary fibers (Lordan et al., 2011) making them increasingly sought for commercial purposes. They are of potential interest to be incorporated in the diet as added-value foods because they are claimed to contain low cholesterol content, fight obesity, reduce blood pressure, tackle free radicals and promote healthy digestion (Plaza et al., 2008); furthermore, some of them have demonstrated biological properties such as antibacterial and anticoagulant activities (Alghazeer, Whida, Abduelrhman, Ammoudi & Azwai, 2013; Chandía & Matsuhiro, 2008). The total concentration of polysaccharides in the seaweeds can range from 4% to 76% of dry weight (Holdt & Kraan, 2011) where agar, algins carrageenans and fucoidans are some of the polysaccharides widely used by man, some with various biological activities (Mak, Hamid, Liu, Lu, & White, 2013; Mak et al., 2014). Protein content is also variable and the highest contents are generally found in green and red seaweeds (10–30% of dry weight) in comparison to brown seaweeds (5–15% of dry weight) (Holdt & Kraan, 2011; Zhou, Robertson, Hamid, Maa, & Lu, 2014). Phospholipids and glycolipids are the major classes of lipids found in seaweeds accounting for 1–5% of cell composition (Chojnacka, Saeid, Witkowska, & Tuhy, 2012);

* Corresponding author.
E-mail address: dinarodrigues@ua.pt (D. Rodrigues).

http://dx.doi.org/10.1016/j.foodchem.2015.03.057
0308-8146/

17–63 mg/g of lipid content has been reported for the brown seaweed *Undaria pinnatifida* (Boulom, Robertson, Hamid, Ma, & Lu, 2014). Phenolic compounds are another important group of chemical compounds in seaweeds (Lordan et al., 2011) varying quantitatively and qualitatively for each specimen of red, brown or green seaweeds. Seaweeds are also recognized as sources rich in several elements such as Ca, Mg, Na, P and K or trace elements such as Zn, I or Mn. This elemental richness is related to their capacity to retain inorganic compounds accounting for up to 36% of dry matter in some species (Lordan et al., 2011).

According to Chojnacka et al. (2012), there are about ten thousand identified algae species, yet only about 5% thereof are used as human food or as animal feed. More than one hundred seaweed species are used worldwide, especially in Asian countries, where they are used as sea vegetables. In Southern Europe the use of edible seaweeds for food purposes is still residual, not yet fitting with the regular consumption habits. However, this trend is changing and currently researchers are searching for, and studying, less-known seaweed species not only because of their potential biological properties due to specific functional compounds but mostly for their nutrient profile, which is being considered as important alternative protein and complex carbohydrate sources (Ibañez & Cifuentes, 2013).

The main objective of the present study was to determine the chemical composition of six different edible species from each of the main seaweed groups (red, brown and green algae) occurring at Buarcos bay (Figueira da Foz, Portugal). The selection was based on representative species from the Central West Portuguese Coast including the less studied *Osmundea pinnatifida*. Information on the proximal composition, fatty acids profile and elemental composition of the six seaweeds is presented as well as the identification of the principal seaweed colloids by FTIR-ATR and FT-Raman.

2. Material and methods

2.1. Specimens of seaweeds

Specimens of red algae (Rhodophyta, Florideophyceae) *O. pinnatifida* (Ceramiales), *Grateloupia turuturu* (Halymeniales) and *Gracilaria gracilis* (Gracilariales), brown algae (Heterokontophyta, Phaeophyceae) *Sargassum muticum* (Fucales) and *Saccorhiza polyschides* (Tilopteridales) and of green algae (Chlorophyta, Ulvophyceae) *Codium tomentosum* (Bryopsidales), were harvested in April 2012 from Buarcos bay (Figueira da Foz, Portugal). The classification of these seaweeds was based on *AlgaeBase* (Guiry & Guiry, 2013). All six selected species are edible being used as food for humans all around the world (Lodeiro et al., 2012; Munier, Dumay, Morançais, Jaouen, & Fleurence, 2013; Roo, Mantri, Ganesan, & Kumar, 2007; SIA, 2015). The seaweeds were first washed with running tap water and then with deionised water to eliminate residues from the thalli surface and then dried in an oven at 60 °C. The dried seaweeds were milled to less than 1.0 mm particle size.

2.2. Chemical characterization of seaweeds

2.2.1. Proximate composition

Content in moisture, organic matter and ash were determined according to AOAC methods, (1990). Nitrogen content was determined by the Kjeldahl method adapted from where protein content is estimated by multiplying the nitrogen content by 6.25 (Denis et al., 2010; Munier et al., 2013) whereas total fat content was determined by Soxhlet extraction. Total sugar content was determined by calculation, i.e. by subtracting protein content and fat content from total organic content. Total polyphenols were extracted from 0.1 g of dry seaweed in 10 mL of ethyl acetate after 30 min of sonication (on a water bath ultrasonicator, Ultrasonik 57H Ney). The extract was filtered with anhydrous sodium sulfate (Sigma) and brought to dryness with a rotary evaporator (Laborato 4000, Heidolph). The residue was re-dissolved in 5 ml of milliQ water and the content of total polyphenols of 2 mL was determined by colorimetric method of Folin–Ciocalteu, using cathecol (0–75 mg/L) as standard and expressed as μg cathecol equivalent per g of dry seaweed.

2.2.2. Analysis of fatty acids

Hexane and methanol were HPLC grade (VWR Scientific, West Chester, PA). Sodium sulphate was analytic grade and purchased to Panreac (Barcelona, Spain). Methyl tricosanoate (99%) and Supelco 37 FAME mix were obtained from Sigma (Sigma–Aldrich, St. Louis, MO, USA). GLC-Nestlé36 was purchased to Nu-Chek Prep, Inc. (Elysian, Minnesota, USA) while butterfat CRM-164 (EU Commission; Brussels, Belgium) was from Fedelco Inc. (Madrid, Spain).

For the analysis of the total fatty acid (FA) composition, 100 mg of sample were accurately weighed and prepared according to Sánchez-Avila, Mata-Granados, Ruiz-Jiménez, and Luque de Castro (2009). For quantification purposes samples were added with 100 μL of methyl tricosanoate (1.28 mg/mL) prior to derivatization. FAME were analyzed in a gas chromatograph HP6890A (Hewlett–Packard, Avondale, PA, USA), equipped with a flame-ionization detector (GLC-FID) and a BPX70 capillary column (50 m × 0.32 mm × 0.25 μm; SGE Europe Ltd, Courtaboeuf, France) according to the conditions described by Vingering and Ledoux (2009). Supelco 37 and CRM-164 were used for identification of fatty acids. GLC-Nestlé36 was assayed for calculation of response factors and detection and quantification limits (LOD: 0.15 μg/mL; LOQ: 0.46 μg/mL).

2.2.3. Elemental composition

2.2.3.1. Microwave-assisted acid digestion procedure. The microwave-assisted digestion proposed by Speedwave MW-3+ (Berghof, Germany) for dried plant samples with some modifications was used for determination of Mo, B, Zn, P, Cd, Co, Ni, Mn, Fe, Mg, Ca, Cu, Na, Al and K in dried seaweed samples. A sample with up to 0.2 g dry seaweed was placed in the digestion vessel and added with 5 mL of concentrated nitric acid. The vessels were capped and placed in a microwave pressure digestor Speedwave MWS-3+ (Berghof) and subjected to microwave radiation at 20 bar according to the following program: room temperature was raised first to 130 °C at 22 °C/min and 30% of irradiation power, then to 160 °C at 6 °C/min and 40% of irradiation power, remaining 5 min at this temperature, and to 170 °C at 5 °C/min and 50% of irradiation power, remaining 5 min at this temperature. The cooling process consisted in decreasing temperature first to 100 °C for 4 min and then to room temperature. After cooling, acid digests were made up to 20 mL with Milli-Q water. Three replicates were performed for each seaweed sample as well as blanks. The content of each element is expressed as the mean plus standard deviation.

2.2.3.2. Determination of 15 elements. The elemental composition was determined using an inductively coupled plasma (ICP) optical emission spectrometer model Optima™ 7000 DV ICP-OES (Dual View, PerkinElmer Life and Analytical Sciences, Shelton, CT, USA) with radial plasma configuration.

Standard plasma conditions were used namely 1300 W for radio-frequency power, 1.5 mL/min pump rate, and 15.0, 0.2 and 0.8 L/min for plasma, auxiliary and nebulizer gas flow, respectively. Detection wavelengths were 202.031, 208.889, 213.857, 214.914,

226.502, 228.616, 231.604, 257.610, 259.939, 279.955, 317.933, 324.752, 330.237, 394.401, 769.896 nm for Mo, B, Zn, P, Cd, Co, Ni, Mn, Fe, Mg, Ca, Cu, Na, Al and K, respectively.

A multi-element standard (Inorganic Ventures) containing up to 10 mg/L of Mo, 2.5 mg/L of B, 15 mg/L of Zn, 750 mg/L of P, 0.2 mg/L of Co and Ni, 1 mg/L of Cd and Cu, 15 mg/L of Mn and Fe, 1000 mg/L of Mg, 3000 mg/L of Ca, 50 mg/L of Na, 5 mg/L of Al and 2000 mg/L of K was used for the preparation of standard solutions in 2% HNO_3. Successive dilutions of the stock reference solution (100, 50 and 10 times) were prepared and used for calibration models and the concentration of each element was determined by direct interpolation in the standard curve within its linear dynamic range. The limits of detection (LODs) were calculated using y = yB + 3SB, where SB is the standard deviation (SD) of the blank signal estimated as sy/x, the residual SD taken from the calibration line, and yB is the blank signal estimated from the intercept, also taken from the calibration line. The LODs were found to be 0.010, 0.0009, 0.010, 0.40, 0.002, 0.002, 0.003, 0.014, 0.16, 0.18, 2.06, 3.32, 1.17, 0.037, 3.75 mg/L for Mo, B, Zn, P, Cd, Co, Ni, Mn, Fe, Mg, Ca, Cu, Na, Al and K, respectively. Acid digests for some seaweed samples were diluted up 400 times to assess the content of some elements in particular for Mg, Na, K and Fe within calibration curve range values.

The accuracy of the method (microwave acid digestion and ICP-OES analysis) was assessed by analysis of certified reference material NIES-03 (Seaweed Chlorella; LGC standards, UK). Five replicates of reference material were subject to microwave digestion and analyzed three times by ICP-OES. Recovery ranged between 89% and 108%.

2.2.4. FTIR-ATR and FT-Raman analysis

Samples of milled, dried algal material were analyzed by Fourier Transform Infrared Spectroscopy with attenuated total reflectance (FTIR-ATR) and Fourier Transform Raman spectroscopy (FT-Raman) according to the method described by Pereira (2006) and by Pereira, Amado, Critchley, van de Velde, and Ribeiro-Claro (2009).

The FTIR spectra of milled dried seaweed were recorded on an Bruker Tensor 27 spectrometer (Bruker Scientific Instruments, Massachusetts, USA), using a Golden Gate single reflection diamond ATR system (Specac Lda, Cranston, USA), with no need for sample preparation. All spectra resulted from the average of two counts, with 128 scans each and a resolution of 2 cm^{-1}.

The room temperature FT-Raman spectra were recorded on a RFS-100 Bruker FT-spectrometer using a Nd:YAG laser with excitation wavelength of 1064 nm. Each spectrum was the average of two repeated measurements, with 150 scans at a resolution of 4 cm^{-1}. For FT-Raman, the samples of seaweeds were first submitted to depigmentation by immersion in cold calcium hypochlorite (4%) for 30–60 s at 4 °C and then washed and dried at 60 °C.

2.3. Statistical analysis

One-way analysis of variance (ANOVA) was carried out with SigmaStat™ (Systat Software, Chicago, IL, USA), to assess differences between seaweed species in terms of proximate or elemental composition at a significance level of $p = 0.05$. The Holm-Sidak method was used for pairwise comparisons at a significance level of $p = 0.05$. FA data in turn, were analyzed using the IBM SPSS Statistics v22 for Mac. Normality and homogeneity were examined and One way ANOVA with the Bonferroni test for post hoc analyses was applied to evaluate statistical differences between seaweeds ($p < 0.05$).

3. Results and discussion

3.1. Proximate composition of seaweeds

The proximate composition of the different edible seaweeds' species is displayed in Table 1. Seaweeds are perishable and deteriorate rapidly within a few days upon harvesting and therefore drying is an essential step to preserve them. Drying decreases the water activity which ultimately retards microbial growth, helps to preserve the desirable qualities and reduces storage volume. In general, moisture content ranged between 8.0 $g/100g_{dry\ seaweed}$ in dried *G. gracilis* to 11.8 $g/100g_{dry\ seaweed}$ in dried *O. pinnatifida*; the moisture content was statistically different ($p < 0.05$) among all species tested except between *O. pinnatifida* and *G. turuturu*.

The protein content, one of the major biochemical components of seaweeds, ranged from 14.4 $g/100g_{dry\ seaweed}$ in *S. polyschides* (brown algae) to 23.8 $g/100g_{dry\ seaweed}$ in *O. pinnatifida* (red algae) being the protein content statistically different ($p < 0.05$) between all seaweed species. Highest values of protein content were found in the red seaweed species (20.2–23.8 $g/100g_{dry\ seaweed}$), followed closely by the green seaweed *C. tomentosum* (18.8 $g/100g_{dry\ seaweed}$) and with lowest content were the brown algae (14.4–16.9 $g/100g_{dry\ seaweed}$) (Table 1). According to Ibañez and Cifuentes (2013), protein content differs between seaweed species and, in general, red (Rhodophyta) and green (Chlorophyta) algae species are characterized by higher protein content in comparison to brown algae species (Phaeophyceae). Similar values were described by Denis et al. (2010) for *G. turuturu* collected in Brittany (France) (23% of protein content) and by Paiva, Lima, Patarra, Neto, and Baptista (2014) for *O. pinnatifida* collected in Azores Archipelago (21% of protein content), respectively. However higher values (27%) are reported for the same species collected in North Yorkshire (UK) by Marsham, Scott, and Tobin (2007). Patarra, Paiva, Neto, Lima, and Baptista (2011) reported 23% of total protein in *G. turuturu* collected in the Azores Archipelago Coast during mid-winter period, which is very similar to that reported herein for *G. turuturu* collected in the Spring, both periods during which algal protein content is at its highest level. Kendel et al. (2013) reported 84 to 120 $g/Kg_{dry\ seaweed}$ of protein content for *S. muticum*, *S. polyschides* and *C. tomentosum* collected in French Brittany. Protein contents found in the red and green seaweeds analyzed are similar to the protein contents of many legumes such as peas or beans (19–22%) or meats (18–25%). Therefore, these algae from the Central Portuguese Coast may very well be used in the formulation of low-cost, protein balanced diets that may alternate with current vegetable protein sources such as legumes and cereals.

Seaweeds are also known for their low fat content which varies significantly throughout the year according to Manivannan, Thirumaran, Devi, Hemalatha, and Anantharaman (2008). A range between 0.6 and 3.6 $g/100g_{dry\ seaweed}$ of total fat was observed in the studied seaweeds (Table 1). The lowest fat contents were reported for the red algae *O. pinnatifida* and *G. gracilaris* which differed significantly from the highest fat content reported for the green algae *C. tomentosum*; no significant differences were found between the fat content of the red and brown algae classes. The analyzed fat contents did not always agree with those of previous studies: lower contents in *O. pinnatifida* (0.9 $g/100g_{dry\ seaweed}$) were found than values of 4.3% and 7.5% fat reported by Marsham et al. (2007) and by Paiva et al. (2014), respectively, whereas higher values of fat content were observed in *C. tomentosum* (3.6 $g/100g_{dry\ seaweed}$) than the 2.5% fat content reported by Manivannan et al. (2008). In the case of *S. muticum* analyzed fat content values were comparable to those found for *S. muticum* collected in French Brittany (19 $g/kg_{dried\ seaweed}$) and reported by Jard et al. (2013). As previously mentioned, sampling period and

Table 1
Proximate composition of seaweeds and their percentage contribution in nutrients intake.

Parameter	*G. gracilis*	*O. pinnatifida*	*G. turuturu*	*S. muticum*	*S. polyschides*	*C. tomentosum*
% moisture ($g/100g_{dry\ seaweed}$)	7.99 ± 0.02 a	11.77 ± 0.01 e	11.68 ± 0.05 e	9.64 ± 0.08 c	10.88 ± 0.04 d	9.0 ± 0.2 b
% total Protein ($g/100g_{dry\ seaweed}$)	20.2 ± 0.6 d	23.8 ± 0.6 f	22.5 ± 0.3 e	16.9 ± 0.2 b	14.44 ± 0.1 a	18.8 ± 0.1 c
% total sugars[1] ($g/100g_{dry\ seaweed}$)	46.6	32.4	43.2	49.3	45.6	32.8
% total fat ($g/100g_{dry\ seaweed}$)	0.60 ± 0.01 a	0.9 ± 0.1 a	2.2 ± 0.1 c	1.45 ± 0.07 b	1.1 ± 0.1 ab	3.6 ± 0.2 d
Total phenolic content (μg cathecol equiv/$g_{dry\ seaweed}$)	228 ± 14 a	337 ± 22 b	208 ± 8 a	499 ± 32 c	224 ± 13 a	920 ± 84 d
% organic matter ($g/100g_{dry\ seaweed}$)	67.21 ± 0.01 d	57.6 ± 0.2 a	67.80 ± 0.06 d	67.41 ± 0.02 d	60.97 ± 0.05 c	55.0 ± 0.7 b
% ash ($g/100g_{dry\ seaweed}$)	24.8 ± 0.03 b	30.62 ± 0.25 a	20.52 ± 0.01 c	22.94 ± 0.06 d	28.15 ± 0.01 e	35.99 ± 0.48 f

Nutrients	g/day	*G. gracilis*		*O. pinnatifida*		*G. turuturu*		*S. muticum*		*S. polyschides*		*C. tomentosum*	
		$g/10g_{portion}$	%RDI[3]	$g/10g_{portion}$	%RDI[3]	$g/10g_{portion}$	%RDI[3]	$g/10g_{portion}$	%RDI[3]	$g/10g_{portion}$	%RDI[3]	$g/10g_{portion}$	%RDI[3]
Protein	50	2.0	4.0	2.4	4.8	2.2	4.5	1.7	3.4	1.4	2.9	1.9	3.8
Total sugars	270	4.7	1.7	3.2	1.2	4.3	1.6	4.9	1.8	4.6	1.7	3.3	1.2
Fat	70	0.06	0.1	0.09	0.1	0.22	0.3	0.15	0.2	0.11	0.11	0.36	0.5
	mg/day[2]	mg/ $10g_{portion}$	%RDI[3]	mg/ $10g_{portion}$	%RDI[3]	mg/ $10g_{portion}$	%RDI[3]	mg/ $10g_{portion}$	%RDI[3]	mg/ $10g_{portion}$	%RDI[3]	mg/ $10g_{portion}$	%RDI[3]
Calcium	800	34.4	4.3	54.1	6.8	26.5	3.3	91.8	11.5	91.1	11.4	51.3	6.4
Potassium	2000	651.0	32.6	261.0	13.0	162.8	8.1	575.6	28.8	765.4	38.3	372.9	18.6
Magnesium	375	17.5	4.7	48.0	12.8	69.5	18.5	150.4	40.1	79.7	21.3	104.6	27.9
Phosphorus	700	22.6	3.2	17.3	2.5	28.1	4.0	22.8	3.3	23.2	3.3	18.0	2.6
Iron	14	0.90	6.5	3.7	26.2	0.50	3.6	1.9	13.4	0.79	5.7	2.83	20.2
Zinc	10	0.25	2.5	0.58	5.8	0.69	6.9	0.25	2.5	0.65	6.5	0.18	1.8
Copper	1	0.04	4.5	0.05	5.0	0.03	3.3	0.05	4.5	0.03	3.4	0.06	6.3
Manganese	2	0.20	9.9	0.12	5.8	0.25	12.6	0.11	5.4	0.08	3.8	0.19	9.4

[1] % total sugars (%) = organic matter (%) – total protein (%) – total fat (%); a–f, in a row: different letters indicate significant differences ($p < 0.05$) between species.
[2] Data from Mišurcová et al. (2011).
[3] Based on daily intake portion of 10 g of dry seaweed.

location may account for such differences. Munier et al. (2013) studied the influence of two sampling sites located in the intertidal zone of Atlantic Coast (Le Croisic and Batz-sur-mer) in Brittany (France) on the biochemical composition of *G. turuturu*. This study demonstrated that the sampling site influenced the biochemical content (protein, lipid and water-soluble carbohydrates) of *G. turuturu;* higher contents of total protein content, lipid and total water-soluble carbohydrates were observed in specimens collected in Le Croisic which had thicker thalli and were less viscous and more greenish than those collected in Batz-sur-mer. According to authors the morphology of thalli could be related to environmental characteristics found in each sampling location.

The total sugar content ranged from 32.4 $g/100g_{dry\ seaweed}$ in *O. pinnatifida* (red algae) to 49.3 $g/100g_{dry\ seaweed}$ in *S. muticum* (brown algae) (Table 1) being of similar order of magnitude to published values by Denis et al. (2010) for *G. turuturu* (43–49%) but higher than those reported by Jard et al. (2013) for *S. polyschides*, *S. muticum* and *C. tomentosum* (159–176 $g/kg_{dry\ seaweed}$) and by Paiva et al. (2014) for *O. pinnatifida*. Once again, greater variability in total sugar content was observed among red algae than among brown algae.

Most of the polyphenols, including phenolic acids or polyphenolic compounds, isolated from marine sources are of macro and micro-algae origin and they have been associated with antioxidant properties; in general, lower degrees of polymerization result in greater antioxidant properties. In this study, the highest total phenolic content was observed in the green algae *C. tomentosum* (920 μg cathecol equiv/$g_{dry\ seaweed}$) followed by brown algae *S. muticum* (499 μg cathecol equiv/$g_{dry\ seaweed}$) and by the red algae *O. pinnatifida* (337 μg cathecol equiv/$g_{dry\ seaweed}$). The phenolic content was statistically different ($p < 0.05$) among all species tested except between *G. gracilis*, *G. turuturu* and *S. polyschides*.

Tanniou et al. (2014) studied the variability of phenolic contents of the invasive brown seaweed *S. muticum* collected in several countries along European Atlantic Coast from Southern Portugal to South Coast of Norway. Interestingly, higher phenolic content was reported in *S. muticum* (2.46 ± 0.16% to 4.28 ± 0.26% dry weight algae) collected in Portugal in comparison to those collected in Norway, Ireland, France or Spain.

Ash percentage was also quite variable among the different species tested ranging from 20.5 $g/100g_{dry\ seaweed}$ in *G. turuturu* to 36 $g/100g_{dry\ seaweed}$ in *C. tomentosum* (Table 1). The differences between the studied seaweed species were all statistically significant ($p < 0.05$) even between red or brown algae. The observed values for ash content are in accordance to published values for *O. pinnatifida* (32.3%) by and for *G. turuturu* (18.5%) by Denis et al. (2010). It is known that higher levels of ash are associated with higher amounts of mineral elements.

3.2. Fatty acids composition of seaweed

Although quantitatively low the qualitative profile of FA composing the total fat content is of particular interest from a nutritional point of view. Indeed, analyzed seaweeds revealed an important, yet complex, fatty acid profile (Table 2). In general, these seaweeds were mainly composed of saturated (SFA) and polyunsaturated FA (PUFA), which ranged from the branched chain C13ai to C22:5 n3 (Docosapentaenoic acid, DPA). Palmitic acid (C16) was the main SFA compound in all samples. This FA reached values of 52.54 $g/100g_{fat}$ in *G. gracilis* while the lowest amount was found in *S. polyschides* (25.49 $g/100g_{fat}$; $p < 0.05$). However, this latter species together with *C. tomentosum* and *G. turuturu* had the highest total FA contents (19.96, 27.58 and 20.89 $\mu g/g_{dry\ seaweed}$ respectively, $p < 0.05$). In all samples except for *O. pinnatifida*, C16 Phy (Phytanic acid) was the third most important SFA after myristic acid (C14). The maximum level of C16 Phy was for *S. polyschides* (3.85 $g/100g_{fat}$). The presence of this compound is interesting since it has been elsewhere suggested to have preventive effects on metabolic dysfunctions due to retinoid X receptor (RXR) and peroxisome proliferator-activated receptor-alpha (PPAR-alpha) agonist activity (Hellgren, 2010).

The content of omega-3 (n3) and omega-6 (n6) varied among the studied seaweed species. The n3 FA accounted for 1.3–31.5%, n6 FA for 6.7–27.4% whereas n9 FA accounted for 6.6–13.5% (Table 2). C20:5 n3 (EPA) was for some seaweed species the most

Table 2
Fatty acid composition (g FA/100g_{fat}) and total content (μg FA/$mg_{dry\ seaweed}$) of the seaweeds.

	G. gracilis		*O. pinnatifida*		*G. turuturu*		*S. muticum*		*S. polyschides*		*C. tomentosum*	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
C13ai	0.30 c	0.09	0.48 c	0.06	0.37 c	0.07	1.05 a	0.09	0.71 b	0.03	0.40 c	0.07
C14	5.57 b	0.01	6.46 a	0.01	2.90 d	0.02	2.94 d	0.01	3.85 c	0.03	1.01 e	0.32
C14:1 c9	0.87 a	0.01	0.08 c	0.01	0.07 c	0.01	0.23 b	0.01	0.07 c	<0.01	0.23 b	0.02
C15	0.51 a	0.01	0.56 a	0.01	0.51 a	0.03	0.31 b	0.01	0.36 b	0.05	0.14 c	0.02
C16	52.54 a	0.03	48.93 b	0.02	35.88 c	0.08	30.33 C	0.03	25.49 d	0.04	31.33 c	0.01
C16:1 c7 n9	0.06 d	<0.01	0.13 c	0.02	0.06 d	0.02	0.20 b	0.00	0.12 c	0.01	0.70 a	0.03
C16 Phy	1.23 c	0.06	0.33 d	0.01	1.29 c	0.03	1.18 c	0.13	3.15 a	0.08	1.62 c	0.06
C16:1 c9	0.29 e	0.07	1.25 c	0.01	0.96 d	0.02	6.08 a	0.13	2.17 b	0.09	1.25 c	0.08
C16:2 c9 t12	<LOD e	–	<LOD e	–	0.04 d	<0.01	0.09 c	0.01	0.22 b	0.01	1.06 a	0.14
C17	0.27 a	0.01	0.12 B c	0.01	0.11 c	<0.01	0.09 c	0.02	0.14 b	0.04	0.10 c	0.01
C16:2 c9 c12	<LOD e	–	0.39 b	<0.01	0.08 c	<0.01	0.67 a	0.02	0.13 c	0.03	<LOD d	–
C17:1 c10	0.13 b	0.01	0.14 b	0.01	0.10 b	<0.01	0.15 ab	0.01	0.17 a	<0.01	0.16 ab	0.02
C16:3 c7 c10 c13	<LOD d	–	<LOD d	–	<LOD d	–	0.22 b	0.02	0.03 c	<0.01	10.77 a	0.01
C18	1.56 a	0.03	0.94 b	0.04	1.10 b	0.02	0.47 c	0.01	0.78 d	0.01	0.62 d	0.01
C18:1 c9 n9	11.13 b	0.39	12.51 a	0.00	6.54 d	0.01	8.69 c	0.02	11.88 b	0.78	12.57 a	0.01
C18:1 c11	1.41 c	0.02	3.90 a	0.02	2.12 b	0.17	0.46 c	0.02	2.32 b	0.07	1.01 d	0.01
C18:2 c9 c12 n6	0.85 d	0.05	1.43 b	0.01	1.79 c	0.05	5.73 a	0.01	5.08 b	0.48	5.12 b	0.01
C18:3 t9 t12 c15 n3	0.22 d	0.03	0.32 c	0.03	0.79 a	0.02	0.51 b	0.02	0.03 e	<0.01	0.06 e	<0.01
C18:3 c6 c9 c12 n6	0.34 d	0.01	0.31 d	0.02	0.07 e	<0.01	8.87 a	0.01	0.65 c	0.03	2.84 b	0.14
C18:3 c9 c12 c15 n3	0.11 b	0.02	<LOD d	–	0.10 b	<0.01	0.34 c	0.01	6.47 b	0.07	17.38 a	0.02
C20	0.11 d	0.01	0.16 c	0.01	0.07 e	<0.01	5.18 a	0.01	0.59 b	<0.01	0.16 c	0.01
C20:1 c9	0.22 d	0.02	0.08 d	<0.01	0.09 d	<0.01	2.09 b	0.01	11.09 a	0.23	1.97 b	0.01
C20:1 c11	0.46 c	0.03	0.55 b	0.02	1.03 a	0.01	0.58 b	0.05	0.07 d	0.01	<LOD e	–
C20:3 c11 c14 c17	0.35 c	0.05	<LOD d	–	0.47 a	0.13	<LOD d	–	0.53 a	0.01	0.51 a	0.03
C20:4 AA n6	18.62 a	0.50	4.92 d	0.01	12.26 a	0.05	12.43 c	0.04	15.01 b	0.03	2.86 e	0.01
C22	<LOD b	–	<LOD b	–	<LOD b	–	<LOD b	–	<LOD b	–	1.73 a	0.01
C22:1 c9	<LOD e	–	<LOD e	–	0.23 c	0.02	2.30 a	0.04	0.54 b	0.01	0.25 c	0.00
C20:5 n3	<LOD f	–	15.58 b	0.14	29.92 a	0.02	7.54 c	0.02	5.77 d	0.02	1.67 e	0.02
C22 c13 c16	<LOD b	–	<LOD b	–	0.22 a	0.01	<LOD b	–	<LOD b	–	<LOD b	–
C24	0.17 b	0.01	<LOD e	–	0.05 d	0.01	0.20 b	0.02	0.11 c	0.01	0.93 a	0.02
C22:5 n6	<LOD c	–	<LOD c	–	<LOD c	–	0.18 b	0.01	0.31 a	0.01	<LOD c	–
C24:1	0.44 a	<0.01	0.13 c	0.03	0.18 b	0.02	<LOD d	–	0.16 bc	0.04	<LOD d	–
C22:5 n3	0.61 a	0.09	<LOD c	–	0.06 b	<0.01	<LOD c	–	<LOD c	–	<LOD c	–
SFA	63.54 a	0.24	58.07 a	0.03	42.74 b	0.19	42.17 b	0.19	36.42 c	0.16	38.88 bc	0.02
MUFA	15.24 d	0.34	18.92 c	0.13	11.54 e	0.03	21.13 b	0.15	29.09 a	0.08	18.51 c	0.08
PUFA	21.22 c	0.58	23.01 c	0.03	45.72 a	0.16	36.70 b	0.01	34.49 b	0.07	42.60 a	0.10
Total n9	11.78 b	0.33	13.10 a	0.11	7.61 d	0.01	9.35 c	0.03	12.20 b	0.89	12.77 ab	1.36
Total n6	20.14 b	0.16	6.68 e	0.02	14.41 c	0.06	27.46 a	0.30	21.48 b	0.13	10.99 d	0.19
Total n3	1.38 e	0.44	16.08 b	0.13	31.56 a	0.11	8.88 d	0.17	13.21 c	0.03	31.57 a	0.72
n6/n3	15.36 a	1.83	0.42 de	0.01	0.46 d	0.05	3.09 b	0.44	1.63 c	0.14	0.35 e	0.02
μg FA/$mg_{dry\ seaweed}$	12.31 d	0.09	16.47 c	0.31	20.89 b	0.59	17.30 c	0.44	19.96 b	0.14	27.58 a	0.15

Data expressed as mean (Mean; $n = 3$) and standard deviation (SD). ai: anteiso. Phy: phytanic acid. AA: arachidonic acid. c/t: cis/trans double bond. SFA/MUFA/PUFA: total of saturated/monounsaturated/polyunsaturated fatty acids; DM: dry matter. a–f: in a row, significant differences among seaweed species.

characteristic PUFA showing 29.92 g/100g_{fat} in *G. turuturu* or 15.58 g/100g_{fat} in *O. pinnatifida*. These FA have gained much attention since they are related to the prevention of cardiovascular (CVD) and coronary (CHD) diseases. The current guidelines recommend an intake of 0.25–2 g of combined eicosapentaenoic acid (C20:5n-3) and DPA (C22:5n-3) (WHO., 2008) but those doses cannot be fully obtained from the analyzed seaweeds. Furthermore, the observed contents of arachidonic acid (AA; 18.62 g/100g_{fat} for *G. gracilis* to 2.86 g/100g_{fat} for *C. tomentosum*) and its association with inflammatory processes open the need to study their possible effects on human metabolism. Furthermore, *S. muticum* and *C. tomentosum* also showed high amounts of γ-linolenic acid (C18:3 c6 c9 c12; 8.87 g/100g_{fat} and 2.84 g/100g_{fat} respectively). On the other hand, the amounts of oleic acid (C18:1 c9) in *O. pinnatifida*, *G. gracilis*, *C. tomentosum* and *S. polyschides* (11.33–12.57/100g_{fat}) may compensate the contents in AA as it has been described to exert anti-inflammatory activities.

Other works comparing the composition of several seaweeds including that from *G. gracilis, G. turuturu* and *O. pinnatifida* reported a qualitative FA profile that is characteristic of these samples due to the presence of C18:3 c9 c12 c15 and AA (Kendel et al., 2013; Patarra, Leite, Pereira, Baptista, & Neto, 2013; Schmid, Guiheneuf, & Stengel, 2014). Those studies also reported similar compositions to that of the current research.

It must not be forgotten that at the current moment the elevated intake of seed oils plays a central role in the unbalance of the n6/n3 ratio and the development of CVD and CHD. According to WHO (2008), the ratio of n6/n3 should be lower than 10 in the diet. Ratio values lower than 1 were observed in *O. pinnatifida*, *G. turuturu* and in *C. tomentosum* (Table 2) making these edible species, already consumed in several countries such as Malaysia, China, Japan or Ireland (Munier et al., 2013; Roo et al., 2007), particularly adequate to be incorporated in a more balanced diet, from a lipid point of view. A ratio of 0.31 was reported by Paiva et al. (2014) for *O. pinnatifida*. A particularly high value for n6/n3 ratio was observed in *G. gracilis* in part due to the absence of EPA.

3.3. Elemental composition of seaweeds

Seaweeds are known to be rich in macro-elements with some content in trace elements. The elemental composition of the six seaweed species was analyzed and is displayed in Fig. 1. The most significant macro-elements present in the seaweeds were K, Na, Mg and Ca, accounting for more than 97% of the total mineral content, demonstrating that seaweeds are a good source of these elements. Phosphorous was present at a fairly constant value (between 1.72 and 2.81 mg/$g_{dry\ seaweed}$) among all 6 species of

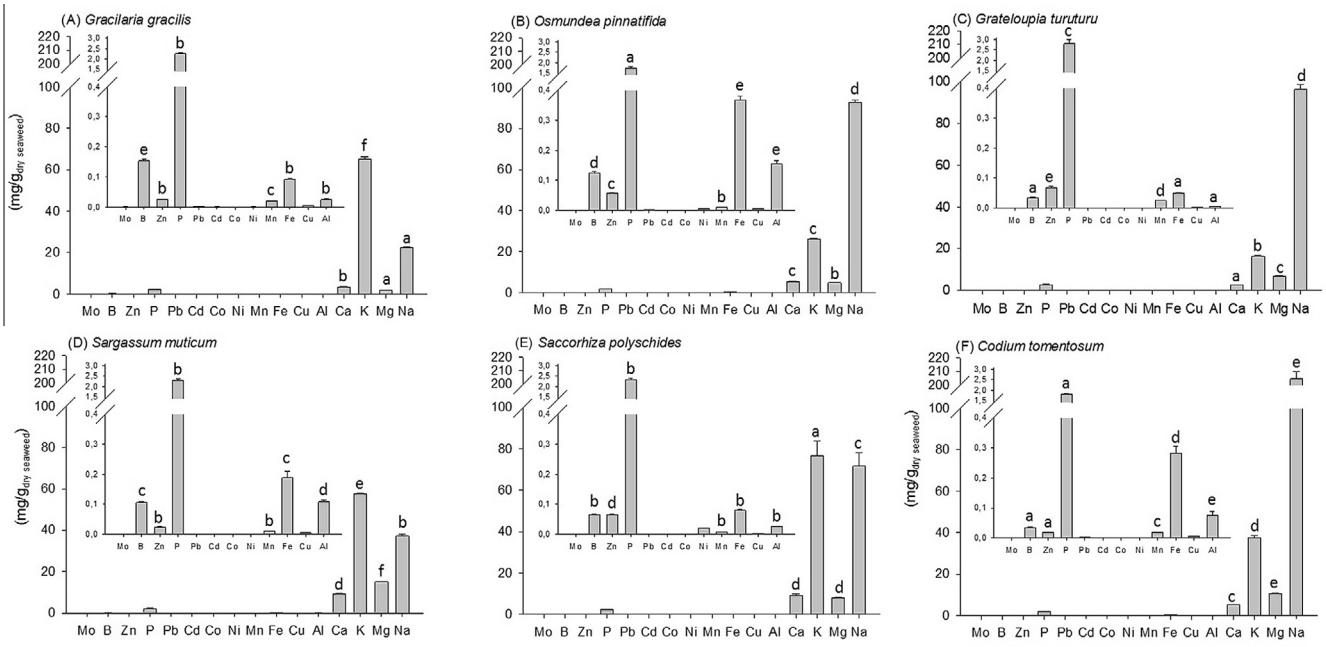

Fig. 1. Element contents ($mg/g_{dry\ seaweed}$) in the different species of red (Rhodophyta) (A, B and C), brown (Phaeophyceae) (D and E) and green (Chlorophyta) (F) seaweeds. For each element, Different letters indicate significant differences ($p < 0.05$) between species.

seaweeds studied but of much lower order of magnitude than the four other minerals previously mentioned.

In what concerns mineral distribution per species, inter and intra algae class variations were observed. For example, K was the most predominant element found in *S. muticum*, *G. gracilis* and *S. polyschides* with contents ranging between 16.28 and 76.54 $mg/g_{dry\ seaweed}$, being statistically different ($p < 0.05$) among all the six species under study. Sodium content also differed significantly between the different seaweed species except between *O. pinnatifida* and *G. turuturu* ($p > 0.05$). In fact, in *O. pinnatifida* and *G. turuturu* Na was the most predominant element ranging between 92.88 and 96.08 $mg/g_{dry\ seaweed}$ yet it attained an extremely high value of 204.52 $mg/g_{dry\ seaweed}$ in *C. tomentosum* (also most predominant element). Sodium/potassium ratios lower than 1 were observed for *G. gracilis* (0.3), *S. muticum* (0.6) and *S. polyschides* (0.9) whereas ratios higher than 1 were observed for *O. pinnatifida* (3.56), *G. turuturu* (5.9) and C. *tomentosum* (5.5), respectively (Fig. 1). A similar tendency was reported by Jard et al. (2013) for *S. muticum* (0.6), *S. polyschides* (0.4) *and C. tomentosum* (10.0). Lower ratio Na/K was reported by Paiva et al. (2014) for *O. pinnatifida* (1.82). Seaweeds with low ratios of Na/K are good possibilities to be used as salt substitutes.

Magnesium is known to be an important mineral for cardiovascular function (Krishnaiah, Sarbatty, Prasad, & Bono, 2008) and its external administration could prevent its intracellular depletion. Magnesium content in the six seaweeds ranged between 1.75 $mg/g_{dry\ seaweed}$ for *G. gracilis* and 15.04 $mg/g_{dry\ seaweed}$ for *S. muticum* (Fig. 1) differing significantly between each species ($p < 0.05$); in general, contents were higher in species of brown and green algae classes than in red algae species.

Calcium values ranged between 2.65 $mg/g_{dry\ seaweed}$ in *G. turuturu* to 9.11–9.18 $mg/g_{dry\ seaweed}$ in brown seaweeds *S. muticum* and in *S. polyschides*, respectively. Calcium content was not statistically different ($p < 0.05$) among *S. muticum* and *S. polyschides* as well as among *C. tomentosum* and *O. pinnatifida*. Calcium is well-known for its importance in terms of health preservation due to its diverse biological roles being essential for structural support, cell adhesiveness, mitosis, blood coagulation, muscle contraction and glandular secretion being the most significant consequence of the low calcium status the occurrence of osteoporosis, which is a disease characterized by reduced bone mass which increases skeletal fragility (Allen, de Benoist, Dary, & Hurrel, 2006).

Phosphorous was the least representative macro-element in quantitative terms, ranging between 1.72 and 2.80 $mg/g_{dry\ seaweed}$. No significant differences for P were observed between *S. polyschides*, *S. muticum* and *G. gracilis* ($p > 0.05$) as well as between *C. tomentosum* and *O. pinnatifida* ($p > 0.05$). 1–2 $g/Kg_{dry\ seaweed}$ of P was reported by Jard et al. (2013) for *S. muticum*, *S. polyschides* and *C. tomentosum*. Phosphorus is part of the skeletal structure and teeth having other important functions such as the contribution to the control of acid-base balance in the blood; in the metabolism of carbohydrates it contributes to the intestinal absorption of glucose by the process of phosphorylation (Pérez, Garaulet, Gil, & Zamora, 2005).

In terms of micro-elements B, Zn, Mn, Fe and Al were the most representative among the six species of seaweeds analyzed. In the six seaweeds species Fe was the most representative among the microelements especially in *S. muticum*, *O. pinnatifida* and *C. tomentosum* ranging from 0.05 and 0.37 $mg/g_{dry\ seaweed}$; values were statistically different among all species analyzed except for comparable contents among *G. gracilis* and *S. polyschides*. Iron is a component of various enzymatic systems and has as its main function the transport of oxygen from the lungs to the tissues: Iron deficiency is one of the most common nutritional disorders worldwide due to several reasons such as bleeding or mal-nutrition balance. It is known that iron deficiency can cause anemia (Allen et al., 2006) and therefore infants under 24 months of age when exposed to iron deficiency can be affected in the development of the neuropsychomotor system, with severe consequences for the future. Zinc element which ranged from 0.02 $mg/g_{dry\ seaweed}$ in *C. tomentosum* to 0.07 $mg/g_{dry\ seaweed}$ in *G. turuturu* is known to enhance the catalytic, structural and regulatory functions, to stabilize membranes, hormones and nucleic acids (Krishnaiah et al., 2008), and is important for cellular growth and differentiation in tissues that have a rapid differentiation and turnover (Allen et al., 2006). The deficiency of Zn could be a limiting factor during pregnancy

affecting normal embryonic and fetal growth in experimental animals, and also the length of gestation.

In general, the six edible seaweeds collected from the Portuguese Coast were of important nutritional value not only from its proximate composition but especially due to its elemental composition. As far as the Portuguese population is concerned these analyzed seaweeds available along the Portuguese Coast offer a wide array of nutrients, at concentrations that may meet with nutritional requirements, particularly in what concerns elements present and in some cases may even allow for application of nutritional claims. Data on daily intake of seaweeds are unavailable for the Portuguese population let alone for other European countries. Nonetheless, if seaweed daily consumption in Asian countries is taken into account then one may consider 8–10 g of seaweed dry matter as an average daily intake (Mišurcová, Machů, & Orsavová, 2011). Based on this reference value Table 1 indicates the possible contribution of analyzed seaweeds to daily nutritional requirements. Whereas such daily intake contributes with a small fraction to protein, carbohydrate and fat requirements (Table 1), in terms of micro-elements some of these seaweeds contribute well over 15% (minimum requirement for nutritional claim) of the recommended daily intakes (RDIs). Variable contribution to the RDI for the elements is observable in Table 1. In terms of macro-elements all species except *G. turututu* are good contributors to potassium RDI, especially *G. gracilis* and *S. polyschides* with values higher than 30%. All selected species, except *G. gracilis*, can be considered as good sources of Mg, especially *S. muticum* (40.1%). On the other hand these species were low providers of Ca and P. Interesting contribution values to Fe RDI were observed in *O. pinnatifida*, *S. muticum* and *G. tutuuru* with values ranging between 13% and 26%. Lower values were, in general, found for the other microelements. Higher values of %RDI for Fe, Zn and Mn were, in general, reported by Mišurcová et al. (2011) but for other brown, red and green algae. According to these authors seaweeds could be utilized as nutraceuticals due to their richness in elements. As previously mentioned, an adequate intake of minerals is essential to prevent nutritional deficiencies and diseases but excessive intakes of trace elements may cause toxicity.

3.4. FTIR-ATR and FT-Raman characterization

Vibrational spectroscopy can reveal detailed information concerning the properties and structure at a molecular level for compounds such as carrageenans and agar present in some red algae (Rhodophyceae), alginic acid/alginates present in some brown algae (Phaeophyceae) (Pereira, 2006). In seaweeds sugars, followed by protein (Table 1), are the major groups of compounds. Carrageenans and agar (galactans) are the main sulphated polysaccharides produced by red seaweeds (Rhodophyta), whereas alginate is mainly found in brown seaweeds (Phaeophyceae). Fucans including compounds such as fucoidin, fucoidan, sargassan, etc. are sulphated polysaccharides that can also be found in brown seaweeds (Shanmugan & Mody, 2000). The major polysaccharides in green algae (Chlorophyta) are, in turn, polydisperse heteropolysaccharides where glucuronoxylorhamnans, glucuronoxylorhamnogalactans or xyloarabinogalactans are the three main groups (Shanmugan & Mody, 2000).

According to Gómez-Ordóñez and Rupérez (2011), based on Mathlouthi and Koenig (1987) verifications, five frequency regions can be differentiated in the normal spectra (4000–650 cm^{-1}) from vibrational structural analysis of carbohydrates for seaweeds: (1) region of O–H and C–H stretching vibrations at 3600–2800 cm^{-1}; (2) region of local symmetry at 1500–1200 cm^{-1}; (3) region of C–O stretching vibration at 1200–950 cm^{-1}; (4) fingerprint or anomeric region at 950–750 cm^{-1}; and (5) skeletal region below 700 cm^{-1}. N–H stretching vibrations at 3700–2900 cm^{-1} as well as from amide I and amide II at 1700–1420 cm^{-1} could be related to proteins (Chopin, Kerin, & Mazerolle, 1999) which were found in a range between 14.3 and 23.8 $g/100g_{dry\ seaweeds}$ in the selected seaweeds.

In Fig. 2, it is possible to observe two bands in the 4000–2000 cm^{-1} common in all studied seaweeds, which is in accordance to data published by Gómez-Ordóñez and Rupérez (2011) for phycocolloid standards (carrageenans, agar and alginate) as well as for seaweeds samples. A broad band at 3280–3350 cm^{-1} and a weaker signal at 2870–2960 cm^{-1} could be assigned to O–H and C–H stretching vibrations but also to N–H stretching vibrations, respectively.

Medium to strong IR absorption bands were observed in all spectra between 900 and 1750 cm^{-1} in FTIR-ATR spectra. According to several authors, bands between 1200 and 970 cm^{-1} are due mainly to C–C and C–O stretching bonds, common to all polysaccharides (Gómez-Ordóñez & Rupérez, 2011). Pereira, Gheda, and Ribeiro-Claro (2013) focused their analysis by FTIR-ATR and by FT-Raman of seaweed polysaccharides mainly in the 600–1500 cm^{-1} spectra range.

In Fig. 3A–C, the 700–1400 cm^{-1} regions of all seaweeds are amplified enabling a more detailed observation of the characteristic bands present in this region. FTIR, FTIR-ATR and FT-Raman band assignments by several authors for red (Rhodophyta) seaweeds polysaccharides (carragenans and agar) and for brown (Phaeophyta) seaweeds polysaccharides (alginates and fucoidans) are listed in Table 3, respectively. The FTIR-ATR spectrum of *G. turuturu* in the region 1400–700 cm^{-1} (Fig. 3A) show some characteristic features related with typical polysaccharides from red seaweeds (Table 3):

(i) a high absorbance band at 1220 and 1150 cm^{-1}, typically more intense for highly sulphated polysaccharides (such as carrageenans) than in less sulphated polysaccharides (such as agar), and at 1012 cm^{-1} which have been assigned to S=O sulphate esters and to C–O and C–C stretching vibrations of pyranose common to all polysaccharides, respectively; Strong absorption at 1220–1260 cm^{-1} have been reported by Yu et al., 2012 for agaran-type polysaccharide isolated from *Grateloupia filicina*.

(ii) 930 and 900 bands in the anomeric region (950–700 cm^{-1}) assigned to the presence of 3,6-anhydro-D-galactose and anomeric C–H of β-galactopyranosyl residues, respectively; the first band has been found in carragenan and agar polysaccharides (Pereira, 2006) whereas the second is more typical in Beta carrageenans (Knutsen, Myslabodski, Larsen, & Usov, 1994; Pereira, 2006);

(iii) a relatively strong band at 819 cm^{-1} which has been assigned to the presence of galactose units sulphated at the C-2 position, typical in lambda carrageenans standards spectra (Pereira et al., 2009) but also observed in agaran-type polysaccharide isolated from *G. filicina* (Yu et al., 2012).

According to several reported studies, natural diversity of polysaccharides among *Grateloupia* spp. exists; polysaccharides isolated from other *Grateloupia* species have showed agaran-carrageenan backbones (*G. indica*, Sen et al., 2002) or agaran-type backbone (*G. filicina*, Yu et al., 2012). Enzymatic digestion of *G. turuturu* confirmed indirectly the presence of agar, kappa and iota carrageenans in their cell walls (Denis, Morançais, Gaudin, & Fleurence, 2009). Therefore, and in accordance to the main bands observed in *G. turuturu* spectrum (Fig. 3A), this red seaweed may be a producer of agaroid-carrageenan hybrid polysaccharides.

The phycocolloid agar is obtained from some families of red algae such as Gracilariaceae and Gelidiaceae; *Gracilaria* species, namely *G. gracilis*, are known to produce agars with relatively high

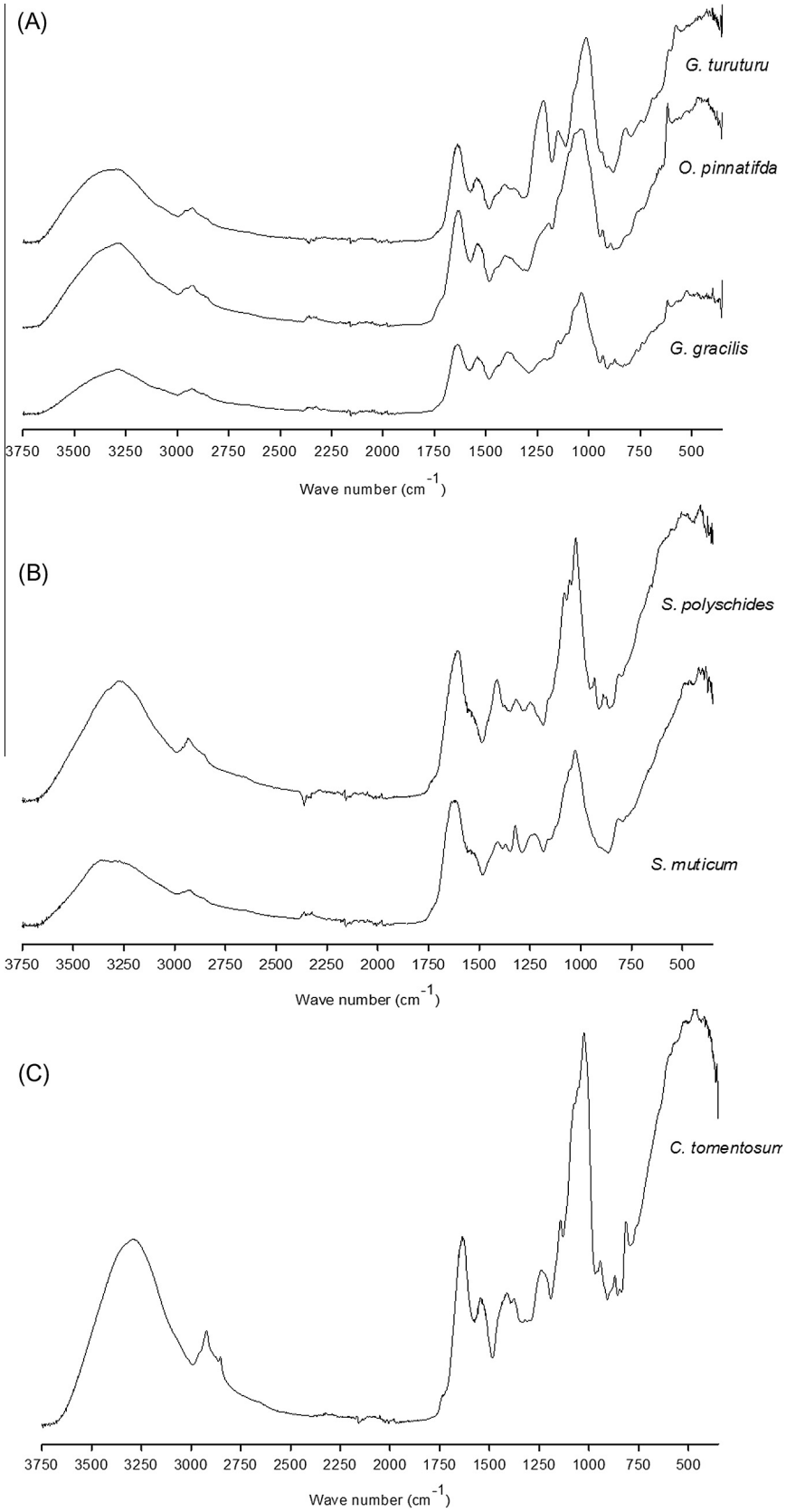

Fig. 2. FTIR-ATR spectra of red (Rhodophyta) (A), brown (Phaeophyceae) (B) and green (Chlorophyta) (C) seaweeds.

sulphate content. According to Pereira et al. (2013), agars differ from carrageenans as they have a L-configuration for the 4-linked galactose residue but they have some structural similarities with carrageenans. In fact the FTIR-ATR spectrum obtained for *G. gracilis* presented several bands in common to the *G. turuturu* spectrum. Some of the main differences were centred on the broad band at

Table 3
FTIR-ATR and FT-Raman band assignment for polysaccharides from red (Rhodophyta) and brown (Phaeophyceae) seaweeds.

Wave numbers (cm^{-1})	Assignment*	Vibrational Spectroscopy	Polysaccharides	References
Red seaweeds				
1210–1260	S=O of sulphate esters	FTIR, FTIR-ATR, FT-Raman	Carrageenan Agar	Knutsen et al., 1994; Pereira, 2006; Gómez-Ordóñez & Rupérez, 2011
1150	Sulphate ester	FTIR-ATR	Carrageenan Agar	Chopin, Kerin, and Mazerolle, 1999
1070–1085, 925–935; 837	C–O of 3,6-anhydro-D-galactose	FTIR, FTIR-AT, FT-Raman	Carrageenan Agar	Knutsen et al., 1994, 2003, 2013; Pereira, 2006; Gómez-Ordóñez & Rupérez, 2011
1010–1030	C–O, C–C stretching vibration of pyranose ring	FTIR, FTIR-AT, FT-Raman	Carrageenan Agar	Pereira et al., 2013
970–975	D-galactose	FTIR; FT-Raman	Carrageenan	Knutsen et al., 1994; Pereira, 2006
905–907, 800–808	C-O-SO_3 on C2 of 3,6-anhydro-galactose	FTIR; FTIR-ATR, FT-Raman	Carrageenan	Knutsen et al., 1994; Pereira, 2006; Gómez-Ordóñez & Rupérez, 2011
890–900	C–H on anomeric carbon of β-galactose (unsulphated β-D-galactose)	FTIR-ATR, FT-Raman	Carrageenan Agar	Pereira et al., 2003, 2009; Pereira, Gheda, and Ribeiro-Claro, 2013
867–871, 810–825	C-O-SO_3 on C_6 of galactose	FTIR; FTIR-ATR, FT-Raman	Carrageenan	Knutsen et al., 1994; Pereira, 2006; Gómez-Ordóñez & Rupérez, 2011
840–850	C-O-SO_3 on C_4 of galactose	FTIR; FT-ATR; FT-Raman	Carrageenan	Knutsen et al., 1994; Pereira et al., 2003; Pereira, 2006; Gómez-Ordóñez & Rupérez, 2011
820–830	C-O-SO_3 on C_2 of galactose	FTIR; FTIR-ATR, FT-Raman	Carrageenan	Knutsen et al., 1994; Pereira, 2006; Gómez-Ordóñez & Rupérez, 2011
740, 770	Skeletal bending of galactose ring	FTIR-ATR, FT-Raman	Carrageenan Agar	Pereira et al., 2003; Pereira, Gheda, and Ribeiro-Claro, 2013
Brown seaweeds				
1195–1260	S=O of sulphate esters	FTIR-ATR	Fucoidan	Gómez-Ordóñez & Rupérez, 2011; Pereira, Gheda, and Ribeiro-Claro, 2013
1290, 1080, 1025, 787	Guluronic acid	FTIR-ATR; FT-Raman	Alginate	Gómez-Ordóñez & Rupérez, 2011; Pereira et al., 2013;
1320,1030, 1019, 808	Mannuronic acid	FTIR-ATR; FT-Raman	Alginate	Gómez-Ordóñez & Rupérez, 2011; Pereira et al., 2013;
948	C–O of uronic acid residues	FTIR-ATR	Alginate	Gómez-Ordóñez & Rupérez, 2011
878	C_1–H of β-mannuronic acid	FTIR-ATR	Alginate	Gómez-Ordóñez & Rupérez, 2011

* Band assignments resulted both from the use of standards (agar and carrageenans for the red seaweeds and alginate for the brown seaweeds) as well as from the red or brown seaweeds studied by the referenced authors.

1220 cm^{-1} and at 1020–1040 cm^{-1} which were extremely more intense in the *G. turuturu* spectrum. The band at 890 cm^{-1} has been assigned to unsulphated β-D-galactose, whereas, band at 825 cm^{-1} has been assigned to α-L-galactose sulphate residues, typical of agar type seaweeds. The strong bands at 740 and 770 cm^{-1} in FT-Raman spectrum and weak in FTIR-ATR spectrum (Fig. 3) are according to those observed by Pereira, Sousa, Coelho, Amado, and Ribeiro-Claro (2003) for red agar-like seaweed. 740 and 770 cm^{-1} bands are assigned to the skeletal bending of the galactose ring (Table 3) whereas 890 cm^{-1} is typical of agar being associated with C–H bending at the anomeric carbon of β-galactose residue (Table 3). The strong bands at 837 and 1079 cm^{-1} in FT-Raman spectrum, absent in FTIR-ATR, have been observed by Pereira et al. (2003) in commercial agar and in *G. gracilis* extracted agar.

Due to the high similarity of the *O. pinnatifida* spectrum with that of *G. gracilis*, especially in terms of such diagnostic agar bands as those at 1220, 1020–1040 and 890 cm^{-1} in FTIR-ATR spectrum and 1079, 890, 837, 770 and 740 cm^{-1} in FT-Raman spectrum (data not shown), it could be indicated that this species probably may be considered an agar-like producer.

The main polysaccharide which have been found in brown seaweeds such as *S. muticum* and *S. polyschides* is alginate known to be a linear copolymer of β-D-mannuronic acid and α-L-guluronic acid (1–4)-linked residues arranged in heteropolymeric and/or homopolymeric blocks (Pereira et al., 2003). The presence of these acids can be evidenced especially by the bands at 1025, 1080 and 787 cm^{-1} assigned to guluronic acid and at 808 and 1320 cm^{-1} assigned to mannuronic acid, respectively (Fig. 3B and Table 3). A strong band at 1025 cm^{-1} in the FTIR-ATR spectrum and weak in FT-Raman spectrum was observed by Pereira et al. (2013) in a commercial alginate and in *S. polyschides*. In addition, this band is indicative that both *S. muticum* and *S. polyschides* are particularly rich in guluronic acid. The FTIR-ATR spectrum of *S. muticum* suggests that this species is richer in mannuronic acid in comparison to *S. polyschides* due to more intense bands at 808 and 1320 cm^{-1}. According to Pereira et al. (2013), *Sargassum vulgare* was characterized by similar amounts of both mannuronate and guluronate residues. The broad band around 1220–1260 cm^{-1} in FTIR-ATR spectrum, assigned to the presence of sulphate ester groups (S=O) which is a characteristic component in fucoidan and other sulphated polysaccharides that can be found in some brown seaweeds, are observable for both *S. polyschides* and S. *muticum* (Fig. 3B). Fucans comprise heteromolecules based on L-fucose, D-xylose, D-glucuronic acid, D-mannose and D-galactose (Shanmugan & Mody, 2000).

(1 → 4)-β-D-mannans have been identified as the predominant skeletal wall polysaccharides in green algae including in the *Codium* genera (Dunn et al., 2007). In terms of matrix cell wall polysaccharides, sulphated xyloarabinogalactans but also a variable range of these polymers such as sulphated galactans, sulphated arabinan, sulphated arabinogalactans and sulphated glucans have been found in green algae (Estevez, Férnandez, Kasulin, Dupree, & Ciancia, 2009). Sulphated arabinan and sulphated arabinogalactan have been identified in *C. tomentosum* (Shanmugan & Mody, 2000). In Fig. 3C more intense bands are observable at 1143, 1078, 1054 and 1025 cm^{-1} which could probably be assigned to (1 → 4)-β-D-mannans. Estevez et al. (2009) reported FTIR spectrum bands at 1151, 1092, 1061 and 1032 cm^{-1} associated to β-mannans in *Codium fragile*; 1151 cm^{-1}

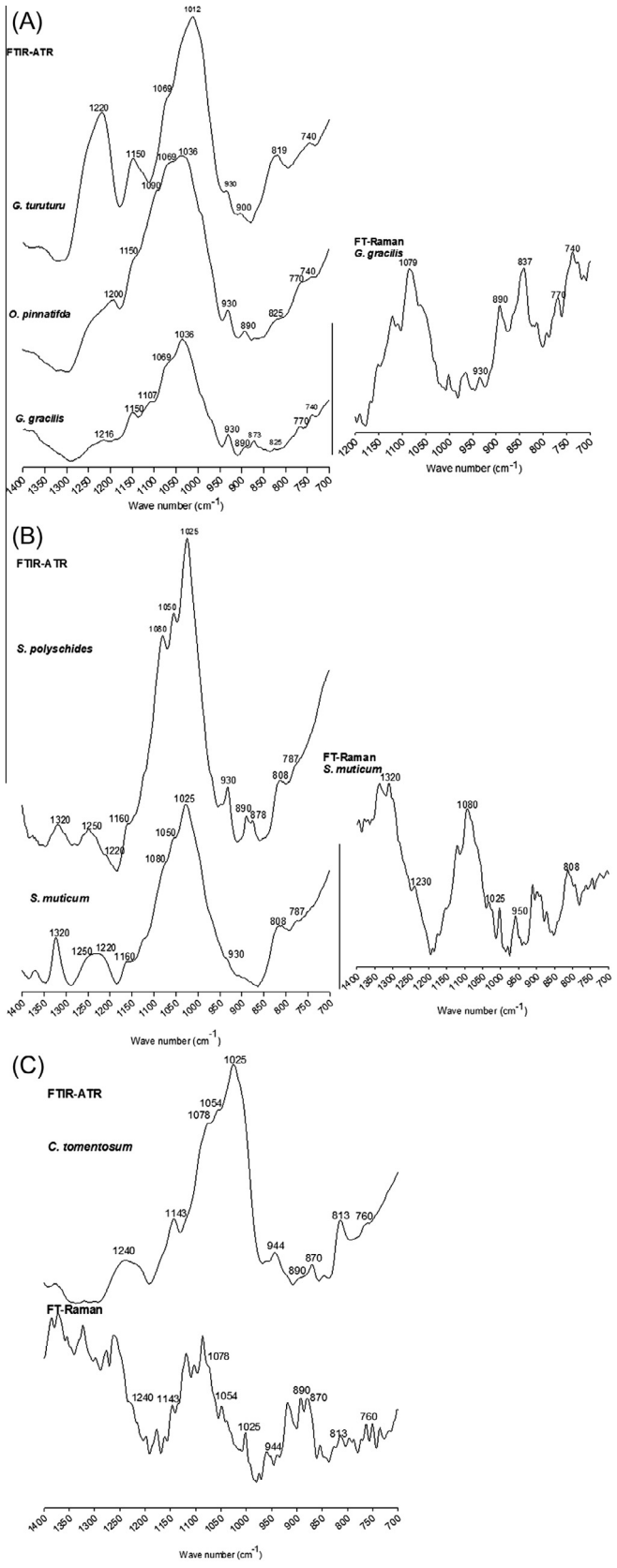

Fig. 3. FTIR-ATR and FT-Raman spectra of red (Rhodophyta) (A), brown (Phaeophyceae) (B) and green (Chlorophyta) (C) seaweeds between 1400 and 700 cm^{-1}.

band has been assigned to the glycosidic C–O–C vibration of (1 → 4)-β-D-mannans whereas the bands at 1061 and 1032 cm^{-1} have been assigned to C–O–C and C–C bonds of mannose unit rings. Bands at 1200–1000 cm^{-1} region have been assigned to sugar rings vibration overlapping with stretching of side groups (C–OH) and glycosidic bonds vibration (C–O–C) in Ulvophyceae green algae (Pereira & Ribeiro-Claro, 2014). A broad weak band around 1240 cm^{-1} is typical of S=O of sulphate esters of sulphated polysaccharides which is observable in the spectrum of C. *tomentosum* (Fig. 3C), indicating the presence of this type of polysaccharide in this green algae. Bands between 945 and 760 cm^{-1} with different intensities in FTIR or FT-Raman spectra (Fig. 3C) are probably due to the presence of sulphated and unsulphated galactose residues. Scarce information on vibrational spectroscopy analysis on *Codium* specimens is found in the literature.

4. Conclusions

The study shows that the six edible seaweed species harvested from the Portuguese West Coast have a good potential for further processing or for direct food and nutraceutical applications given their very good nutrient profiles. The proximate and elemental composition varied significantly among brown, red and green seaweeds as well as within species in each main class. Red algae species registered the highest protein content but the lowest fat and sugar content. On the other hand, comparatively green and brown algae stood out for their highest fat and sugar content, respectively. The low fat content (0.6–3.6%) found among the studied seaweeds was coupled to a specific FA profile rich in palmitic acid, araquidonic acid and EPA; the presence of phytanic acid was also worth noting. Higher total phenolic content was observed in the green algae (*C. tomentosum*) followed by brown algae (*S. muticum*) and the red algae (*O. pinnatifida*). In general, seaweeds were characterized by high levels of minerals. Higher levels of ash were associated with higher amounts of elements content especially of macro-elements. Some of the selected seaweeds (*S. muticum*, *S. polyschides* and *C. tomentosum*) may be included in human diet to help solve problems with mineral deficiency, in particular, Ca, K, Mg and Fe, since they revealed to be good sources of these elements contributing significantly to the daily requirements intake of several countries. According to FTIR-ATR and FT-Raman spectra, *G. gracilis* and *O. pinnatifida* were mostly agar producers whereas *G. turuturu* was associated *to* agaroid-carrageenan hybrid polysaccharides. In the brown algae, *S. muticum* and *S. polyschides,* alginates and fucoidans were the main representative polysaccharides and in *C. tomentosum* (1 → 4)-β-D-mannans, sulphated and unsulphated galactose residues were evidenced. The presence of these polysaccharides further upholds the interest in exploring these algae for applications in health-related fields, for example, drug or nutraceutical delivery approaches.

Acknowledgments

This work was supported by Portuguese Science Foundation (FCT – Fundação para a Ciência e Tecnologia) through individual research Grants references SFRH/BPD/73781/2010, SFRH/BD/77647/2011 and SFRH/BPD/65410/2009 under QREN – POPH funds, co-financed by the European Social Fund and Portuguese National Funds from MCTES and projects PEst-C/MAR/LA0017/2013 and PEst-OE/EQB/LA0016/2013 supported by European Funds through COMPETE and by National Funds through the FCT.

References

Alghazeer, R., Whida, F., Abduelrhman, E., Ammoudi, F., & Azwai, S. (2013). Screening of antibacterial activity in marine green, red and brown macroalgae from the western coast of Libya. *Natural Science, 5*, 7–14.

Allen, L., de Benoist, B., Dary, O., & Hurrel, R. (2006). Guidelines on food fortification with micronutrientes. World Health Organization and Food and Agriculture Organization of the United Nations, ISBN 92 4 159401 2.

Boulom, S., Robertson, J., Hamid, N., Ma, Q., & Lu, J. (2014). Seasonal changes in lipid, fatty acid, α-tocopherol and phytosterol contents of seaweed, *Undaria pinnatifida*, in the Marlborough Sounds, New Zealand. *Food Chemistry, 161*, 261–269.

Chandía, N. P., & Matsuhiro, B. (2008). Characterization of a fucoidan from *Lessonia vadosa* (Phaeophyta) and its anticoagulant and elicitor properties. *International Journal of Biological Macromolecules, 42*, 235–240.

Chojnacka, K., Saeid, A., Witkowska, Z., & Tuhy, L. (2012). Biologically active compounds in seaweed extracts - the prospects for the application. The Open Conference Proceedings Journal, 3 (Suppl 1–M4), 20–28.

Chopin, T., Kerin, B. F., & Mazerolle, R. (1999). Phycocolloid chemistry as a taxonomic indicator of phylogeny in the Gigartinalis, Rhodophyceae: A review and current developments using Fourier transform infrared diffuse reflectance spectroscopy. *Phycological Research, 47*, 167–198.

Denis, C., Morançais, M., Gaudin, P., & Fleurence, J. (2009). Effect of enzymatic digestion on thallus degradation and extraction of hydrosoluble compounds from *Grateloupia turuturu*. *Botanica Marina, 52*, 262–267.

Denis, C., Morançais, M., Li, M., Deniaud, E., Gaudin, P., & Wielgosz-Collin, G. (2010). Study of the chemical composition of edible red macroalgae *Grateloupia turuturu* from Brittany (France). *Food Chemistry, 119*, 913–917.

Dunn, E. K., Shoue, D. A., Huang, X., Kline, R. E., Mackay, A. L., Carpita, N. C., et al. (2007). Spectroscopic and biochemical analysis of regions of the cell wall of the unicellular "Mannan Weed", *Acetabularia acetabulum*. *Plant Cell Physiology, 48*, 122–133.

Estevez, J. M., Férnandez, P. V., Kasulin, L., Dupree, P., & Ciancia, M. (2009). Chemical and in situ characterization of macromolecular components of the cell walls from the green seaweed *Codium fragile*. *Glycobiology, 19*, 212–228.

Gómez-Ordóñez, E., & Rupérez, P. (2011). FTIR-ATR spectroscopy as a tool for polysaccharide identification in edible brown and red seaweeds. *Food Hydrocolloids, 25*, 1514–1520.

Guiry, M. D., & Guiry, G. M. (2013). *AlgaeBase*. World-wide electronic publication, National University of Ireland, Galway. http://www.algaebase.org, searched on 06 June 2013.

Hellgren, L. I. (2010). Phytanic acid–an overlooked bioactive fatty acid in dairy fat? *Annals of the New York Academy of Sciences, 1190*, 42–49.

Holdt, S. L., & Kraan, S. (2011). Bioactive compounds in seaweeds: Functional food aplications and legislation. *Journal of Applied Phycology, 23*, 543–597.

Ibañez, E., & Cifuentes, A. (2013). Benefits of using algae as natural sources of functional ingredients. *Journal of Science Food and Agricultural, 93*, 703–709.

Jard, G., Marfaing, H., Carrère, H., Delgenes, J. P., Steyer, J. P., & Dumas, C. (2013). French Brittany macroalgae screening: Composition and methane potential for potential alternative sources of energy and products. *Bioresource Technology, 144*, 492–498.

Kendel, M., Couzinet-Mossion, A., Viau, M., Fleurence, J., Barnathan, G., & Wielgosz-Collin, G. (2013). Seasonal composition of lipids, fatty acids, and sterols in the edible red alga *Grateloupia turuturu*. *Journal of Applied Phycology, 25*(2), 425–432.

Knutsen, S.-H., Myslabodski, D. E., Larsen, B., & Usov, A. I. (1994). A modified system of nomenclature for red algal galactans. *Botanica Marina, 37*, 163–170.

Krishnaiah, D., Sarbatty, R., Prasad, D. M. R., & Bono, A. (2008). Mineral content of some seaweeds from Sabah's South China sea. *Asian Journal of Scientific research, 1*, 166–170.

Lodeiro, P., Lopez-Garcia, M., Herrero, L., Barriada, J. L., Herrero, R., Cremades, J., et al. (2012). A physicochemical study of Al(+3) interactions with edible seaweed biomass in acidic waters. *Journal of Food Science, 77*, 1750–3841.

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

Mak, W., Hamid, N., Liu, T., Lu, J., & White, W. L. (2013). Fucoidan from New Zealand *Undaria pinnatifida*: Monthly variations and determination of antioxidant activities. *Carbohydrate Polymers, 95*, 606–614.

Mak, W., KelvinWang, S., Liu, T., Hamid, N., Li, Y., Lu, J., et al. (2014). Anti-proliferation potential and content of fucoidan extracted from sporophyll of New Zealand *Undaria pinnatifida*. *Frontiers in Nutrition, 1*, 1–10.

Manivannan, K., Thirumaran, G., Devi, G. K., Hemalatha, A., & Anantharaman, P. (2008). Biochemical composition of seaweeds from Mandapam Coastal Regions along Southeast Coast of India. *American-Eurasian Journal of Botany, 1*, 32–37.

Marsham, S., Scott, G. W., & Tobin, M. L. (2007). Comparison of nutritive chemistry of a range of temperate seaweeds. *Food chemistry, 1001*, 1331–1336.

Mathlouthi, M., & Koenig, J. L. (1987). Vibrational spectra of carbohydrates. *Advances in Carbohydrate Chemistry and Biochemistry, 44*, 82–89.

Mišurcová, L., Machů, L., & Orsavová, J. (2011). Seaweed Minerals as Nutraceuticals. In Steve. Taylor (Ed.), *Marine medicinal foods: Implications and applications, macro and microalgae: 64 (Advances in Food and Nutrition Research* (pp. 371–390). USA: Elsevier Inc.

Munier, M., Dumay, J., Morançais, M., Jaouen, P., & Fleurence, J. (2013). Variation in the Biochemical Composition of the Edible Seaweed Grateloupia turuturu Yamada Harvested from Two Sampling Sites on the Brittany Coast (France): The Influence of Storage Method on the Extraction of the Seaweed Pigment R-Phycoerythrin. Journal of Chemistry, 2013, Article ID 568548, 8 pages.

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.

Patarra, R. F., Leite, J., Pereira, R., Baptista, J., & Neto, A. I. (2013). Fatty acid composition of selected macrophytes. *Natural Product Research, 27*(7), 665–669.

Patarra, R. F., Paiva, L., Neto, A. I., Lima, E., & Baptista, J. (2011). Nutritional value of selected macroalgae. *Journal of Applied Phycology, 23*, 205–208.

Pereira, L. (2006). Identification of phycocolloids by vibrational spectroscopy. In Alan T. Critchley, Masao Ohno and Danilo B Largo (Eds.) World Seaweed Resources – An authoritative reference system. ETI Information Services Ltd., Hybrid Windows and Mac DVD-ROM; ISBN: 90-75000-80-4.

Pereira, L. & Ribeiro-Claro, P. J. A. (2014). Analysis by vibrational spectroscopy of seaweed with potential use in food, pharmaceutical and cosmetic industries. In: L. Pereira, L. J., Patrício, J., & Neto, J. M. (Eds.), Marine Algae – Biodiversity, Taxonomy, Environmental Assessment and Biotechnology. Cap. 7. Science Publishers. An Imprint of CRC Press / Taylor & Francis Group. p. 225–247. ISBN 9781466581678.

Pereira, L., Gheda, S. F., & Ribeiro-Claro, P. J. A. (2013). Analysis by vibrational spectroscopy of seaweed polysachrides with potential use in food, pharmaceutical and cosmetic industries. International Journal of Carbohydrate Chemistry, 2013, Article ID 537202, 7 pages.

Pereira, L., Amado, A. M., Critchley, A. T., van de Velde, F., & Ribeiro-Claro, P. J. A. (2009). Identification of selected seaweed polysacharide (phycocolloids) by vibrational spectroscopy (FTIR-ATR and FT-Raman). *Food Hydrocolloids, 23*, 1903–1909.

Pereira, L., Sousa, A., Coelho, H., Amado, A. M., & Ribeiro-Claro, P. J. A. (2003). Use of FTIR, FT-Raman and 13C-NMR spectroscopy for identification of some seaweed phycocolloids. *Biomolecular Engineering, 20*, 223–228.

Pérez, F., Garaulet, M., Gil, A., & Zamora, S. (2005). Calcio, fósforo, magnesio y flúor. Metabolismo óseo y su regulación, In: A. Gil (Ed.), Tratado de Nutrición, Vol. I Grupo Acción Médica, Madrid, pp. 897–925.

Plaza, M., Cifuentes, A., & Ibánez, E. (2008). In the search of new functional food ingredients from algae. *Trends Food Science Technology, 19*, 31–39.

Roo, P. V. S., Mantri, V. A., Ganesan, K., & Kumar, K. S. (2007). Chapter 5 – Seaweeds as a Human Diet: An Emerging Trend in the New Millennium. In R. K. Gupta & V. D. Pandey (Eds.), *Advances in Applied Phycology* (pp. 85–96). New Delhi: Daya Publishing House.

Sánchez-Avila, N., Mata-Granados, J. M., Ruiz-Jiménez, J., & Luque de Castro, M. D. (2009). Fast, sensitive and highly discriminant gas chromatography-mass spectrometry method for profiling analysis of fatty acids in serum. *Journal of Chromatography A, 1216*(40), 6864–6872. http://dx.doi.org/10.1016/j.chroma.2009.08.045.

Schmid, M., Guiheneuf, F., & Stengel, D. B. (2014). Fatty acid contents and profiles of 16 macroalgae collected from the Irish Coast at two seasons. *Journal of Applied Phycology, 26*(1), 451–463.

Sen, A. K., Das, A. K., Sarkar, K. K., Takano, R., Kamei, K., & Hara, S. (2002). An agaroid-carrageenan hybrid type backbone structure for the antithrombotic sulfated polysaccharide from *Grateloupia indica* Boergensen (Halymeniales, Rhodophyta). *Botanica Marina, 45*, 331–338.

SIA, (2015). *Sargassum muticum* – Seaweed Industry Association, available online at: https://www.seaweedindustry.com/seaweed/type/sargassum-muticum.

Shanmugan, M., & Mody, K. H. (2000). Heparinoid-active sulphated polysaccharides from marine algae as potential blood anticoagulant agents. *Current Science, 79*, 1672–1683.

Tanniou, A., Vandanjon, L., Incera, M., Leon, E. S., Husa, V., Le Grand, J., et al. (2014). Assessment of the spatial variability of phenolic contens and associated bioactivities in the invasive alga *Sargassum muticum* along its European range from Norway to Portugal. *Journal of Applied Phycology, 26*, 1215–1230.

Vingering, N., & Ledoux, M. (2009). Use of BPX-70 60-m GC columns for screening the fatty acid composition of industrial cookies. *European Journal of Lipid Science and Technology, 111*(7), 669–677.

WHO. (2008). Population nutrient intake goals for preventing diet-related chronic diseases. Recommendations for preventing dental diseases. In: World Health Organization. Diet, nutrition and the prevention of chronic diseases. Report of a joint WHO/FAO expert consulta. WHO Technical Report Series), 916.

Yu, Q., Yan, J., Wang, S., Ji, L., Ding, K., Vella, C., et al. (2012). Antiangiogenic effects of GFP08, an agaran-type polysaccharide isolated from *Grateloupia filicina*. *Glycobiology, 22*, 1343–1352.

Zhou, A. Y., Robertson, J., Hamid, N., Maa, Q., & Lu, J. (2014). Changes in total nitrogen and amino acid composition of New Zealand *Undaria pinnatifida* with growth, location and plant parts. *Food Chemistry*. http://dx.doi.org/10.1016/j.foodchem.2014.06.016.