J Appl Phycol (2017) 29:2427–2437
DOI 10.1007/s10811-017-1074-x

22ND INTERNATIONAL SEAWEED SYMPOSIUM, COPENHAGEN

Biochemical composition, nutritional value, and antioxidant properties of seven seaweed species from the Madeira Archipelago

N. Nunes[1,2] · **S. Ferraz**[1] · **S. Valente**[2] · **Maria Carmo Barreto**[3] · **M. A. A. Pinheiro de Carvalho**[1,4]

Received: 29 July 2016 / Revised and accepted: 24 January 2017 / Published online: 10 February 2017

Abstract Biochemical composition and antioxidant activity were assessed in seven seaweeds from Madeira Archipelago, namely, the chlorophyte (*Ulva lactuca*), the rhodophytes (*Asparagopsis taxiformis, Chondrus crispus, Galaxaura rugosa, Grateloupia lanceola* and *Nemalion elminthoides*), and the phaeophyte (*Zonaria tournefortii*). Seaweed mineral content varied from 16.60 to 84.16 g (100 g)$^{-1}$ dry weight (dw). Organic matter, composed by fiber and matrix polysaccharides (8.33 to 54.04 g (100 g)$^{-1}$ dw), starch (1.95 to 25.41 g (100 g)$^{-1}$ dw), protein (2.80 to 17.55 g (100 g)$^{-1}$ dw), and fat (1.46 to 12.04 g (100 g)$^{-1}$ dw), was also determined. *Asparagopsis taxiformis* was found to have substantial quantities of protein, fat, fiber, and matricial polysaccharides, compared to the other analyzed seaweeds. Analysis of antioxidant components included the measurement of chlorophyll *a* (28.81 to 244.3 g (100 g)$^{-1}$ dw), total carotenoids (0 to 297.8 g (100 g)$^{-1}$ dw), total phenolic compounds (0 to 2154 mg GAE (100 g)$^{-1}$ dw), and total flavonols (7.27 to 604.8 mg QE (100 g)$^{-1}$ dw). *Zonaria tournefortii* was found to possess the highest contents of chlorophyll *a*, total carotenoids, total phenolic content (TPC), and antioxidant activity, determined through ferric reduction antioxidant potential (FRAP), ferrous ion chelating (FIC), free radical-scavenging assay (FRSA), and β-carotene bleaching (β-CB). Statistical analysis showed 38 significant correlations between various biochemical and antioxidant parameters or activity and determined that fat content showed the highest number of correlations. Overall, this study gives a better understanding of Madeira autochthonous seaweeds in their potential of being introduced as a raw material for nutrient supplementation in various food products or to produce functional foods using seaweed natural properties.

Keywords Total carotenoids · Antioxidant activity · Nutritional and biochemical evaluation · TPC · Flavonols · Chlorophyll *a*

Introduction

Portugal has an exclusive economic zone of 1,720,560 km^2 of sea and only 91,763 km^2 of land area, representing an enormous potential for sea economy development. The Madeira archipelago where this study was carried out has 10,823 km^2 of sea and only 810 km^2 of land, showing a similar potential (Portuguese Navy 2015). The increase of world population and constraint determined by global climate change and limitation of terrestrial resources for food and energy supply raise serious concerns about global food security (Rosegrant and Cline 2003) and lead us to explore new food sources. A worldwide quest to explore and utilize non-conventional food sources, of both terrestrial and marine origin, to improve the nutritional quality of food is underway (Kumar et al. 2011). To achieve this goal, a deeper understanding of seaweed resources, their biochemical and antioxidant composition, is required.

✉ N. Nunes
nunonunes96@gmail.com

[1] ISOPlexis Genebank, University of Madeira, Campus da Penteada, 9050-290 Funchal, Madeira, Portugal

[2] UBQ II, Unidade de Bioquímica, Lda. Rua Visconde de Anadia, Edifício Anadia 5° Andar CC, 9050-020 Funchal, Madeira, Portugal

[3] CE3C–Centre for Ecology, Evolution and Environmental Changes/Azorean Biodiversity Group and Departamento de Ciências Tecnológicas e Desenvolvimento, University of Azores, 9501-801 Ponta Delgada, Portugal

[4] ICAAM, University of Évora, Apartado 94, 7006-554 Évora, Portugal

Seaweed as an alternative food source depends of its biochemical composition, nutrients, and calories supplied for the human diet. Seaweeds represent an excellent raw material of nutrients and bioactive compounds such as minerals, dietary fiber, protein, essential fatty acids, vitamins, and carotenoids (Hold and Kraan 2011; Kılınç et al. 2013). Seaweed protein content varies greatly depending on the species and factors such as season and environmental conditions (Dawczynski et al. 2007). Seaweed is a source of dietary fiber, largely soluble and known to prevent constipation, colon cancer, cardiovascular disease, and obesity (Dreher 1987; Jiménez-Escrig et al. 2013). Protein from seaweed contains all essential amino acids, although some seasonal variations in their concentrations can be observed (Galland-Irmouli et al. 1999). Fatty acids of certain seaweeds are predominantly unsaturated and show antiviral activity (Kendel et al. 2015). Seaweeds are a natural source of water-soluble and liposoluble vitamins, such as thiamine and riboflavin, β-carotene, and tocopherols, which may reduce the risk of heart disease, thrombosis, and atherosclerosis (Mishra et al. 1993).

Carotenoids and phenolics are plant and seaweed antioxidants that can rapidly neutralize the free radicals and retard or decrease the extent of oxidative deterioration (Miyashita 2014; Rengasamy et al. 2015). The reactive oxygen species (ROS) formed in human tissues can promote an extensive oxidative damage that leads to age-related degenerative processes, cancer, and a wide range of other human diseases (Aruoma 1999). For this work, we selected *Ulva lactuca*, *Asparagopsis taxiformis*, *Chondrus crispus*, *Galaxaura rugosa*, *Grateloupia lanceola*, *Nemalion elminthoides*, and *Zonaria tournefortii*. Various bioactive compounds have been determined in these seaweeds, showing antifouling, antibacterial, antifungal, antiviral, antioxidant, and antiinflammatory properties, as well as antitumor and anticoagulant activities (Pereira 2015). Several of these seaweeds are known to be consumed by humans such as *A. taxiformis*, *N. elminthoides*, *C. crispus*, and *Ulva* spp., where no special regulation exists (Mahadevan 2015), but only *C. crispus* and *Ulva* spp. are permitted by French legislation and recognized as food (Holdt and Kraan 2011). Due to their commercial value, *U. lactuca* and *C. crispus* have been integrated in seaweed cultivation efforts, implementing different strategies and techniques for bioremediation or yield increase (Zertuche-González et al. 2001; Ale et al. 2011; Nielsen et al. 2012; Castelar et al. 2014).

In this work, seaweeds were assayed for their biochemical composition to assess moisture content, total mineral, protein, starch, fat, fiber, and matrix polysaccharides. Nutritional value was also determined in some, with a history of human consumption or related to these, calculating the percent of contribution to the recommended dose intake (RDI) regarding protein, carbohydrates, and fat. For antioxidant compound analysis, chlorophyll *a*, total carotenoids, total phenolic, and flavonols were evaluated. To assess the antioxidant activity, two single-electron transfer tests (ferric-reducing antioxidant power (FRAP) and free radical-scavenging assay (FRSA)), one hydrogen atom transfer (β-carotene bleaching (β-CB)), and one chelating assay (ferrous ion chelating (FIC)) were included. This is the first study carried out in Madeira to evaluate autochthonous seaweeds in their potential of being introduced as alternative or functional foods in the agri-food industry.

Materials and methods

Seaweeds were collected in Madeiran south coastline from intertidal zone to a maximum of a 10-m-depth dive. Seven of the most common species were included in this study, namely, *Ulva lactuca* Linnaeus, *Asparagopsis taxiformis* (Delile) Trevisan de Saint-Léon, *Chondrus crispus* Stackhouse, *Galaxaura rugosa* (J. Ellis & Solander) J.V. Lamouroux, *Grateloupia lanceola* (J. Agardh) J. Agardh, *Nemalion elminthoides* (Velley) Batters, and *Zonaria tournefortii* (J.V. Lamouroux) Montagne. Samples were transported in seawater and gently rinsed with filtered freshwater. Afterwards, a primary drying was applied in which seaweeds were frozen at −35 °C and lyophilized, under reduced pressure (4×10^{-4} mbar) with a cooling trap set at −56 °C for 5 days. Samples were milled to 200-mesh particle size, vacuum packed, and stored in the dark until use. All samples were analyzed in triplicates in all tests carried out.

Chemicals

Methanol, trichloroacetic acid, chloroform, boric acid, potassium sulfate, and sulfuric acid (95–97%) were from Sigma-Aldrich. Folin Ciocalteu reagent, aluminum chloride, sodium acetate, monopotassium phosphate, dipotassium phosphate, ethylenediamine tetraacetic acid (EDTA), anthrone, and sodium carbonate were from Fluka. Gallic acid, quercetin, ferric chloride hexahydrate, butylated hydroxytoluene (BHT), linoleic acid, and β-carotene were from Sigma. 2,2-Diphenyl-1-picrylhydrazyl (DPPH), hexacyanoferrate (III) potassium, ferroin, and selenium were from Aldrich. Ascorbic acid, soluble starch, and ferric sulfate were from Merck. Tween 20 was purchased from Pharmacia Biotech and hydrochloric acid (37%) from Riedel de Haen. All chemicals were of analytical grade.

Sample chemical analysis

Moisture content was determined according with AOAC 925.10 (2000). Ash was determined through AOAC 930.22 (2005). Lipids were quantified as described by Folch et al. (1957), with the modifications suggested by Bligh and Dyer (1959). Protein content was determined as described by AOAC 978.04 (2005), adjusting the nitrogen to protein conversion factor to 5.58 for *U. lactuca* (Shuuluka et al. 2013), 5.38 for brown seaweeds, and 4.59 for red seaweeds (Lourenço et al. 2002), except for *A. taxiformis* where a factor of 4.51 was used (Diniz et al. 2011). Starch analysis was conducted according to the Hodge and Hofreiter method (Hodge and Hofreiter 1962). Fiber and matrix polysaccharides were calculated as the difference between 100% of dry matter and the sum of other parameter values.

For the antioxidant quantification and activity experiments, 1 g of dry seaweed was weighed and mixed with 25 mL of 50% methanol (*v*/*v*), under continuously stirring for 1 h. Extracts were sonicated and centrifuged, 10 min, at 2935×*g*. The supernatant was stored and pellet used in a second extraction. The supernatants were combined, brought up to 50 mL with 50% methanol (*v*/*v*), and stored at −20 °C. Total phenolic content (TPC) using Folin Ciocalteu method was conducted as described by Chew et al. (2008), and chlorophyll *a* and total carotenoids were determined according to Wellburn (1994) and Kumar et al. (2010). Flavonol quantification was conducted according with the method developed by Kumaran and Karunakaran (2006). The free radical-scavenging assay was determined using DPPH described by Yen and Chen (1995) and Duan et al. (2006), and FRAP assay was performed following Oyaizu (1986) and Yuan et al. (2005). FIC assay was based on Decker and Welch (1990) and Chew et al. (2008); β-CB assay was conducted as described by Velioglu et al. (1998) and Ismail and Tan (2002).

Statistical analysis

To analyze data, the statistical program SPSS 23.0 for Windows was used. All values are expressed as mean of three replicate determinations ± standard deviation. Data were analyzed using one-way analysis of variance (ANOVA), following the determination of homoscedasticity, and followed by Pearson's test ($p \leq 0.01$) to assess correlations between means and Tukey's test ($p \leq 0.01$) to determine statistical variance.

Results

The biochemical evaluation of the seven seaweeds analyzed is presented in Table 1. Values for all biochemical parameters analyzed were expressed on a dry weight basis. Moisture content varied between 1.47 g in *G. rugosa* and 4.50 g (100 g)$^{-1}$ in *A. taxiformis*; *U. lactuca* had a moisture content of 3.41 g (100 g)$^{-1}$ and *Z. tournefortii* 4.13 g (100 g)$^{-1}$. Total mineral (ash) content showed great variation depending on the seaweed. Red seaweeds varied their content between 16.60 g in *G. lanceola* and 84.16 g (100 g)$^{-1}$ dw for *G. rugosa*. The protein content in red seaweed ranged from 2.80 g in *G. rugosa* and 17.55 g (100 g)$^{-1}$ dw in *A. taxiformis*; *U. lactuca* had 7.16 g and *Z. tournefortii* 9.44 g (100 g)$^{-1}$ dw. Starch content alternated in red seaweeds from a minimum of 1.95 g in *G. rugosa* to 25.41 g (100 g)$^{-1}$ dw in *G. lanceola*; *U. lactuca* had 9.34 g and *Z. tournefortii* 4.07 g (100 g)$^{-1}$ dw. Fiber and matrix polysaccharide contents varied in red seaweed from 8.33 g in *G. rugosa* to 47.96 g (100 g)$^{-1}$ dw in *G. lanceola*; *U. lactuca* contained 54.04 g and *Z. tournefortii* 44.50 g (100 g)$^{-1}$ dw. Lipid content showed a variation between 1.46 g in *G. rugosa* and 6.62 g (100 g)$^{-1}$ dw in *A. taxiformis*; *U. lactuca* had 2.36 g and *Z. tournefortii* contained 12.04 g (100 g)$^{-1}$ dw.

The antioxidant quantification and potential of the seven seaweeds are given in the Table 2. Chlorophyll *a* content varied between 28.81 mg in *A. taxiformis* and 184.5 mg (100 g)$^{-1}$ dw in *G. lanceola* in red seaweed; *U. lactuca* presented a value of 92.72 mg and *Z. tournefortii* 244.3 mg (100 g)$^{-1}$ dw. Also, a wide variation in carotenoid content was observed; in red seaweed, these values alternated between 8.99 mg in *N. elminthoides* and 131.1 mg (100 g)$^{-1}$ dw in *G. lanceola* with no significant amounts detected in *G. rugosa*. *U. lactuca* had 20.41 mg (100 g)$^{-1}$ and *Z. tournefortii* had the highest value, 297.8 mg (100 g)$^{-1}$ dw. TPCs showed as well high degree of variability. Red seaweeds diverged from 25.79 mg in *G. lanceola* to 65.52 mg gallic acid equivalent (GAE) (100 g)$^{-1}$ dw in *N. elminthoides* with again no significant amounts detected in *G. rugosa*. *Ulva lactuca* presented 55.61 mg GAE (100 g)$^{-1}$ and *Z. tournefortii* showed the highest TPC content of 2155 mg GAE (100 g)$^{-1}$ dw. Flavonol content varied from 7.27 mg in *G. rugosa* to 206.1 mg quercetin equivalent (QE)(100 g)$^{-1}$ dw in *G. lanceola* for red seaweeds; the highest result was in *U. lactuca* 604.8 mg QE (100 g)$^{-1}$ and *Z. tournefortii* presented a value of 157 mg QE (100 g)$^{-1}$ dw.

Antioxidant activities are given in Table 3. FRAP assay showed dispersed results that ranged from 71.69 mg in *A. taxiformis* to 1896 mg ascorbic acid equivalent (AAE) (100 g)$^{-1}$ dw in *G. lanceola* for red seaweeds; *U. lactuca* had 238.8 mg AAE (100 g)$^{-1}$ dw and *Z. tournefortii* developed the highest value 6078 mg AAE (100 g)$^{-1}$ dw. In FIC assay, red seaweed varied their chelating potential from 4.00% in *G. lanceola* to 45.64% in *G. rugosa*; *U. lactuca* had 4.28% and *Z. tournefortii*, a brown seaweed, had the highest chelating activity, 77.93%. FRSA in red seaweeds showed values alternating from 18.69 mg in *G. rugosa* to 63.97 mg AAE (100 g)$^{-1}$ dw in *C. crispus*; it was determined in *U. lactuca* 59.97 mg AAE (100 g)$^{-1}$ dw, and *Z. tournefortii* presented the

Table 1 Biochemical content, color, and uses

Seaweeds	Color	Uses (Pereira 2015)	Moisture g (100 g)$^{-1}$ dw	Total minerals g (100 g)$^{-1}$ dw	Protein g (100 g)$^{-1}$ dw	Starch g (100 g)$^{-1}$ dw	Fiber and matrix polysaccharides g (100 g)$^{-1}$ dw	Fat g (100 g)$^{-1}$ dw
Asparagopsis taxiformis	Red	Seasoning, source of pharmaceutical and bioactive compounds	4.50 ± 0.20 a	23.76 ± 0.48 a,e	17.55 ± 0.11 a	8.03 ± 0.38 a	32.47 ± 1.04 a	6.62 ± 0.54 a
Chondrus crispus	Red	Extraction of polysaccharide carrageenan	3.26 ± 0.11 a,b	23.11 ± 0.13 a	6.71 ± 0.07 b	18.23 ± 0.46 b	46.39 ± 0.79 b,d	2.46 ± 0.14 b,c
Galaxaura rugosa	Red	Extracts with antiviral, antifungal, antiinflammatory, and antimicrobial activities	1.47 ± 0.30 b	84.16 ± 0.08 b	2.80 ± 0.06 c	1.95 ± 0.11 c	8.33 ± 0.08 c	1.46 ± 0.06 b
Grateloupia lanceola	Red	–	3.00 ± 0.91 a,b	16.60 ± 0.03 c	4.97 ± 0.02 d	25.41 ± 0.91 d	47.96 ± 1.00 d	2.97 ± 0.12 c
Nemalion elminthoides	Red	Utilized as food and extracts with antiherpetic activity	4.10 ± 0.50 a	60.64 ± 0.02 d	3.80 ± 0.05 e	5.36 ± 0.21 e	26.07 ± 0.12 e	2.17 ± 0.13 b,c
Ulva lactuca	Green	Used as food worldwide, in cosmetics and extracts have biological activity	3.41 ± 1.05 a	25.18 ± 0.48 e	7.16 ± 0.03 f	9.34 ± 0.87 a	54.04 ± 0.53 f	2.36 ± 0.12 b,c
Zonaria tournefortii	Brown	Antioxidant and antitumor activity in extracts	4.13 ± 0.05 a	25.18 ± 1.10 e	9.44 ± 0.06 g	4.07 ± 0.41 e	44.50 ± 1.77 b	12.04 ± 0.81 d

Data are mean ± standard deviation in gram per 100 g of seaweed on a dry weight basis. All determinations were carried out in triplicate. Different letters within the same column indicate significant differences ($p \leq 0.01$)

highest value of 3928 mg AAE (100 g)$^{-1}$ dw. The β-CB assay was also carried out, and in red seaweeds, values ranged from 3.01% in *A. taxiformis* to 12.12% in *C. crispus*; *U. lactuca* showed 2.88% and *Z. tournefortii* presented again the highest value, 95.03%. The β-CB assay was followed spectrophotometrically for 120 min and compared with BHT standard at 0.010 mg mL^{-1}. The results presented in Fig. 1 show *G. rugosa*, *A. taxiformis*, *N. elminthoides*, and *U. lactuca*, in decreasing order of antioxidant activity and the last seaweed comparable with control, with no antioxidant mechanism to protect linoleic acid. *Zonaria tournefortii*, *C. crispus*, and *G. lanceola* showed the highest antioxidant potential, with *Z. tournefortii* showing higher antioxidant activity than BHT (0.010 mg mL^{-1}) at the end of the 120 min of the assay.

All the seaweeds listed below were statistically different (Tukey's test; $p \leq 0.01$) from each other for each given parameter, and the names of the seaweeds appear from highest to the lowest content. Three red seaweeds, namely, *G. rugosa*, *N. elminthoides*, and *G. lanceola*, were statistically different in their total mineral content. Protein quantity in all seaweeds was statistically different, with *A. taxiformis* having the highest content and *G. rugosa* the lowest. Starch content of *G. lanceola*, *C. crispus*, and *G. rugosa* also was statistically different. *Ulva lactuca*, *A. taxiformis*, *N. elminthoides*, and *G. rugosa* have statistically different fiber content. The higher fat content in *Z. tournefortii* and *A. taxiformis* was also statistically different. Regarding the antioxidant compounds, *Z. tournefortii*, *G. lanceola*, and *C. crispus* displayed

Table 2 Antioxidant compounds

Seaweeds	Chlorophyll *a* mg (100 g)$^{-1}$ dw	Carotenoids mg (100 g)$^{-1}$ dw	TPC mg GAE (100 g)$^{-1}$ dw	Flavonols mg QE (100 g)$^{-1}$ dw
Asparagopsis taxiformis	28.81 ± 3.25 a	13.14 ± 2.63 a,b	57.63 ± 3.92 a	19.26 ± 0.95 a
Chondrus crispus	62.51 ± 4.09 b	21.25 ± 1.56 b	36.28 ± 4.64 a	79.17 ± 1.44 b
Galaxaura rugosa	32.30 ± 0.52 a	ND	ND	7.27 ± 1.30 a
Grateloupia lanceola	184.55 ± 5.90 c	131.13 ± 5.23 c	25.79 ± 2.13 a	206.06 ± 9.04 c
Nemalion elminthoides	89.09 ± 6.27 d	8.99 ± 1.15 a,b	65.52 ± 8.20 a	85.76 ± 9.63 b
Ulva lactuca	92.72 ± 2.88 d	20.41 ± 1.68 b	55.61 ± 4.13 a	604.77 ± 15.73 d
Zonaria tournefortii	244.25 ± 8.48 e	297.77 ± 10.14 d	2154.57 ± 119.27 b	156.99 ± 11.37 e

Data are mean ± standard deviation in milligram per 100 g of seaweed on a dry weight basis. All determinations were carried out in triplicate. Different letters within the same column indicate significant differences ($p \leq 0.01$)

ND not detected

Table 3 Antioxidant activity

Seaweeds	FRAP mg AAE $(100\ g)^{-1}$ in dw	FIC % chelating activity	FRSA (AEAC) mg AAE $(100\ g)^{-1}$ in dw	β-CB (% antioxidant activity) in dw
Asparagopsis taxiformis	71.69 ± 2.11 a	16.95 ± 1.18 a	21.29 ± 1.21 a	3.01 ± 0.14 a
Chondrus crispus	100.96 ± 9.16 a	40.38 ± 1.39 b	63.97 ± 1.32 a	12.12 ± 1.15 b
Galaxaura rugosa	149.15 ± 4.99 a,b	45.64 ± 0.81 b	18.69 ± 1.65 a	9.04 ± 0.65 b,c
Grateloupia lanceola	1895.51 ± 29.51 c	4.00 ± 0.28 c	31.64 ± 0.99 a	6.24 ± 0.20 a,c
Nemalion elminthoides	107.26 ± 3.95 a	12.57 ± 1.10 a	43.46 ± 1.27 a	4.17 ± 0.34 a
Ulva lactuca	238.79 ± 9.86 b	4.28 ± 0.86 c	59.97 ± 2.97 a	2.88 ± 0.04 a
Zonaria tournefortii	6078.29 ± 89.63 d	77.93 ± 4.62 d	3927.83 ± 47.59 b	95.03 ± 3.50 d

Data are mean ± standard deviation in milligram per 100 g of seaweed on a dry weight basis or percentage. All determinations were carried out in triplicate. Different letters within the same column indicate significant differences ($p \leq 0.01$)

statistical differences in their chlorophyll *a* content. *G. lanceola* and *Z. tournefortii* carotenoid contents were statistically distinct from all the other seaweeds, and only *Z. tournefortii* showed this distinction for TPC content. For flavonols, *U. lactuca*, *G. lanceola*, and *Z. tournefortii* were statistically different from each other and all the other seaweeds used in this work. For the antioxidant activity, *Z. tournefortii* and *G. lanceola* were found to have statistical different values for FRAP assay, and only *Z. tournefortii* continued to demonstrate its dissimilarity for the other parameters, such as FIC, FRSA, and β-CB assays. The Pearson's coefficient test ($p \leq 0.01$) was used to determine the existence of correlations between composition and antioxidant parameters, as shown in Table 4. Thirty-eight significant correlations between different biochemical and antioxidant parameters or activity were identified. Seven correlations occur between biochemical compositional parameters, and three of these correlations were negative, involving total minerals with starch, moisture content, and fiber. The correlation between minerals and fiber content presented a $R^2 = -0.905$. The total content of lipids is the biochemical parameter that shows the highest number of correlations with antioxidant compounds or activity, with nine significant positive correlations, of which five have an R^2 ranging from 0.800 to 0.899 with carotenoids and antioxidant activity assays (TPC, FRSA, FRAP, and β-CB). Twenty-four correlations showed values of linearity higher than $R^2 = 0.700$, and 23 of them are between antioxidant compounds and activity parameters. The antioxidant compounds analyzed, TPC, carotenoids, and chlorophyll *a*, showed 11 positive correlations with antioxidant capacity assays performed in this work, with 10 of these correlations with an R^2 higher than 0.700. Carotenoids correlate with FRSA, measuring scavenging of free radicals ($R^2 = 0.912$). Total phenolic compounds appear correlated with total carotenoids ($R^2 = 0.908$) and to a lesser extend to chlorophyll *a* ($R^2 = 0.759$). TPC also is highly correlated with antioxidant activities such as FRSA ($R^2 = 0.998$), β-CB ($R^2 = 0.990$), and FRAP ($R^2 = 0.951$). FRAP assay showed a positive

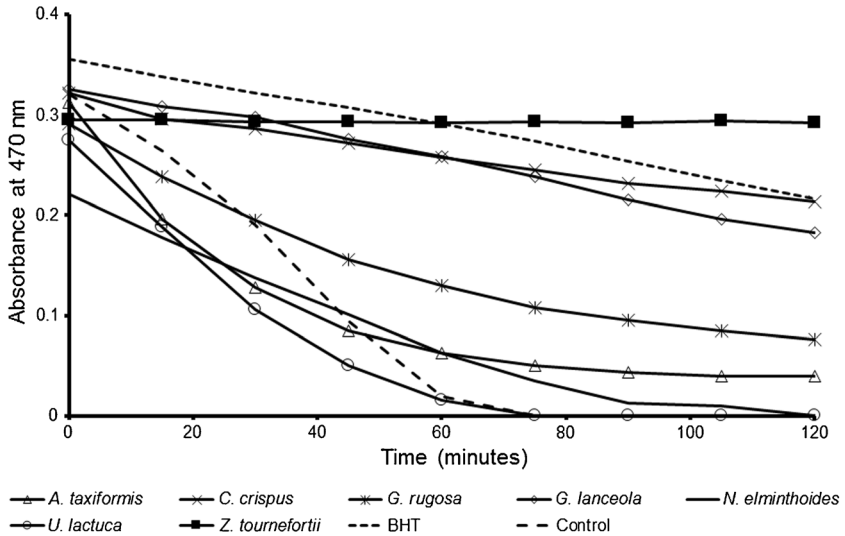

Fig. 1 - β-carotene bleaching assay plot of seaweeds methanol extracts, control and BHT (0.010 mg mL) to determine the antioxidant activity during -1 120 minutes

Table 4 Statistical analysis using Pearson's correlation to determine relationships between different parameters

	Moisture	Starch	Minerals	Fiber	Fat	Protein	Chlorophyll *a*	Carotenoids	Flavonols	TPC	FRSA	FRAP	FIC	β-CB
Moisture			− e		+ d	+ e				+ e	+ e			+ e
Starch			− d	+ e										
Minerals	− e	− d		− a										
Fiber		+ e	− a						+ d					
Fat	+ d					+ e	+ d	+ b		+ b	+ b	+ b	+ d	+ b
Protein	+ e				+ e									
Chlorophyll *a*					+ d			+ a		+ c	+ c	+ b		+ c
Carotenoids					+ b		+ a			+ a	+ a	+ a	+ d	+ a
Flavonols				+ d										
TPC	+ e				+ b		+ c	+ a			+ a	+ a	+ c	+ a
FRSA	+ e				+ b		+ c	+ a		+ a		+ a	+ c	+ a
FRAP					+ b		+ b	+ a		+ a	+ a		+ d	+ a
FIC					+ d			+ d		+ c	+ c	+ d		+ b
β-CB	+ e				+ b		+ c	+ a		+ a	+ a	+ a	+ b	

Statistical significance at 0.01 level. Signaling + or − reveals the type of relation between parameters in their positive or negative correlation, respectively. Letters indicate the interval of the linearity correlation (R^2)—a (1.000–0.900), b (0.899–0.800), c (0.799–0.700), d (0.699–0.600), and e (0.599–0.500)

correlation with total carotenoids ($R^2 = 0.992$) and chlorophyll *a* ($R^2 = 0.934$).

Discussion

The biochemical composition results, presented in Table 1, demonstrate the variability between seaweeds in each given parameter. Seaweed dehydration and processing into flour have successfully developed moisture content less than 13 g (100 g)$^{-1}$ in all seaweeds processed and according to Van Hal (2000), the lower limit for development of microorganisms. Total mineral (ash) content shows great variability, and *G. rugosa* presented the highest value, 84.16 g (100 g)$^{-1}$ dw, due to its calcareous composition. *A. taxiformis* showed almost twice the value demonstrated by El-Baroty et al. (2007), 23.76 (100 g)$^{-1}$ dw. *G. lanceola* gave similar results (16.60 (100 g)$^{-1}$ dw) the same genus, *Grateloupia turuturu*, studied by Denis et al. (2010) and Munier et al. (2013). The green seaweed, *U. lactuca*, gave equivalent results as those of Mageswaran and Sivasubramaniam (1984), 25.18 (100 g)$^{-1}$ dw, but more than twice those of Ortiz et al. (2006), probably due to different sea conditions or seasonality. Protein quantification showed that *A. taxiformis*, a red seaweed, had the highest content of all the seaweed used in this study, 17.55 (100 g)$^{-1}$ dw, potentially providing 31.34% of the RDI (Table 5). Seaweeds are only considered staple food in few countries and regions, but this raw material has the potential of being introduced as an ingredient in various food-related items, increasing nutritional quality and helping some food products to meet the RDI. Dawczynski et al. (2007) and Diniz et al. (2011) also showed that protein of red seaweeds contains all essential amino acids and has a high essential amino acid index (EAAI), rich in lysine, but can be poor in some amino acids such as methionine or tryptophan. The *G. lanceola* used in this work had an up to four times lower protein content (4.97 g (100 g)$^{-1}$ dw) than that reported in other studies of this genus (*G. turuturu*) (Denis et al. 2010; Chandraprabha et al. 2012; Munier et al. 2013). Using same genera but different species for these comparisons may probably be the cause for these high degrees of discrepancy, but few published works exist on the same species and analysis, forcing a genus-based comparison in most cases. Interestingly, protein content fluctuations have also been shown between studies of the same species (Hardouin et al. 2013). In the present study, *U. lactuca* had a content of 7.16 g (100 g)$^{-1}$ dw, whereas in the studies of Mageswaran and Sivasubramaniam (1984), Ortiz et al. (2006), Manivannan et al. (2008), and Yaich et al. (2011), it varies from 3.30 to 27.20 g (100 g)$^{-1}$ dw.

Grateloupia lanceola and *C. crispus* showed the highest starch content, 25.45 and 18.23 g (100 g)$^{-1}$ dw, respectively. Although starch content is lower than some terrestrial crops, these algae can be used to fortify foodstuff as a source of energy, where other food sources are not available all year around. Another species in this genus, *G. turuturu*, is known to be consumed by humans (Denis et al. 2010) and has been reported to contain only 5.52 g (100 g)$^{-1}$ dw of starch (Chandraprabha et al. 2012). These differences can be attributed to seasonal starch fluctuations.

Fiber content showed great variability between seaweeds used in this work, ranging from 8.33 to 54.04 g (100 g)$^{-1}$ dw

Table 5 Mean values for some nutritional parameters to seaweeds known to be edible and percentage of recommended dose intake (RDI) that each seaweed can deliver when consumed

	Protein (g)		Carbohydrate (g)		Fat (g)		
Recommended dose intake (RDI)[a]	56		130		70		
Species	Protein g (100 g)$^{-1}$ dw	%RDI	Carbohydrate g (100 g)$^{-1}$ dw	%RDI	Fat g (100 g)$^{-1}$ dw	%RDI	Energy (kcal)
Asparagopsis taxiformis[b]	17.55	31.34	40.50	31.15	6.62	9.46	181.84
Chondrus crispus[b,c]	6.71	11.98	64.61	49.70	2.46	3.51	116.73
Grateloupia lanceola[d]	4.97	8.88	73.37	56.44	2.97	4.24	122.07
Nemalion elminthoides[b]	3.80	6.79	31.43	24.18	2.17	3.10	68.15
Ulva lactuca[b,c]	7.16	12.79	63.38	48.75	2.36	3.37	115.21

[a] Data from Trumbo et al. (2002)

[b] Seaweed known to be consumed by humans (Pereira 2015)

[c] Permitted seaweed for human consumption in Europe (Meland and Rebours 2012)

[d] Seaweed from the same genus as seaweeds known to be consumed by humans (Athukorala et al. 2003; Munier et al. 2013; Seo et al. 2013)

in *G. rugosa* and *U. lactuca*, respectively. Yaich et al. (2011) and Ortiz et al. (2006) reported similar values for *U. lactuca*. Fiber consists of different polysaccharide fractions, such as hemicelluloses, celluloses, lignins, oligosaccharides, or pectins. Some of these are used in the food industry as texture modifiers and known to decrease the risks of some human diseases, such as coronary disease. Considering human or animal consumption, fiber and matrix polysaccharides are a positive feature in seaweeds. High content in consumed seaweeds can be positively correlated with low incidence of colorectal cancer (Hoshiyama et al. 1993). Indigestible viscous seaweed polysaccharides such as alginates, carrageenans, and funorans have shown positive effects on serum lipid levels in rats (Jiménez-Escrig and Sánchez-Muniz 2000).

Fat content showed some variability, ranging from 1.46 and 12.04 g (100 g)$^{-1}$ dw, between *G. rugosa* and *Z. tournefortii*. *Asparagopsis taxiformis* had the second highest fat content among the seaweed studied (6.62 g (100 g)$^{-1}$ dw), contributing a 9.46% RDI (Table 5) when consumed by humans. This seaweed is known as "limu kohu" in Hawaii and is consumed as a staple food and is part of their tradition (Burreson et al. 1976). El-Baroty et al. (2007) analyzed the lipid composition concluding that ω-3 linolenic acid is the major fatty acid of the lipid fraction in *A. taxiformis*. This fatty acid has the ability to prevent cardiovascular diseases (Mozaffarian 2005). *Grateloupia lanceola* fat content (2.97 g (100 g)$^{-1}$ dw) was similar to *G. turuturu* (2.81 and 5.44 g (100 g)$^{-1}$ dw) from two separate locations (Munier et al. 2013). The fat content of *U. lactuca* was lower (2.36 g (100 g)$^{-1}$ dw) than that reported by Yaich et al. (2011) (7.87 g (100 g)$^{-1}$ dw), higher than that found by Ortiz et al. (2006) (0.30 g (100 g)$^{-1}$ dw), but similar to using *Ulva armoricana* (2.62 g (100 g)$^{-1}$ dw) (Kendel et al. 2015). According to Manivannan et al. (2008), seaweeds are known for their low fat content, which varies significantly throughout the year. This biochemical parameter showed the highest number of correlations with antioxidant compounds (TPC, carotenoids, chlorophyll *a*) and activity (FRSA, β-CB, FRAP), suggesting that the chemical components that provide part of the antioxidant capabilities are of lipophilic nature.

Antioxidant quantification of the seven seaweeds is given in Table 2 and includes the determination of chlorophyll *a*, total carotenoids, TPCs, and flavonols. Brown seaweeds contain chlorophylls *a* and *c*, red seaweed only chlorophyll *a*, and green seaweed chlorophylls *a* and *b* (Takaichi 2013). Chlorophyll *a* content ranged from 28.81 mg (100 g)$^{-1}$ dw in *A. taxiformis* to 244.3 mg (100 g)$^{-1}$ dw in *Z. tournefortii*. The chlorophyll *a* content of *U. lactuca* (92.72 mg (100 g)$^{-1}$ dw) was higher than that reported by Abd El-Baky et al. (2008) (18.00 and 28.00 mg (100 g)$^{-1}$ dw). For the remaining seaweeds, our measurements represent the first results for chlorophyll *a*. Chlorophylls in consumed seaweed have bioactivity as an antioxidant (Lanfer-Marquez et al. 2005) and in processed food can be converted to pigments such as pheophytin, pyropheophytin, and pheophorbide, known to prevent cancer (Holdt and Kraan 2011). Carotenoids, terpenoid pigments, and their oxygenated derivatives (xanthophylls) act as antioxidants, neutralizing ROS during metabolic processes (von Elbe and Schwartz 1996). Although seaweeds synthesize β-carotene, brown seaweeds synthesize mainly fucoxanthin and violaxanthin. Red seaweeds produce essentially lutein, α-carotene, and zeaxanthin. Green seaweeds produce mostly lutein, violaxanthin, neoxanthin, and zeaxanthin (Holdt and Kraan 2011; Pereira 2015). In this work, seaweeds demonstrated a wide range in carotenoid content, with the highest value of 297.8 mg (100 g)$^{-1}$ dw in *Z. tournefortii* and the lowest to below our

detection limit in *G. rugosa*. *Asparagopsis taxiformis* had a carotenoid content (13.14 mg (100 g)$^{-1}$ dw) more than 10 times lower that found by Ragonese et al. (2014), 137.2 mg (100 g)$^{-1}$ dw, showing enormous variability between seaweed of the same species and suggesting that carotenoid content is more likely influenced by external factors than by species or genus variability. For *G. lanceola*, carotenoid content reached 131.1 mg (100 g)$^{-1}$ dw, which is more than twice the published content of *G. turuturu* (60.00 mg (100 g)$^{-1}$ dw; Chandraprabha et al. 2012). *Ulva lactuca* carotenoid content was similar to the values determined by Abd El-Baky et al. (2008). Carotenoid content is very important due to their nutraceutical and antioxidative properties, showing bioactivity in the prevention of pathologies caused by oxidative stress (Okuzumi et al. 1993). In this work, total carotenoids were highly correlated with the FRAP assay (R^2 = 0.992), possibly due to higher contents of astaxanthin, fucoxanthin, lutein, and zeaxanthin (Rodrigues et al. 2012), acting as iron-reducing antioxidants. Carotenoid content also correlates with FRSA (R^2 = 0.912), a mechanism that applies electron transfer to convert free radicals into more stable compounds (Matanjun et al. 2008).

Phenolic compounds are a major molecular group, contributing to the tissue antioxidant activity (Chan et al. 2007). They are important in defending against invading bacteria, wounding, or excessive radiation (Cox et al. 2010). *Z. tournefortii* showed the highest TPC content in the present study (2155 mg GAE (100 g)$^{-1}$ dw), approximately twice the amount reported by Chkhikvishvili and Ramazanov (2000) (1060 mg GAE (100 g)$^{-1}$ dw). Brown seaweed TPC content consists mainly of polyphenols such as fucol, fucophlorethol, fucodiphloroethol G, and ergosterol and phenol compounds, namely, phlorotannins (Holdt and Kraan 2011). Protocatechnic, gentisic, and hydroxybenzoic phenolic acids were determined at higher quantities in red and green seaweeds by Farvin and Jacobsen (2013), who evaluated 11 different phenolic acids in 16 seaweeds from the Danish coast. In their work, using ethanol for extraction, they obtained 236.5 mg GAE (100 g)$^{-1}$ dw in *U. lactuca*, representing 4.3-fold more than the TPC exhibited in this work (55.61 mg GAE (100 g)$^{-1}$ dw), using 50% methanol. *Grateloupia filicina*, a source of food in Korea and Japan, was found to contain 198.0 mg (100 g)$^{-1}$ dw total phenolics (Athukorala et al. 2003), seven times greater than the *G. lanceola* used in this work (25.79 mg (100 g)$^{-1}$dw; Athukorala et al. 2003), demonstrating high variability within same genus. Similarly, *N. elminthoides*, from the Yucatan Peninsula (Mexico), showed a TPC content of 1870 mg (100 g)$^{-1}$dw (Athukorala et al. 2003; Zubia et al. 2007), presenting incredibly higher results than the same seaweed analyzed in this work (65.52 mg (100 g)$^{-1}$dw; Athukorala et al. 2003). This discrepancy can be attributed to extrinsic factors that influence the TPC content greatly, namely, UV radiation, grazing, bacterial infection, and epiphytism (Tanniou et al. 2014). According to Pereira (2005), the Madeira archipelago has lower limits of solar irradiance (5 MJ m^{-2}) than Yucatan Peninsula (10 MJ m^{-2}; Quej et al. 2017), suggesting a possible reason for the TPC variation within the same species of seaweed. Statistical analysis revealed a high correlation between TPC with total carotenoids (R^2 = 0.908); chlorophyll *a* (R^2 = 0.759); and antioxidant activities such as FRSA (R^2 = 0.998), β-CB (R^2 = 0.990), and FRAP (R^2 = 0.951). The high correlation between TPC and FRSA in seaweeds was also demonstrated in the work of Ragan and Glombitza (1986), evidencing the relationship of the polyphenolic content in seaweed and the activity of free radical scavengers. The positive correlation between TPC and β-CB suggests a contribution of lipophilic compounds to antioxidant activity, also demonstrated by Chew et al. (2008). These findings are in agreement with the work of Matanjun et al. (2008), who determined the phenolic content and lipophilic antioxidant activity in eight species of seaweed. Flavonols and flavonol glycosides, a class of compounds of phenolic origin, are known to be scavengers of ROS and inhibitors of lipid peroxidation (Cox et al. 2010). According to Yoshie-Stark et al. (2003), who analyzed 27 seaweeds from the Sea of Japan, flavonols such as morin were identified in all seaweeds and myricetin was mainly present in brown and red seaweeds. Flavonol glycosides such as rutin were in higher content in red seaweeds and quercitrin only present in brown seaweeds. Flavonol content in the present study varied from 7.27 mg QE (100 g)$^{-1}$ dw in *G. rugosa* to 604.8 mg QE (100 g)$^{-1}$ dw in *U. lactuca*. For *U. lactuca*, contrasting values of flavonol content have been published, 800.0 mg (Sarojini et al. 2012), 135.0 mg (Meenakshi et al. 2009), and 2.36 mg QE (100 g) $^{-1}$ dw (Elmegeed et al. 2014). Although flavonol contents determined in this work were similar to the values reported by Sarojini et al. (2012), they greatly differ from other data published, highlighting the variability between distinct areas, climate conditions, and seasonality. Statistical evaluation showed that flavonols did not correlate with TPC or with β-CB, suggesting that flavonols are a minor constituent in phenolic compounds and do not play a significant role in lipid antioxidant protection in the seaweeds used in this work.

Antioxidant activity was also measured (Table 3), which includes the determination of primary antioxidant capacity with FRAP assay and FRSA using mechanisms based on the single-electron transfer (SET) (Prior et al. 2005). The β-CB assay, a scavenging peroxyl radical test, uses mechanisms of hydrogen atom transfer (HAT) for proton relocation (Dawidowicz and Olszowy 2010). And FIC assay measures the ability of secondary antioxidants to inhibit oxidation through an indirect approach, in this case chelating metal (Kristinsson 2014). FRAP activity measures the ability of antioxidant compounds to reduce iron ions involved in the

Fenton and Haber-Weiss reaction (Li et al. 2006). Results obtained for this assay range from 71.69 mg in *A. taxiformis* to 6078 mg AAE (100 g)$^{-1}$ dw in *Z. tournefortii*. For *U. lactuca*, the value was 238.8 mg AAE (100 g)$^{-1}$ dw, being relatively similar to the work of Stern et al. (1996), 310.0 mg AAE (100 g)$^{-1}$ dw. FIC assay also showed a high variability in the results, ranging from 4.28% in *U. lactuca* to 77.93% in *Z. tournefortii*. Phlorotannins usually present in brown seaweeds are responsible for the strong chelation of heavy metals, developing strong FIC activity demonstrated by *Z. tournefortii* (Toth and Pavia 2000). However, the chelating activity determined by Wang et al. (2009) reached 40% in *U. lactuca*, presenting almost 10 times higher than we have obtained. They also tested *C. crispus* that showed 30% chelating activity, lower than our results. Metal ion-chelating activity of seaweed extract has higher binding ability, preventing the generation and free movement of these oxidative radicals in the tissues (Kumar et al. 2010). FRSA measures the ability of seaweed antioxidant compounds to scavenge and neutralize ROS and proton radicals, generated in tissues as a result of oxidative stress (Chew et al. 2008). The lowest scavenging activity was developed by *G. rugosa* (18.69 mg AAE (100 g)$^{-1}$ dw) and the highest by *Z. tournefortii* (3928 mg AAE (100 g)$^{-1}$ dw). Statistical analysis, applying a Pearson's correlation test (Table 4), determined a high positive relationship between FRSA and β-CB ($R^2 = 0.995$), also documented by Zhang et al. (2007), using 28 seaweed species from Qingdao, China, indicating that free radical-scavenging activity is mainly due to lipophilic compounds present in seaweed. For β-CB activity of lipophilic antioxidants that protect unsaturated fatty acid of peroxidation by ROS, a great variability between seaweeds is also found, ranging from 2.88% for *U. lactuca* to 95.03% for *Z. tournefortii*.

In summary, this work increases our knowledge about the biochemical composition, nutritional value, antioxidant potential, and activity of seven seaweeds from Madeira. *Asparagopsis taxiformis* showed the highest protein content and other nutritional factors. Together with its history of human consumption, a selection of this seaweed as a raw material for food supplementation assessments was suggested. *Grateloupia lanceola* had the highest starch and fiber content among red seaweeds, and being from same genus as *G. turuturu* and *G. filicina*, it could be suitable for human consumption and be introduced in food-related products. For *Z. tournefortii*, an understudied brown seaweed, important results were obtained, such as its highest content of fat and antioxidant components and activity. It potentially can be suitable for antioxidant extraction and purification for food applications, but more studies should be carried to determined specific components that are responsible for its antioxidant activity. Significant correlations between different biochemical and antioxidant parameters or activity provided a better perception of how biochemical, antioxidant components or activity are related and the degree of relationship. Interestingly, fat content is highly correlated with antioxidant compounds and activity, suggesting that most of the antioxidant capabilities of seaweeds are of lipophilic origin.

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