

Nutritional Benefits of Edible Macroalgae from the Central Portuguese Coast: Inclusion of Low-Calorie 'Sea Vegetables' in Human Diet

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Abstract

In this work, major nutrients were determined in edible green (*Codium tomentosum*, *Ulva rigida*), red (*Gracilaria gracilis*, *Osmundea pinnatifida*, *Porphyra* sp), and brown macroalgae (*Saccorhiza polyschides*, *Undaria pinnatifida*) collected from the central Portuguese coast. The results showed that selected species had the macroelements (Ca+Mg+Na+K+P) ranged from 85 (*Porphyra* sp) to 226mg g⁻¹ (*Codium tomentosum*), and the oligoelements (Cu+Fe+Mn+Se+Zn) ranged from 60 (*U. pinnatifida*) to 655µg g⁻¹ (*O. pinnatifida*), thus representing a sustainable source of minerals. The low values of Na/K (0.3-3.7) showed that selected species can be of special nutritional interest for patients with hypertension. The highest contents of Fe (552µg g⁻¹) found in *O. pinnatifida* and Se (30µg g⁻¹) in *S. polyschides*, suggest the increased proportion of these oligoelements, by the inclusion of macroalgal species in the diet, rather than some traditionally known food. The total content of proteins (15.5-24%), carbohydrates (52-62%), crude fibres (14-32%), and lipids (0.9-4.8%) indicated that the selected macroalgae were relatively low-calorie food. Analyzed red species were characterized by the higher content of proteins (24% in *Porphyra* sp), whereas brown species showed increased levels of crude fibres (32% in *S. polyschides*). The caloric values ranged from 172 (*S. polyschides*) to 256kcal 100g⁻¹ (*Porphyra* sp) and they were negatively correlated (R = -0.81) with the ash content in studied species. The correlation between calories and ash indicated the influence of (thallus) morphology of species on their mineral and nutritional composition. Based on their particular nutritional profiles, the studied species can be recommended for selective inclusion in the human diet, ensuring high-quality and health-promoting food.

Keywords: Edible macroalgae; *Osmundea pinnatifida*; *Saccorhiza polyschides*; Na/K value; Selenium; Low-calorie food

Introduction

Edible macroalgae (seaweeds) are an important sustainable source of food, nutrients, and food-flavoring products for direct human consumption, accounting for 83-90% of their total production [1]. The species of each taxonomic group of macroalgae, green (*Chlorophytae*), red (*Rhodophytae*), and brown (*Phaeophyceae*), have variable ranges of nutritional components, including macroelements (Ca, Mg, Na, K, P), some oligoelements (e.g., Cu, Fe, I, Mn, Se, Zn), proteins, carbohydrates, and lipids [2-12]. Most polysaccharides are constituents of the cell wall, these are not digested and function as dietary fibres, important for digestion [13,14]. The nutritional composition of macroalgae depends on numerous factors, both biotic (the morphology, the

maturity, physical properties of macroalgae, etc.), and abiotic, i.e., environmental conditions [3,11,15].

China is the largest global producer of cultivated macroalgae, of which red and brown species account for more than 93% of those used in nutrition [1]. The main commercial species are red *Gracilaria* spp., used as a salad vegetable, *Porphyra* spp. (nori), commonly used in Japanese sushi, and brown *Saccharina japonica* (kelp), *Undaria* spp (wakame), contributing to 92% of the global production in 2015 [1]. In addition to these species, the two main commercial species used for direct human consumption are green *Ulva* spp (sea lettuce) and *Codium* spp [1,16]. In Asia, these species are considered important food resources (good source of

minerals, proteins, and fibres), commonly eaten raw or dried (in salads) or cooked (in soups and stews) [17].

The use of macroalgae in the human diet has strong roots in Asian countries, and their highest consumption was estimated in Japan (at approximately 9.6g a day), slightly more than the size of a typical portion in other Asian cuisines (8.0g per day) [4,18]. Recently, demands for macroalgae as food have been spread to Western countries (Canada, the USA, Nordic countries, Ireland, Iceland, France, Portugal, Spain, etc.), but their consumption is still less than 1% of the fully harvested macroalgae at national levels [19]. The increase in demand for the introduction of macroalgae in gastronomy (phycogastronomy), over the last fifty years has surpassed the capacity to supply the requirements from natural resources. In Canada, the USA, Iceland, and Ireland, different types of macroalgae have traditionally been consumed, thus continuously developing this market. The government and commercial organizations in some Nordic countries and France promoted phycogastronomy by gradually introducing macroalgae in restaurant menus, but still not considering macroalgae as fully-fledged vegetables [20,21]. With its large maritime area, Portugal has a considerable variety of available macroalgal species, and some of them are potential food resources [22,23].

However, so far only a few data on the nutritional composition of several edible species from the Portuguese coasts was reported [8,9,11]. To the best of our knowledge, their complete nutrition profiles, including the caloric values, are not available in the current scientific literature.

In this work, the concentrations of macroelements (Ca, Mg, Na, K, P), oligoelements (Cu, Fe, Mn, Se, Zn), biomolecules

(proteins, carbohydrates, crude fibres, lipids) are reported in edible macroalgae collected from the central Portuguese coast (Cape Mondego). In addition to species with wide applications in Asian gastronomy (*Codium tomentosum*, *Ulva rigida*, *Gracilaria gracilis*, *Porphyra* sp, *Undaria pinnatifida*), red species *Osmundea pinnatifida* and brown *Saccorhiza polyschides* were also selected for the present work because of their abundance along the Portuguese coastline. The nutritional potentials of analyzed species are critically commented on and some recommendations for their inclusion in the regular diet (particularly in Western countries) as low-calorie 'sea vegetables' have been also considered.

Materials and Methods

Reagents

All reagents used were of analytical grade from commercial suppliers, as purchased. The standard stock solutions of elements were purchased from Merck Inorganic Ventures, Spectrosol, Alfa Aesar, Plasma Cal, Sigma Aldrich, and VWR chemicals. All solutions were obtained by using ultrapure Milli-Q water (18.2MΩ cm⁻¹), from Millipore Purification System (Watermax W1, Diwer Technologies, Portugal).

Collection and pretreatment of macroalgae

Seven edible macroalgal species were collected from the central Portuguese coast, at the Cape Mondego (40° 11' N, 08° 54' W) (Figure 1). The area of Cape Mondego was classified as a Natural Monument in 2007, and it is integrated into the Network of Protected Areas in Portugal [24].

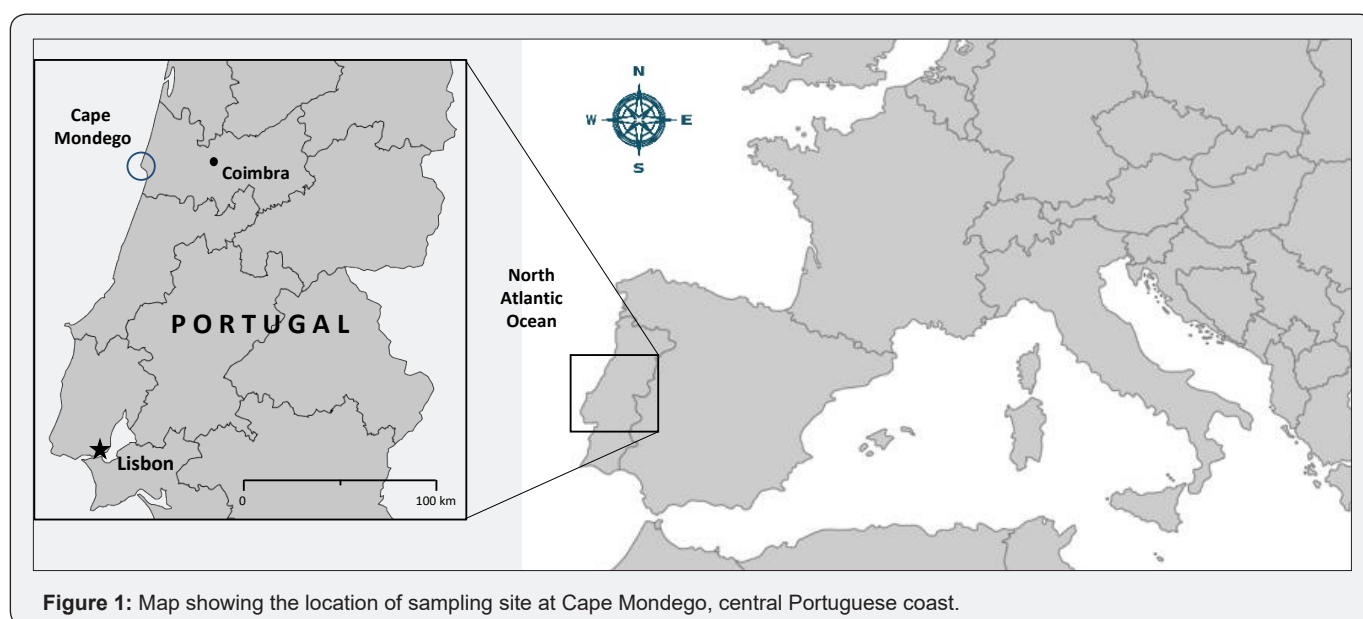


Figure 1: Map showing the location of sampling site at Cape Mondego, central Portuguese coast.

Seven species were sampled, of which two green (*Codium tomentosum*, *Ulva rigida*), three red (*Gracilaria gracilis*, *Osmundea pinnatifida*, *Porphyra* sp), and two brown (*Saccorhiza polyschides*,

Undaria pinnatifida). The species were collected in 2019, in two seasons, from spring (*G. gracilis*, *O. pinnatifida*), to late summer/early autumn (*C. tomentosum*, *U. rigida*, *Porphyra* sp, *S. polyschides*,

U. pinnatifida), according to their abundance (Figure 2). Two species, red *O. pinnatifida* and brown *S. polyschides* lesser-known in global gastronomy, were also selected for the present work due

to their abundance along the Portuguese coastline. All species were collected at the mature phase, following the best ecological practices and favourable weather conditions [12].



Figure 2: Representative images of *G. gracilis* (A), *O. pinnatifida* (B), and *S. polyschides* (C) collected at Cape Mondego, central Portuguese coast.

After harvesting samples were transported in thermal boxes to the laboratory. The samples were washed with distilled water to remove salts, detrital particles, and epiphytes. Then, samples were lyophilized at -50°C for 48h (CoolSafe 9L, LaboGene™, Denmark). The lyophilized biomasses were finely ground automatically, and the resulting grains were sifted through the laboratory's stainless-steel sieve (120 mesh, 0.12mm aperture). The obtained homogeneous fine fractions were kept in labeled recipients and stored at -18°C before the analysis.

Determination of ash content and minerals in macroalgae

The total ash, corresponding to the inorganic material of the sample, was determined gravimetrically after incineration of the lyophilized sample (1.0g) at 550°C for 8h [25]. All results are expressed as a mean mass fraction in % ($\text{g } 100\text{g}^{-1}$, referred to sample dry weight, dw), based on three replicate measurements ($n = 3$). The standard deviation (SD) was low ($\text{SD} < 0.4\%$) for determining ash content in all samples.

The macroelements (Ca, K, Mg, Na, P) were analysed using the inductively coupled plasma - optical emission spectroscopy (ICP-OES, Jobin Yvon model Activa M), whereas oligoelements (Cu, Fe, Mn, Se, Zn) and potentially toxic elements (Cd, Hg, Pb) were determined by inductively coupled plasma - mass spectrometry (ICP-MS, Quadrupole Thermo Scientific X Series), as previously described [26,27]. Before ICP-analyses, approximately 0.2g of lyophilized sample was mixed with 2.0mL of HNO_3 in a Teflon vessel, and heated in a microwave (CEM MARS 5) according to the following digestion program: within the initial 10min, the temperature increased to 180°C and remained constant for the next 10min. After cooling, 0.5mL of H_2O_2 was added to the solution, following a new digestion cycle as described. Then, the sample was evaporated to 0.5mL and Milli-Q water was added to the volume of 25mL. The samples were diluted in 2% HNO_3 to avoid matrix interferences due to salinity.

Limit of detection (LOD) for Ca, K, Na, and P was 0.006mg g^{-1} , while for Mg was 0.0012mg g^{-1} . LOD for Cu, Se, and Zn was $0.25\mu\text{g}$

g^{-1} , whereas for Mn and Fe was 0.62 and $1.2\mu\text{g g}^{-1}$, respectively. Cadmium, Pb and Hg had LODs equal to 0.012 and $0.031\mu\text{g g}^{-1}$, respectively. Quality control of the method was assured by the same analytical procedure performed with blanks, sample duplicates ($\text{SD} < 10\%$), and the certified reference materials (CRMs) DORM-4 ($n = 24$), and TORT-2 ($n = 6$). The CRMs were used throughout the analytical procedures for the quantification of elements and the control of the quality and traceability of results, as a calibration measurement standard. Obtained recoveries were high, ranging from 91 to 106%. All results are expressed as a mean concentration in mg g^{-1} (Ca, K, Mg, Na, P) or $\mu\text{g g}^{-1}$ (Cd, Cu, Fe, Hg, Mn, Pb, Se, Zn), referred to sample dry weight, dw.

The concentrations of potentially toxic elements Cd ($0.08\text{--}0.63\mu\text{g g}^{-1}$ dw), Hg (not detected), and Pb ($0.1\text{--}0.7\mu\text{g g}^{-1}$ dw) in macroalgae were below the threshold limits regulated in the EU ($< 3.0\mu\text{g g}^{-1}$ dw), thus designating selected macroalgae as edible species [1,28].

Determination of biomolecules in macroalgae

The protein content was determined by the method of Kjeldahl [25,29]. The organic nitrogen conversion factor of 6.25 was used to calculate the total protein content [30].

The lipids in macroalgae were quantified by the modified method based on extraction using a mixture of chloroform, methanol and water [6,31]. Briefly, 0.25 g of the lyophilized sample was mixed with 5.5 mL of solution chloroform/methanol/water (1.5:1.2:1, v/v/v), in a glass tube (6 mL). The tube was shaken in the vortex (1 min) and subsequently the separation of phases was achieved by centrifugation ($1700\times g$ for 15 min). The chloroform phase was transferred to the vial (previously weighted) and the methanol/water layer was washed with a new portion of chloroform (2.0 mL). The combined chloroform phases were evaporated under a nitrogen stream to dryness. The total lipid content was calculated gravimetrically.

The crude (water-insoluble) fibre content was determined by the Weende method [30]. Briefly, a portion of 1.0 g of lyophilized sample was boiled in diluted solutions of H_2SO_4 and KOH, and after, the content of crude fibre was determined gravimetrically.

All results are expressed as a mean mass fraction in % ($\text{g } 100\text{g}^{-1}$, dw), based on three replicate measurements ($n = 3$). For quantified proteins, lipids, and crude fibres SD was lower than 1.0, 0.8, and 3.0%, respectively.

Evaluation of total carbohydrates and caloric values in macroalgae

The total carbohydrate content (expressed in %, dw) was calculated as the weight difference based on the ash, protein, and lipids in the lyophilized sample. Based on results for total protein, lipids, and carbohydrates (less the crude fibre), the energy value of macroalgae was calculated as the sum of these parameters, multiplied by a factor of 4, 9, and 4, respectively [32]. The results

are expressed in $\text{kcal } 100\text{g}^{-1}$, referred to sample dw.

Data analyses

The relationship between concentrations was assessed by the correlation coefficient (R) at a significant level of 95% ($p = 0.05$). Comparison of mean values of nutritional composition between macroalgal species was carried out by ANOVA test ($p = 0.05$). Principal component analysis (PCA) was performed on data to summarize the information of nutritional profiles in studied species. 2-component PCA was applied using the software Unscrambler (v. 10.4, Camo, ASA, Norway). PCA used the nonlinear iterative partial least squares (NIPALS) algorithm and Hotelling's T^2 distribution to calculate the outliers ($p = 0.05$).

Results and Discussion

Ash and mineral contents in edible macroalgae

As all the macroalgae were harvested from a single site (Cape Mondego), species collected during each season were exposed to the same environmental conditions. *G. gracilis* and *O. pinnatifida* were sampled in spring, and the other species in late summer/early autumn of 2019. However, the concentrations of macroelements and oligoelements did not vary significantly between the two sampling seasons. This can be justified for a low variability of the physico-chemical properties in a site (e.g., the temperature of seawater, pH, and salinity). During harvesting seasons, in spring and late summer/early autumn, the seawater temperature was 16°C , pH 7.6 and 8.0, and salinity $31\text{--}35\text{ng L}^{-1}$ and $34\text{--}36\text{ng L}^{-1}$, respectively [12].

The concentrations of ash and minerals (macroelements and oligoelements) in analysed Portuguese macroalgae are presented in Table 1. The ash content ranged from 21% (*C. tomentosum*, *U. pinnatifida*) to 29% (*S. polyschides*). The differences in ash content between the studied species, which were not statistically different, and they corresponded to varying levels of minerals. Similar results for the ash content (20–31%) were obtained for the same edible species (*U. rigida*, *G. gracilis*, *O. pinnatifida*, *Porphyra* sp., *S. polyschides*, and *U. pinnatifida*) collected at different coastline sites in NW Portugal, Spain, and New Zealand [9,33,34].

Although the sum of macroelements (Ca, K, Mg, Na, and P), accounted for $> 99.6\%$ of the total minerals, their individual concentrations varied between the species. In all species, Mg ($3.3\text{--}19\text{mg g}^{-1}$) showed higher concentrations than Ca ($1.7\text{--}14\text{mg g}^{-1}$), with an exception for *S. polyschides* in which both elements had similar values. Within three phyla, the contents of Mg were higher in green species, probably due to higher levels of chlorophylls (with Mg ion in the center of the porphyrin ring). Potassium and Na were also in varying concentrations in all species, i.e., $20\text{--}106\text{mg g}^{-1}$ and $27\text{--}136\text{mg g}^{-1}$, respectively. The sum of K and Na represented 70.3–94.3% of the total concentration of macroelements in analyzed samples (Figure 3). The lowest concentrations of K+Na were found in *Porphyra* sp (7.1mg g^{-1}) whereas the highest in *C.*

tomentosum (195mg g⁻¹) and *U. pinnatifida* (17.4mg g⁻¹). The Na/K value was less than unity only in *G. gracilis* (Na/K = 0.3) and *S. polyschides* (Na/K = 0.9), whereas in the rest of samples this ratio was between 1.3 and 3.7, which is similar to other results previously reported [7-9,35].

Table 1: Concentration of ash (expressed as %, w/w), macroelements, Ca, K, Mg, Na, and P (expressed as mg g⁻¹), and oligoelements, Cu, Fe, Mn, Se, and Zn (expressed as µg g⁻¹) in edible macroalgae from central Portuguese coast.

Macroalgae	Ash	Ca	K	Mg	Na	P	Cu	Fe	Mn	Se	Zn
	%	mg g ⁻¹					µg g ⁻¹				
Chlorophyta (green)											
<i>Codium tomentosum</i> (Spongweed)	21	9.4	59	19	136	2.9	2.4	178	23	18	7
<i>Ulva rigida</i> (Sea lettuce)	21	6.9	20	30	74	2.5	3.6	383	18	15	10
Rhodophyta (red)											
<i>Gracilaria gracilis</i> (Slender wet weed)	28	1.7	106	3.3	27	2.9	2.3	123	15	18	17
<i>Osmundea pinnatifida</i> (Pepper dulse)	21	5.5	53	9.9	103	2.8	3.1	552	23	14	63
<i>Porphyra</i> sp (Nori, Laver)	22	2	29	6.3	42	6	5.9	110	53	2.9	61
Phaeophyceae (brown)											
<i>Saccorhiza polyschides</i> (Furbelows)	29	14	85	13.6	77	3.2	1.4	46	6	30	31
<i>Undaria pinnatifida</i> (Wakame)	21	9.7	76	13.5	97	6.8	1.4	29	4	15	10

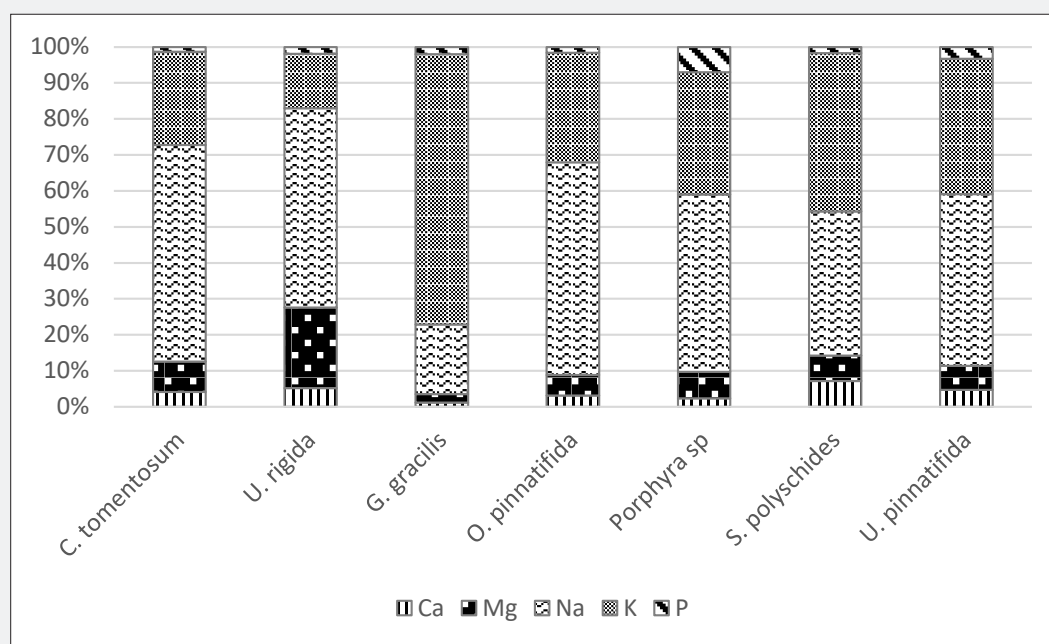


Figure 3: The relative percentage of macroelements in edible macroalgae from central Portuguese coast.

Phosphorous was present at a fairly low level (1.3-7.1% of the sum of macroelements), in all seven species ranging from 2.5 (*U. rigida*) to 6.8mg g⁻¹ (in *U. pinnatifida*). The concentrations of P were of the same order of magnitude as Ca, giving the value of Ca/P higher than unity in all species, with an exception in *G. gracilis* (Ca/P = 0.6) and *Porphyra* sp (Ca/P = 0.3). The results obtained were similar to those previously reported for the similar species of the same genus, *Porphyra columbina*, collected from the central pristine beach in Argentina [36].

The highest amounts for the sum of Ca+K+Mg+Na were found in *C. tomentosum* (223mg g⁻¹) and *U. pinnatifida* (197mg g⁻¹). In *O. pinnatifida* (172mg g⁻¹) and *S. polyschides* (189mg g⁻¹) similar values were observed, while *U. rigida* and *G. gracilis* contained less amounts of macroelements (131 and 138mg g⁻¹, respectively). The species *Porphyra* sp had the smallest sum of the macroelements (79mg g⁻¹). Similar composition of macroelements was observed in the edible macroalgae from the French coastline [16].

Among oligoelements, Cu content was in the lowest range ($1.4\text{--}5.9\mu\text{g g}^{-1}$) whereas Fe was present in a broad interval of concentrations, from $29\mu\text{g g}^{-1}$ (*U. pinnatifida*) to $552\mu\text{g g}^{-1}$ (*O. pinnatifida*). Manganese, Se, and Zn were 3-22 orders of magnitude higher than Cu in edible species studied, i.e., up to 53, 30, and $63\mu\text{g g}^{-1}$, respectively. Manganese was found at the highest level in *Porphyry* sp ($53\mu\text{g g}^{-1}$), whereas in the same species the lowest content of Se ($2.9\mu\text{g g}^{-1}$) was registered. In the rest of the species had Se ranged from 14 to $18\mu\text{g g}^{-1}$, and its maximum level was found in *S. polyschides* ($30\mu\text{g g}^{-1}$). The highest Zn content was obtained in red species *O. pinnatifida* ($63\mu\text{g g}^{-1}$) and *Porphyra* sp ($61\mu\text{g g}^{-1}$). Copper was positively correlated with Mn ($R = 0.95$), and negatively with Se ($R = -0.83$).

Biomolecules in edible macroalgae

In general, studied macroalgae showed relatively high contents of proteins and carbohydrates, whereas their total lipid level was very low (Table 2). The protein content ranged 15-17%, 18-24%, and 16% in studied green, red, and brown species, respectively. Since proteins are mostly composed of glutamate and aspartate, both amino acids that contribute to the umami taste [4,37,38], the maximum protein levels obtained in red species, correspond to their highest umami amino acid levels, previously found in red

species (*G. gracilis*, *O. pinnatifida*) [38]. The high levels of proteins found in *O. pinnatifida* (20%) and *Porphyra* sp (24%) were similar to other results obtained for these edible species from the Azores archipelago and the Central and NW Portuguese coast [8,9,11]. Similar results were obtained in the edible species of *Porphyra* collected from a pristine beach within San Jorge Gulf, Argentina [36], NW Iberian coast [2], and in coastlines of Japan, Korea, and China [3]. These results may suggest similar natural characteristics of marine environments, predominantly oligotrophic with good availability of nitrogen to algal populations. Moreover, all results were obtained after the same methodology applied in protein determination (Kjeldahl's method with the use of N-conversion factor of 6.25).

The total carbohydrates showed the highest values (60-62%) in green species, while the red and brown macroalgae contained lower levels (52-58%). Similar results were obtained in previous studies with several edible macroalgal species from Chile [31]. Red species showed the lowest, whereas brown species were characterized by the highest fibre contents (14-18% and 22-32%, respectively) (Table 2). Due to the lack of fibre content results in previous studies with macroalgae from the Portuguese coastal environments, the comparisons of results are unattainable.

Table 2: The total protein, carbohydrate, lipid, and crude fibre contents (expressed as %) and caloric values (kcal 100g^{-1}) of edible macroalgae from central Portuguese coast.

Species	Proteins	Carbohydrates	Lipids	Crude Fibres	Calories
	%				kcal 100g^{-1}
Chlorophyta (green)					
<i>Codium tomentosum</i> (Spongweed)	17	60	1.4	17	255
<i>Ulva rigida</i> (Sea lettuce)	15	62	1	21	237
Rhodophyta (red)					
<i>Gracilaria gracilis</i> (Slender wet weed)	18	53	0.9	14	237
<i>Osmundea pinnatifida</i> (Pepper dulse)	20	57	1.8	18	252
<i>Porphyra</i> sp (Nori, Laver)	24	52	2.1	17	256
Phaeophyceae (brown)					
<i>Saccorhiza polyschides</i> (Furbelows)	16	52	3.3	32	172
<i>Undaria pinnatifida</i> (Wakame)	16	58	4.8	22	253

All species showed very low total lipid content (Table 2), and this can be explained by the harvesting season. Namely, the lowest lipid percentage is typical in summer and autumn, whereas the highest values occur in winter [39]. Green and red species showed very low lipid contents, ranging from 0.9% to 2.1%, and slightly higher values were found in brown macroalgae (3.3-4.8%). Low lipid ranges were obtained for the same species of macroalgae by other authors [2,5,7-9,14,33,34].

The caloric values of the studied species varied significantly, from $172\text{kcal }100\text{g}^{-1}$ (*S. polyschides*) to $256\text{kcal }100\text{g}^{-1}$ (*Porphyra* sp). The calories in macroalgae were statistically negatively

correlated to their ash content ($R = -0.81$). Therefore, a high ash and Ca content in *S. polyschides*, derived from precipitated calcium carbonate in the thick thallus of this species (Figure 2 & 3) resulted in the low caloric value, whereas *Porphyra* sp has a foliose thallus, with lower ash and Ca values and therefore, having a higher caloric content.

The caloric values obtained in this work were lower in comparison to the scarce data reported for the same species, *O. pinnatifida* and *Porphyra* sp, collected in North Yorkshire, United Kingdom, which caloric values were respectively 325 and $437\text{kcal }100\text{g}^{-1}$ [40]. This difference in results can be associated not only

to slightly different contents of biomolecules (proteins, lipids, carbohydrates, etc.) in species grown in different environmental conditions but also due to the different methodologies used i.e., the latter values were determined directly by using a bomb calorimeter [40].

Clustering of edible macroalgae based on their nutritional profile

A two-component PCA allowed a good distinction (79%) between the studied species belonging to the three taxonomic phyla (Figure 4). The first principal component (PC-1) accounted for 44% of the variance in data, while the second component (PC-2) explained 35% of the variance. PC-1 represents gradient

opposing high concentrations of Se, crude fibre (with positive scores), with that of Fe (with negative score). PC-2 mainly reflects variations in Mg, Na (with positive scores), versus Cu, Mn, Zn (with negative scores). Both green species (*C. tomentosum* and *U. rigida*) were positively correlated with Mg, Na, Fe, and carbohydrates, whereas all studied red species were correlated to the P, most of oligoelements (e.g., Cu, Mn, Zn), as well as proteins. The brown species (*S. polyschides* and *U. pinnatifida*), also clustered separately, showed a higher affinity for Ca, Se, lipids, and crude fibres. Data for *S. polyschides* are strongly displaced along the PC-1, and this is likely to be caused by its relatively high Ca, ash, and crude fibre contents, and low caloric values.

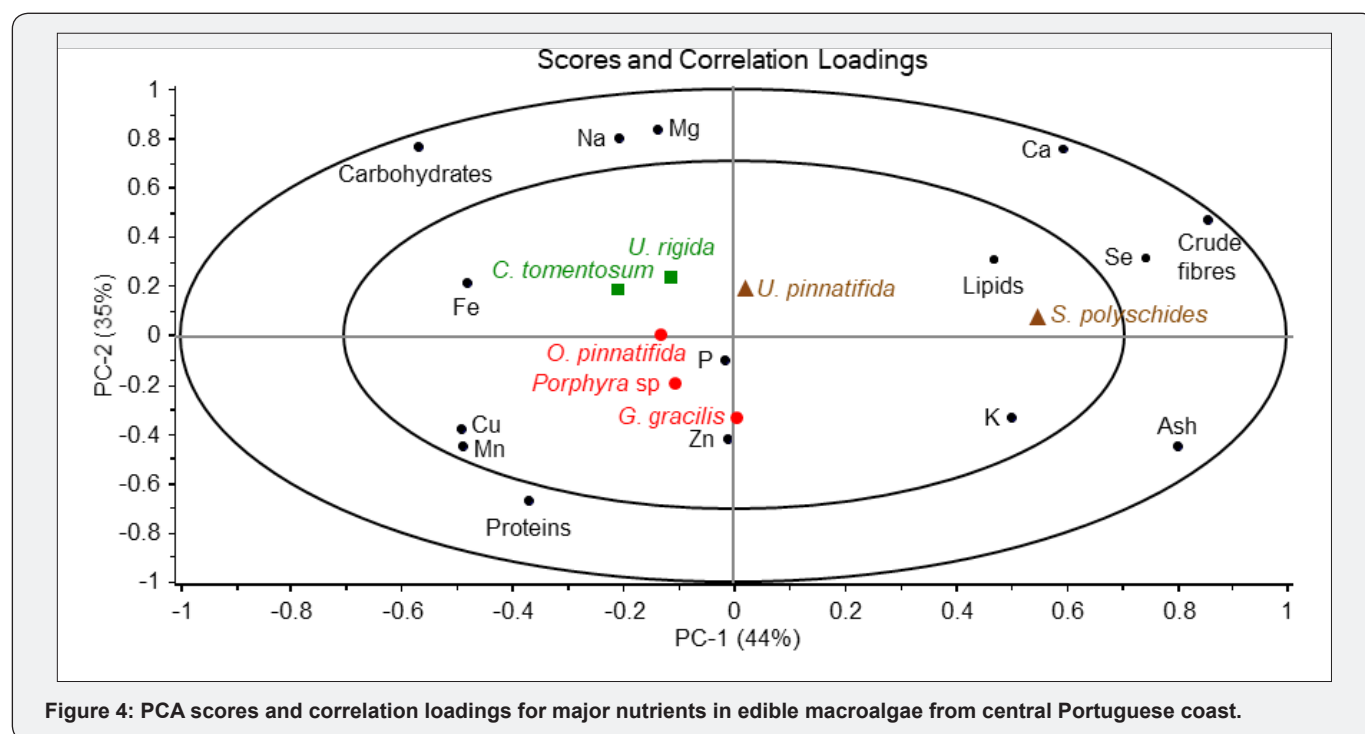


Figure 4: PCA scores and correlation loadings for major nutrients in edible macroalgae from central Portuguese coast.

The results presented in PCA plots can serve as a database of a smaller set of nutritional components in selected edible species. According to their specific nutritional composition (e.g., *O. pinnatifida* had highest Fe, Zn; *S. polyschides* had highest Ca, Se, ash, and crude fibres), each species can be introduced selectively as unique dietary supplement in the diet.

Inclusion of macroalgae from the Cape Mondego in human diet

Results obtained for both minerals and biomolecules (proteins, carbohydrates) in the studied edible macroalgal species can be considered for their introduction into the regular human diet with balanced nutritional requirements. The sum of macroelements (Ca+K+Mg+Na+P), ranging from 85mg g⁻¹ (*Porphyra* sp) to 226mg g⁻¹ (*C. tomentosum*), and oligoelements (Cu+Fe+Mn+Se+Zn), ranging from 60µg g⁻¹ (*U. pinnatifida*) to 655µg g⁻¹ (*O. pinnatifida*),

were higher than in some terrestrial vegetables, such as carrots, tomatoes, or potatoes [7]. These results indicate that macroalgae can provide a sustainable source of minerals (macroelements and oligoelements), by their diverse applications in the diet.

Consumption of some macroalgae, can provide significant health effects in terms of increased oligoelements levels. As an example, a portion of 10g (dw) of *O. pinnatifida* contains 5.5mg of Fe, and thus its addition as a supplement (e.g., to bread, crackers, or biscuits) can increase the proportion of iron in food thus helping in the treatment of iron deficiency anemia [41,42]. Therefore, the addition of *O. pinnatifida* to food, can be an alternative to the French baguette supplemented with *Ulva* sp or *Palmaria palmata* (Fleurence, 2016). The content of Se in all studied species (except in *Porphyra* sp) was in higher levels (14-30µg g⁻¹), than in some traditionally known food rich in Se (e.g., Brazil nuts contain 0.8-

6.9 $\mu\text{g g}^{-1}$ [43]. According to the European Food Safety Authority, for the regulation of immune and reproductive systems, the adequate daily intake of Se was set at 70 μg for adults [28], and this amount can be achieved by the inclusion of smaller portions of macroalgae in diet. Nevertheless, it is important to analyse the levels of Se species in macroalgae and for instance, their changes after applying various methods of cooking (e.g., steaming, water blanching, boiling, etc.). The obtained results for Se species can provide information on the optimal use of macroalgal species as food ingredients (e.g., in broths and soups).

The favorable nutrition effects of macroalgae can also be very beneficial for patients with hypertension who require special medical treatments. The Na/K ratio is important to control hypertension for patients suffering from an excessive secretion of K [35]. In the studied samples, the low level of Na/K values (0.3-3.7) can be of special nutritional interest, significantly lower than in common food, such as olives (Na/K~44) [36]. Therefore, the consumption of selected species can provide balanced Na/K diets. As an example, macroalgae can be incorporated in meat products, with less salt than the conventional recipes, provided a promising foodstuff with functional, health and sensory attributes [44].

The recent global estimate for protein sources highlights their higher demand by 2050 [45]. Given the variability of the content of total proteins in macroalgae, they can be an alternative to non-animal protein sources in the future [46]. The total protein content in studied red macroalgae (18-24%) is comparable to plants with a high-protein content, such as carrots, tomatoes, peas, or beans (11-28%) [9,36,47]. Hence, macroalgae can substitute or complement, the role of legumes and cereals to get protein-balanced diets. However, the protein digestibility can differ between the species, and this should be studied in more details. Some results showed that red species had higher protein digestibility than green species, while brown macroalgae had the lowest digestibility [48]. The study of digestibility of proteins in *U. pinnatifida* showed they were high, i.e., 70% in humans [49].

A portion of 10.0g (dw) of green, red, or brown macroalgae can provide 6.1, 5.4, or 5.5g of total carbohydrates, respectively, which corresponds to a similar portion of peas, tomatoes, and carrots (4.7, 5.8, or 6.3g, respectively) [47]. Compared to terrestrial plants, which contain crude fibres in quantities varying from 8.3% (apples) to 17.5% (cabbage), the studied macroalgae (*U. rigida*, *S. polyschides*, *U. pinnatifida*) contained higher amounts of crude fibres (21-32%), representing a valuable source of fibre, which recommended daily intake (25g for adults) can be achieved by consuming of food supplemented with selected species [14,28].

Macroalgae-derived lipids are known to be highly digestible and this was reported for *U. pinnatifida* species from Japan, which lipids are 98% digestible in adults [50]. Hence, the inclusion of macroalgae in diet can provide moderate lipid intake and moderate prevention of weight gain, compatible with good health.

Conclusion

The edible green, red, and brown macroalgae examined in this study, have considerable nutritional potentials, with better mineral profiles than some terrestrial plants. Together with high protein, carbohydrate, and low lipid levels, studied species from Cape Mondego, in the central Portuguese coastline, represent healthy, low-calorie 'sea vegetables'. Given their different nutritional profiles, species belonging to green, red, and brown phylum can be included in diversified macroalgal-based food (e.g., appetizers, snacks, salads, meals, desserts, beverages, etc.) both healthy and attractive to consumers.

The application of edible macroalgae, in particular in Western gastronomy, should be fully exploited, to fit gastronomic purposes. Future studies are important for the nutritional evaluation of various edible species and their digestibility, as well as possible effects as a functional food.

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