Vitamins & Minerals
ISSN: 2376-1318

Research Article | Open Access

Antioxidant Substances and Trace Element Content in Macroalgae from a Subtropical Lagoon in the West Coast of the Baja California Peninsula

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Abstract

Macroalgae play an important role in the ecology of the marine environment. They form the base of the food webs, and represent the major food source for a variety of organisms. In order to assess their potential nutritional value, the antioxidant and trace element content (vitamin C, total polyphenols, zinc, iron, cooper, selenium, cadmium and lead) of eight macroalgae species, three red (*Hypnea spinella*, *Gracilaria textorii* and *G. vermicullophyla*), four green (*Caulerpa sertularioides, Codium simulans, C. amplivesiculatum* and *Ulva lactuca*) and one brown (*Dictyota flabellata*) macroalgae, were determined. The concentration ranges found were as follows: zinc, 19.1-7.4; iron, 638.4-89.2; copper, 3.9-0.9; selenium, 0.32-10; cadmium, 4.33-0.02, and lead 8.8-0.39 mg kg^{-1} dry weight. Green macroalgae showed higher concentrations of iron and zinc. Total polyphenol content ranged from 29.6 to 70.3 mg 100 g^{-1} dry weight; brown macroalgae showed higher polyphenol concentration. *C. simulans* and *C. amplivesiculatum* had higher vitamin C content (3.16 ± 0.52 mg g^{-1} and 6.08 ± 0.69 mg g^{-1} dry weight, respectively) than the other species. Comparison with several commonly consumed vegetables and fruits suggest that these macroalgae are likely a good alternative source of antioxidants and micronutrients for consumers.

Keywords: Antioxidants; Macroalgae; Micronutrients; Polyphenols; Trace elements; Vitamin C

Abbreviations: ROS: Reactive oxygen species; LDL: Low density lipoproteins; $O_2^{\bullet-}$: Superoxide radical; H_2O_2: Hydrogen peroxide; $OH^{\bullet}$: Hydroxyl radical; $^1O_2$: Singlet oxygen; Cu: Copper; Fe: Iron; Pb: Lead; Cd: Cadmium; Zn: Zinc; Se: Selenium; GAE: Gallic acid equivalents; FAO/WHO: Food and Agriculture Organization/World Health Organization; RNI: Recommended nutrient intake

Introduction

In many countries, fresh marine macroalgae are used as food by coastal communities, and considered as a traditional food item due to their nutritional value and characteristic taste [1-3]. Macroalgae are valuable sources of proteins, polysaccharides, and fiber; but they are also rich in antioxidants and micronutrients, such as vitamins and trace elements [4-6].

Antioxidant vitamins and trace elements are usually obtained from the diet, since some organisms are unable to synthesize them. The beneficial effects of antioxidants are due to their capacity to scavenge and neutralize reactive oxygen species (ROS) [7]. An excessive ROS production and/or low antioxidant defense can cause oxidative damage to biomolecules, such as proteins, lipids and DNA [8,9]. Antioxidants may reduce ROS production by scavenging free radicals through various mechanisms [7,10]. Some trace elements contribute to the function of endogenous antioxidant enzymes by acting as cofactors [11,12]. Most polyphenols can act as chain breakers or radical scavengers and prevent the oxidation of low density lipoproteins (LDL) [12,13]. Vitamin C is a scavenger against superoxide radical ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^{\bullet}$), and singlet oxygen ($^1O_2$), which would otherwise react to form lipid peroxides; vitamin C also reduces the tocopheroxyl radical formed by interaction of α-tocopherol with lipid peroxides in cell membranes [7,14]. This demonstrates the wide variety of reactive intermediates vitamin C interacts with, to maintain the function of cellular components.

Marine macroalgae are a potentially good source of micronutrients and may be beneficial for human health, given their reportedly high vitamin and trace element content [1,15,16]. Most published studies on macroalgae are focused on ecological aspects and chemical composition (protein, carbohydrate, ash and calories). However, little is known about macroalgae antioxidant vitamins and micronutrient composition [5,17,18]. The objective of this study was to assess the antioxidant vitamin and trace element content of eight macroalgae species, from a subtropical and unpolluted lagoon in the west coast of the Baja California peninsula. Lead and cadmium concentrations were also assessed, given their toxicological potential. This information may be useful in the search for alternative and supplementary food items in the Baja California peninsula.

Materials and Methods

Eight species of macroalgae belonging to the Chlorophyta, Rhodophyta and Ochrophyta Pheaophyceae divisions were collected from Bahia Magdalena, Baja California Sur, Mexico (24°15´N and 25°20´N and 111°30´W and 112°15' W). Bahia Magdalena is a shallow lagoon with high productivity resulting from seasonal marine upwelling [19]. Macroalgae were collected in November of 2009, February, April and June of 2010. Samples were carried on ice, stored in black bags to the laboratory, where macroalgae were washed with filtered, cold seawater to remove all epiphytes and other debris. The samples used for trace element analyses were dried in an oven at 60°C for 48 h. The dried samples were then ground into a fine powder using a coffee grinder, and stored until analyzed. Dried samples destined for copper (Cu), iron (Fe), lead (Pb), cadmium (Cd) and zinc (Zn) analyses were digested in

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Received November 12, 2012; **Accepted** November 24, 2012; **Published** November 26, 2012

Citation: Tenorio-Rodríguez PA, Méndez-Rodríguez LC, Serviere-Zaragoza E, O´Hara T, Zenteno-Savín T (2013) Antioxidant Substances and Trace Element Content in Macroalgae from a Subtropical Lagoon in the West Coast of the Baja California Peninsula. Vitam Trace Elem 2:108.

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acid-washed Teflon tubes, with concentrated nitric acid (HNO_3) in a microwave oven (Mars 5X, CEM, Matthew, NC, USA), and analyzed by atomic absorption (GBC Scientific equipment, AVANTA, Dandegong, Australia), using an air-acetylene flame. Selenium (Se) determinations were performed at the Wildlife Toxicology Laboratory, University of Alaska Fairbanks. Samples were digested twice, first with HNO_3, and then with HCl (37%) in a microwave (3000 Microwave Multiwave Sample Preparation System, Anton Para, Austria). Se concentration was analyzed by atomic absorption spectrometry (AAnalyst 800 PerkinElmer Instruments, Shelton, CT, USA), with a flow injection system, according to the procedure described by Knott et al. [20] and Barrera-García et al. [21]. The results are expressed in mg kg^{-1} dry weight (d.w.).

The vitamin C (L-ascrobic acid) content of macroalgae was determined by high-performance liquid chromatography (HPLC, Waters, Milford, MA, USA) [22,23]. Each sample (0.1 g) was homogenized with metaphosporic acid (3% w/v), incubated for 40 min at 4°C in the dark and centrifuged for 15 min at 23,985 g at 4°C. Samples were then filtered through a 0.45 µm filter prior to injection. Ten µL of each sample were injected into the HPLC. The mobile phase was a mixture of water, sulfuric acid (pH 2.4), and acetonitrile (100%, HPLC grade). The flow rate was 1 mL min-1, and the detection wavelength was set at 245 nm. The results are expressed in mg vitamin C g^{-1} d.w.

The Folin-Ciocalteu colorimetric method, with modifications, was used to quantify total polyphenol concentration in macroalgae samples [24,25]. Briefly, 1 g of fresh sample was ground with a mixture of water:methanol:acetone (2:3:5 v/v), using a mortar and pestle over ice. Samples were incubated in a water bath at 65°C with agitation for 1 h. Extracts were centrifuged at 15,292 g for 10 min, and the supernatant was collected. Na_2CO_3 was added and samples were incubated for 1 h at room temperature. The absorbance at 750 nm was recorded in a microplate reader (BioRad™ 550, Hercules, CA, USA), and compared to a gallic acid calibration curve. The results are expressed as gallic acid equivalents (GAE) in mg 100 g^{-1} fresh weight (f.w.).

Normality and homogeneity of variance were determined by using Shapiro-Wilks and Bartlett tests. All variables were log-transformed in order to normalize the data. Differences between groups and species were analyzed by using ANOVA or Student t-tests, with Tukey post-hoc tests [26]. Results are expressed as mean ± SE, and differences were considered statistically significant at $p<0.05$ level. Statistica 8.0 (StatSoft Inc. Tulsa, OK, USA) software was used to perform all statistical analyses.

Results

The trace element content of eight macroalgae species is shown in table 1. Fe concentration in all macroalgae species studied ranged from 89.2 to 638.4 mg kg^{-1} d.w. Green macroalgae had the highest Fe content (p=0.013), with *C. simulans* and *C. amplivesiculatum* showing the highest values. Zn levels ranged from 7.4 mg kg^{-1} d.w. in *H. spinella* to 19.1 mg kg^{-1} d.w. in *U. lactuca*. Cu concentrations ranged from 0.9 mg kg^{-1} in *H. spinella* to 3.9 mg kg^{-1} in *C. sertularioides*. Se, Cd and Pb levels ranged between 0.10-0.32 mg kg^{-1}, 0.01-4.33 mg kg^{-1}, and 8.8-0.54 mg kg^{-1}, respectively (Table 1). No differences in trace element content were found between groups or among species.

The mean content of total polyphenols in all macroalgae species studied, ranged from 29.6 to 70.3 mg of GAE 100 g^{-1} w.w. (Figure 1). Total polyphenol content was higher in green and brown macroalgae, compared to red macroalgae (p=0.00002). In general, the brown macroalga *D. flabellata* and the green macroalga *C. sertularioides* had higher total polyphenol concentrations, in comparison with the rest of the species (70.3 ± 7.6 and 68.6 ± 6.03 mg of GAE 100 g^{-1} w.w., respectively). *U. lactuca* had the lowest total polyphenol content (29.6 ± 1.5 mg of GAE 100 g^{-1} w.w.).

Vitamin C concentration in eight macroalgae species from Bahia Magdalena, Baja California Sur is shown in figure 2. Vitamin C content in all macroalgae species analyzed ranged from 1.24 to 6.08 mg g^{-1} d.w. Vitamin C concentration was higher in green and brown macroalgae, compared to red macroalgae (p=0.002). The highest vitamin C concentration was found in the green macroalga *C. amplivesiculatum* (6.08 ± 0.69 mg g^{-1} d.w.) (p=0.001), while the red macroalga *H. spinella* had the lowest vitamin C content (1.24 ± 0.16 mg g^{-1} d.w.).

Discussion

The majority of the essential minerals and trace elements needed for human nutrition can be found in macroalgae; therefore, macroalgae could be regarded as a valuable resource. Trace elements such as Fe, Cu and Se are considered essential for biological processes, including growth, reproduction, hormone metabolism and antioxidant defense [27-29]. Cd and Pb are a potential health hazard and are commonly measured, as indicators of environmental pollution [30,31]. All macroalgae species analyzed in the present study showed lower concentrations of Cd than previously reported (0.71 mg kg^{-1} [32]; 1.9 mg kg^{-1} [31]; 3.70 mg kg^{-1} [33]; 4.8 mg kg^{-1} [34]). The mean Pb concentration in macroalgae species analyzed was 3.46 mg kg^{-1}. Similar Pb concentrations were reported for macroalgae (3.50 mg kg^{-1} [33], and

Species	n	Zn	Fe	Cu	Se	Cd	Pb
Chlorophyta *Caulerpa sertularioides* (S.G. Gmelin) M.A. Howe	5	16.8 ± 3.9	420.9 ± 107.9	3.9 ± 1.84	0.32 ± 0.09	0.38 ± 0.18	2.71 ± 1.01
Codium amplivesiculatum Setchell & N.L. Gardner	3	17.1 ± 4.9	638.4 ± 176.4	2.2 ± 0.66	0.19 ± 0.07	0.02 ± 0.00	0.39 ± 0.28
Codium simulans Setchell & N.L. Gardner	5	18.2 ± 6.5	447.6 ± 176.7	1.7 ± 0.52	0.19 ± 0.01	1.11 ± 0.39	8.28 ± 2.44
Ulva lactuca Linnaeus	3	19.1 ± 7.5	213.1 ± 72.3	2.3 ± 2.2	0.11 ± 0.04	0.02 ± 0.01	1.68 ± 0.46
Ochrophyta-Phaeophyceae *Dictyota flabellata* (F.S.Collins) Setchell & N.L.Gardner*	2	14.0	560.1	2.0		4.33	8.8
Rhodophyta *Gracilaria vermiculophylla* (Ohmi) Papenfus	7	12.2 ± 4.8	190.3 ± 51.5	1.0 ± 0.41	0.10 ± 0.02	0.10 ± 0.09	2.25 ± 1.05
Gracilaria textorii (Suringar) De Toni	4	15.8 ± 4.3	89.2 ± 24.4	1.2 ± 0.18	0.15 ± 0.01	1.09 ± 0.54	1.47 ± 0.77
Hypnea spinella (C. Agardh) Kützing	3	7.4 ± 0.21	409.6 ± 27.2	0.9 ± 0.05	0.10 ± 0.01	1.01*	2.1*

Mean values ± standard error
*Standard error not calculated

Table 1: Trace element concentrations (mg kg^{-1}) in macroalgae species from Bahia Magdalena, Baja California Sur, Mexico.

2.84 mg kg^{-1} [35]); however, Mok et al. [36] reported lower Pb levels (0.89 mg kg^{-1}). Se content in macroalgae has been reported to be in the range of 0.1-6 µg g^{-1} [32], which is similar to what was found in the present study. Concentrations of trace elements similar or lower than those found in eight species of macroalgae in this study have been reported for vegetables: 323 µg Fe g^{-1} in lettuce, 182.8 µg Fe g^{-1} in parsley, 166.4 µg Fe g^{-1} in spinach [37], 1.02-0.33 mg Cu kg^{-1} in spinach, and 1.11-0.51 mg Cu kg^{-1} in tomato [38]. The concentrations of these elements in macroalgae depend on the surrounding environment. Natural enrichment of trace elements in the Baja California peninsula has been suggested to occur due to natural phenomena, such as currents, upwelling and various geological sources [39,40].

Concentrations of vitamin C in most of the analyzed macroalgae species in this study were similar or higher than those reported for congeneric species in Hawaii (3.0, 2.2, and 1.3 mg g^{-1} d.w. in *Ulva*

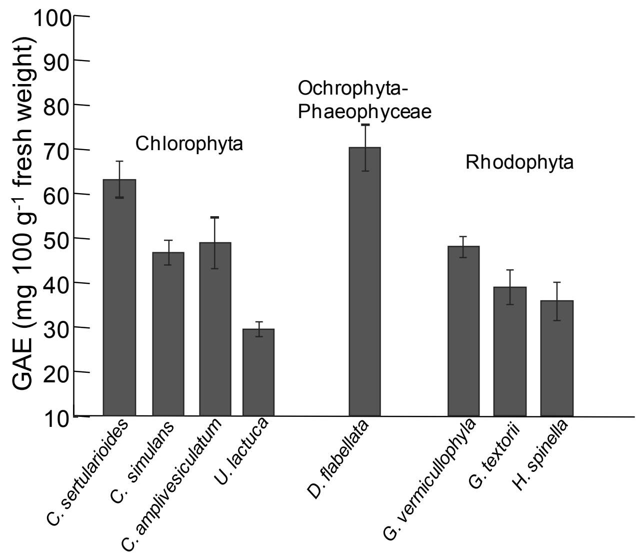

Figure 1: Total polyphenol content (mg 100 g^{-1} fw) in macroalgae species from Bahia Magdalena, Baja California Sur, Mexico, grouped by division. Data are presented as mean ± SE.

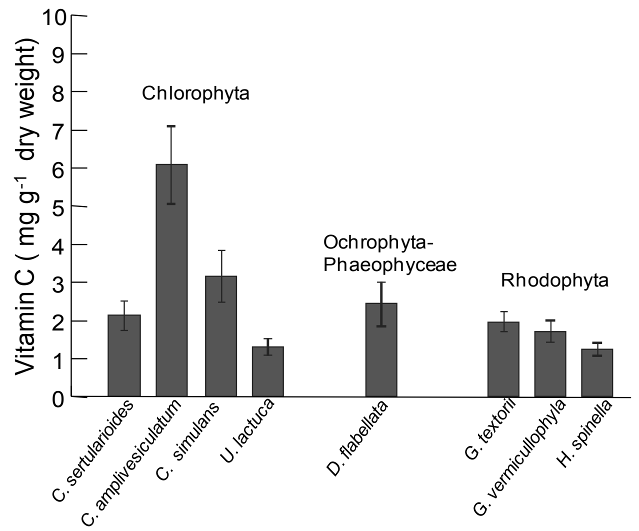

Figure 2: Vitamin C content (mg g^{-1} dw) in macroalgae species from Bahia Magdalena, Baja California Sur, Mexico, grouped by division. Data are presented as mean ± SE.

Micronutrient	Recommended daily intake	Reference
Fe	48 mg	Joint FAO/WHO Expert Committee on Food Additives, 1999
Cu	3 mg	Joint FAO/WHO Expert Committee on Food Additives, 1999
Zn	60 mg	Joint FAO/WHO Expert consultation 1998
Se	29 µg	Joint FAO/WHO Expert consultation 1998
Cd	70 µg	Joint FAO/WHO Expert Committee on Food Additives, 1999
Pb	214 µg	Joint FAO/WHO Expert Committee on Food Additives, 1999
Vitamin C	45 mg	Joint FAO/WHO Expert consultation 1998

Table 2: Recommended daily dose of trace elements and antioxidants.

flexuosa (as *Enteromorpha flexuosa*), *Ulva fasciata* and *Monostroma oxyspermum,* respectively, [5,41], along the coast of India (0.00244 mg g^{-1} d.w. in Ulva reticulate) [42], or the central part of the Baja California Peninsula (3.4 mg g^{-1} d.w. in *Eisenia arborea*) [43]. However, the two *Codium* species analyzed in this study had higher vitamin C content (mean of 4.62 mg g^{-1} d.w.) than the values reported for other green macroalgae species [5,44]. Overall, vitamin C concentrations in the macroalgae species analyzed in this study were comparable with those reported in guava (1.44 mg g^{-1}), papaya (1.08 mg g^{-1}) and red peppers (1.51 mg g^{-1}), and were higher than in oranges (0.67 mg g^{-1}), or broccoli (0.89 mg g^{-1}) [45,46]. The intake of vitamin C is essential since the human body is unable to synthesize it. It is effective in ameliorating symptoms of the common cold; moreover, evidence shows that vitamin C reduces mortality from heart disease, and can prevent oxidative stress in the process of immune response [47,48].

Total poly phenol content in the brown macroalga *D. flabellata* and in the green macroalga *C. sertularioides* was higher than in other species analyzed in this study. Previous studies similarly reported that polyphenol content in brown marine algae is higher than that in red marine algae [49,50]. Phenolic compounds are widely distributed in all macroalgae. These compounds are good antioxidants, since they can act as metal chelators and ROS scavengers trapping the lipid alkoxyl radical preventing lipid peroxidation. The beneficial effects of polyphenols on human health and their contribution to protect against chronic diseases, such as neurodegenerative disorders and cancer, have been extensively documented [51,52]. In this study, total polyphenol content in the macroalgae species analyzed was higher than that reported for several vegetables and fruits, such as avocado (3.6 mg 100 g^{-1} f.w.), kiwifruit (28.1 mg 100 g^{-1} f.w.), broccoli (98.9 mg 100 g^{-1} f.w.), pear (69.2 mg 100 g^{-1} f.w.), mango (68.1 mg 100 g^{-1} f.w.), and tomato (13.7 mg 100 g^{-1} f.w.) [53].

In order to evaluate the food safety of the analyzed samples, regulation in other countries was consulted, since Mexico has not yet adopted official trace element limits regarding macroalgae used for human consumption. Cd concentration in G. *textorii*, *H. spinella, C. simulans* and *D. flabellata* and Pb content in *C. simulans* and *D. flabellata* exceeded the permissible limit, considered by the French Legislation (0.5 mg Cd kg^{-1} and 5 mg Pb kg^{-1} d.w.). However, the Food and Agriculture Organization/World Health Organization (FAO/WHO) [54-56] have jointly recommended daily doses for trace elements and vitamins, based on the body weight of an average adult (60 kg body weight). Recommended nutrient intake (RNI) values are shown in table 2. The Committee on Medical Aspects of Food and Nutrition Policy [57], suggested macroalgae consumption of 8 g per day is a typical daily portion consumed in the Asian cuisine. Considering these references, and the maximum content of Pb and Cd detected in *D. flabellata* (8.8 mg kg^{-1} and 4.33 mg kg^{-1} d.w, respectively), these macroalgae would contribute 33% Pb and 49% Cd of the recommended daily dose by FAO/WHO. These contributions from a single macroalgae species could be considered to be high. However, trace element levels in all the macroalgae species analyzed in this study are below the permissible limits established by FAO/WHO.

The trace element and antioxidant content found in eight species of macroalgae in Bahia Magdalena, Baja California Sur, suggest that these species are safe and potentially exploitable for human consumption, and that macroalgae could be an alternative source of antioxidants and trace elements to help meet the recommended daily intake of some trace elements and vitamin C. However, in brown macroalgae, the content of elements, such as Cd and Pb should be monitored to ensure chemical safety.

Acknowledgments

Authors acknowledge financial support from CIBNOR (PC2.0, PC0.10), and the assistance of H. Berviera Leon and J. Angulo Calvillo in the field, A. Mazariegos in macroalgae identification, and N. Olguín-Monroy, B. Acosta, G. Peña Armenta, O. Lugo-Lugo and J.M. Castellini in quantitative analysis.

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