Journal of Nutrition & Food Sciences
ISSN: 2155-9600

Garcia, J Nutr Food Sci 2016, 6:3
DOI: 10.4172/2155-9600.1000505

Research Article Open Access

Nutritional Potential of Four Seaweed Species Collected in the Barbate Estuary (Gulf of Cadiz, Spain)

Garcia JS*, Palacios V and Roldán A

Department of Chemical Engineering and Food Technology, University of Cádiz, PB 40, Poligono Rio San Pedro, Puerto Real 11510, Cádiz, Spain

***Corresponding author:** Garcia JS, Department of Chemical Engineering and Food Technology, Faculty of Sciences, University of Cádiz, PB 40, Poligono Rio San Pedro, Puerto Real 11510, Cádiz, Spain, Tel: 0034692037874; E-mail: fini.sanchez@uca.es

Received date: Apr 06, 2016; **Accepted date:** May 17, 2016; **Published date:** May 26, 2016

Abstract

The Gulf of Cádiz has great socio-economic importance given its high fishing activity and high diversity and abundance of marine resources including a wide variety of seaweeds not used as food. Four macro-algae (*Ulva* spp., *Codium* spp., *Halymenia floresia* and *Saccorhiza polyschides*) collected in the Gulf of Cádiz near the Barbate estuary were analyzed for moisture, proteins, lipids, carbohydrates, ash, dietary fiber, caloric content, minerals, amino acids and fatty acids. The algae showed variations in all of the parameters analyzed. However, high concentrations of lipids and macro and micro-minerals and low concentrations of fiber and carbohydrates were related to the salinity, water temperature and nitrate contents of the collection areas. All four species had a high nutritional value, and these macro-algae have great potential for human consumption.

Keywords: Seaweed; *Ulva* spp.; *Codium* spp.; *Halymenia floresia*; *Saccorhiza polyschides*; Chemical composition

Introduction

Seaweeds have a long tradition in Asia as a food source, are commonly used as sushi wrapping, seasonings, condiments and vegetables and can constitute between 10 and 25% of food intake by most Japanese [1,2]. The production of macroalgae is limited to the brown species, Kombu, Wakame, Hijiki and Nori, marketed in China, Japan and Korea. However, in recent years, interest in these products and their consumption has increased in countries such as Chile, Brazil, Mexico, France, Germany and Spain, thereby increasing their collection and cultivation. World production is estimated at just over 25,000,000 tons of dry weight and total consumption of raw materials amounting to some 150,000 tons [3].

The abundance, diversity and commercial value of algae give it a large number of industrial applications, such as sources of human and animal food [4], biologically active phytochemicals (e.g., carotenoids, phycobilins, fatty acids, polysaccharides, vitamins and sterols) [5] and phycocolloids (e.g., alginates, carrageenan and agar) [6]. In the food industry, algae are currently used as raw materials for food in raw salads, soups, cookies, meals and condiments [7]. Algae can be used as a nutritional or functional ingredient to supplement food with natural antioxidants, antimicrobials or texturing agents, including chitin, chitosan, omega-3 oils, carotenoids, vitamins and minerals [4-6]. Thus, algae are used to develop new fortified foods such as bakery, dairy confectionary and pasta products [5].

The nutritional composition of macroalgae has been extensively studied [7-10]. These authors have highlighted its high water content, which varies between 70% and 90% of the fresh weight, protein content between 7% and 47%, carbohydrate content between 4% and 70%, lipids between 0.2% and 20% and ash content between 11% and 43%. Moreover, algae are appealing because of their high dietary fiber content (30-70%) which is higher than that found in common fruits and vegetables (1-5%) [8,10,11].

Macroalgae species are generally rich in essential amino acids, such as glycine, arginine, alanine, aspartic acid and glutamic acid [11], and in 3 and 6 PUFAs, of which the most abundant are C16:0, C18:19, C20:46, and C20:53 [12-14]. Macroalgae contain higher amounts of both macrominerals (Na, K, Ca and Mg) and trace elements (Fe, Zn, Cu and Mn) [15], but their concentrations vary by species and geographical origin [7,10,16,17].

Macroalgae are considered to be a highly nutritious food source, but their nutritional composition varies with species, habitat, growth stage, geographical origin, season and environmental conditions in the collection area (i.e., temperature, salinity and nutrients) [7,8,11,15,18-20].

The Gulf of Cádiz (southwestern coast of Spain) is an important fishing ground with a high diversity and productivity of exploited species [21]. This area connects the Atlantic Ocean with the Mediterranean Sea, and there are important estuaries and salt marshes associated with the mouths of rivers, such as the Guadiana, Piedras, Tinto-Odiel, Guadalquivir, Guadalete and Barbate, that feed in freshwater and other dissolved or suspended substances from the continent and increase the physiographic biodiversity of this area [22]. Moreover, the combination of warm nutrient-rich waters and wind regimes favors the diversity and abundance of marine resources [23], including a wide variety of seaweeds.

Although the Gulf of Cádiz marine ecosystem has great socio-economic importance given its high fishing activity [23], macroalgae is not yet collected and exploited. In fact, there is a lack of knowledge about the species that develop in this area, their characteristics and their food use.

The main goal of the present work was to determine the potential and value of Gulf of Cadiz macroalgae for human consumption. *Ulva* spp., *Codium* spp. (Chlorophyceae), *Halymenia floresia* (Rhodophyceae) and *Saccorhiza polyschides* (Phaeophyceae) collected

in the Gulf of Cádiz near the Barbate estuary were evaluated in terms of their nutritional composition (i.e., moisture, proteins, carbohydrates, lipids, ash, dietary fiber, minerals, free amino acids and fatty acids) and caloric value and were compared to other species reported in the literature.

Materials and Methods

Raw materials

Macroalgae samples, *Ulva* spp., *Codium* spp., *Halymenia floresia* and *Saccorhiza polyschides*, were obtained from staff at Innova Vegetalia del Mar, S.L., who had experience in diving and seaweed harvesting. The samples were manually collected in autumn (2011) at 12-15 m depth near the Barbate estuary, Gulf of Cádiz, Spain (36°16´N, 5°89´W). This area is characterized by salinity values between 36.2 and 36.45 and temperatures between 19°C and 22°C at 20 m depth [24].

After collection, macroalgae samples were rinsed with seawater, tap water and distilled water. For all analyses except the moisture analysis, samples were freeze-dried and milled into a fine powder to homogenize them prior to use. The homogenized macroalgae samples were stored in a desiccator until analysis. The moisture content was determined by drying below 100°C in an oven to a constant weight [25].

Analytical methods

Chemical analysis of seaweeds

Protein content: Protein content was determined using the Kjeldahl method in Marsham. Samples were digested in an automated digester, model DK6 (Velp Scientific, Italy), and distilled by steam distillation, model UDK127 (Velp Scientific, Italy). The protein content was calculated using a nitrogen conversion factor of 6.25 (following previous studies of seaweeds, as Norziah and Ching [11], and Ortiz et al. [8], and the results were expressed as a percentage of dry weight.

Carbohydrate content: Carbohydrates were determined using the Clegg manual anthrone method [25]. Following extraction, the sample was reacted with anthrone reagent (0.1%) and a high concentration of sulfuric acid (96%). The absorbance was read at 630 nm on a spectrophotometer, model Genesys10v (Thermo Fisher Scientific Inc., USA). The amount of carbohydrate was estimated using a standard curve of d-glucose.

Dietary fiber: Fiber content was determined by acid digestion using the Scharrer–Kürschner method [25].

Fat and fatty acid content: Fat content was determined by the Soxhlet method [25] using an automated SER 148 Soxhlet extraction unit (Velp Scientific, Italy) and n-hexane as the extraction solvent.

Fatty acids were determined by gas-liquid chromatography after derivatization to methyl esters (FAMEs) according to the IUPAC standard method [26]. Analysis of FAMEs was performed on a Hewlett-Packard 5890 Series II GC with an FID detector and a capillary column: Teknokroma TBR-WAX (30 m × 0.25 m × DF ID 0.25 m). The column temperature was programed from 190°C to 250°C at 2°C/min. Reference fatty acid methyl esters (FAMEs) of marine products (SIGMA, Spain) were used to identify and quantify FAMEs by comparing the retention times of the peaks with those of the standards.

Ash and mineral contents: Ash content was determined by incineration of 1 g of freeze-dried sample in a muffle oven at 550°C [25]. The ashes were dissolved in HNO_3 [27], and the mineral constituents (i.e., Ca, K, Na, Mg, Fe, Zn and Cu) were determined by inductively coupled plasma atomic emission spectroscopy (Iris Intrepid ICP-AES, Thermo Scientific, Maryland, USA).

Caloric value determination: The caloric value was calculated using energy conversion factors specified in EU Directive 90/496, as shown in Livesey et al. [28].

Free amino acid analysis: Amino acids were determined by high-performance liquid chromatography (HPLC) according to the method described in Pereira et al. [29] with slight modifications. The samples extracts were derivatized pre-column using OPA (Panreac, Spain) as the derivatization reagent. The chromatographic conditions were as follows: flow rate of 1 ml/min; LiChrospher 100 RP-18 (5 µm) column thermostated at room temperature; solvents, A, sodium acetate buffer (50 mM, pH 6.8): methanol (9:1) with 2% tetrahydrofuran and B, methanol with 0.5% tetrahydrofuran. The gradient consisted of 85-72% of A to B for 3 min, 56% A (25 min), 44% A (35 min) and 20% A (45 min). Fluorometric detection was performed at excitation and emission wavelengths of 324 and 420 nm, respectively, using an Intelligent Fluorescence detector, model FP-2020 (Jasco Europe s.r.l., Italy). Identification and quantification of amino acids were performed by comparing the retention times of the peaks with those of standards (Sigma Chemicals).

Expression of data and statistical analysis

All analytical determinations were performed at least in triplicate. The data were expressed as means ± standard deviations, and reported on a dry matter basis.

Results and Discussion

Chemical composition

The proximal composition of the macroalgae considered in this study is shown in Table 1. The macroalgae had different compositions that were primarily species-specific.

Components	*Ulva spp.*	*Codium spp.*	*H. floresia*	*S. polyschides*
Moisture[b]	84.3 ± 0.3	98.6 ± 1.2	91.9 ± 0.7	94.3 ± 0.9
Protein	7.2 ± 0.0	15.6 ± 1.4	9.4 ± 0.0	7.3 ± 0.1
Lipid	3.4 ± 0.4	7.1 ± 0.1	12.3 ± 1.1	8.2 ± 0.1
Fibre	9.5 ± 0.7	1.4 ± 0.1	1.7 ± 0.0	16.6 ± 0.4
Carbohydrate	11.7 ± 0.4	8.8 ± 0.4	15.5 ± 1.5	6.3 ± 0.1
Ash	34.2 ± 0.7	30.7 ± 1.1	50.2 ± 1.1	30.7 ± 0.8
Caloric value[c]	106.5 ± 1.3	161.4 ± 2.3	210.6 ± 2.1	128.2 ± 0.9

Table 1: Chemical characteristics and caloric value of seaweeds studied (g/100 g on dry weight)[a]. [a]All values given are means of three determinations (X ± SD); SD: standard deviation. [b] Moisture content is expressed as percentage of fresh weigh. [c]Caloric value is expressed as kcal/100 g of dry weight.

The moisture content of fresh macroalgae ranged from 84.3% (*Ulva* spp.) to 98.6% (*Codium* spp.). *Ulva* spp., whose structure is laminar, had the lowest value, while *Codium* spp., with a tubular structure and water-holding capacity, had the highest water content. *Saccorhiza polyschides* and *Halymenia floresia*, which have laminar structures, had similar moisture contents (94.2 and 91.9%, respectively). The moisture content, of other species studied by Dawczynski et al. [9], including *Porphyra* sp., *Undaria pinnatifida* and *Laminaria* sp., ranged from 89.3-93.5%. These species have the same structure as *Saccorhiza polyschides* and *Halymenia floresia* and had similar moisture contents. Therefore, this content appears to be related to the morphology and structure of the species.

The protein contents ranged from 7.2-15.6% and were much higher in *Codium* spp. (15.6%). The rest of the macroalgae contained similar values ranging from 7-9%. These results were similar to other studies in other species. Gressler et al. [29] reported protein contents in *G. domingensis*, *G. birdiae*, *L. filiformis* and *L. intricate* macroalgae that ranged from 4.6-18.3%. Gómez-Ordoñez et al. [10] reported contents from 10.9-25.7% in brown macroalgae and 15.5-21.3% in red species. Protein content may vary according to the species, geographic area, season and environmental conditions [10,30]. The macroalgae studied were collected in the same geographic area, season and environmental conditions; thus, the protein content appears to be related to the species in this study. Of all the species evaluated, *Codium* spp. had the highest protein content, which could be related to its enhanced ability to assimilate nitrogen [31,32].

Lipid contents in all of the species studied in this work were higher than 7%, except in *Ulva* spp. (3.4%). These values are higher than that determined by Wong & Cheung [33] (1.42-1.64%), Ortiz et al. [8] (0.3-0.8%) and Gómez-Ordoñez et al. [10] (0.3-0.9%), but similar to those reported by other authors [7,9,20]. A review by Miyashita et al. showed that lipids vary in brown macroalgae based on species, geography, season, temperature, salinity and light intensity, as well as interactions between these factors. These authors reported that tropical species have significantly lower lipid contents than cold-water species. Additionally, Floreto et al. [34,35] showed that high levels and a lack of nitrate and high salinity and light intensity led to high lipid levels. Therefore, the high fat content in the macroalgae of the present study could be related to the salinity (36.4%), water temperature (19.5-20.4°C) or nitrate content (45M NO_3^-; [36]) of the collection area However, the observed differences between the macroalgae in the present study were species-specific.

Carbohydrate contents ranged from 6.3-15.5% and were higher in *Ulva* spp. (11.7%) and *Halymenia floresia* (15.5%). These values were similar to that reported by Wong & Cheung [33] for *H. japonica*, *H. charoides* and *U. lactuca* (4.3, 7.02 and 14.6%, respectively). However, Ortiz et al. [8] found higher values in *Durvillaea antarctica* (70.9%), *Ulva lactuca* (61.5%) and *Ulva fasciata* (34.8%). According to Macler [37], there is an inverse relationship between the N content of water and the carbohydrate content of macroalgae. Therefore, the high nitrogen content in the collection area [36] could explain the low levels of carbohydrates found in the four species studied.

Most previous studies have found that fiber is the most abundant component of seaweeds [9,10,30,33]. However, in the present work, fiber contents were less than 20% in all macroalgae. In fact, the highest value was observed in *Saccorhiza polyschides* (16.6%) followed by *Ulva* spp. (9.5%), and the lowest values were in *Codium* spp. and *Halymenia floresia* (1.4% and 1.7%, respectively). Norziah and Ching [11] observed that the fiber content may be related to geography, water temperature, and collection time and collection depth. More specific studies [10] have shown that the fiber content decreases in nitrogen-rich areas. Seaweeds collected in the studied area had significantly lower fiber contents than samples reported in other studies, a finding that could again be related to the high nitrogen content in the collection area [36].

Ash contents of *Ulva* spp., *Codium* spp. and *Saccorhiza polyschides* were high and ranged from 30.7-50.2% (*Codium* spp. and *S. polyschides*) (*H. floresia*). These results were similar to those obtained by Gressler et al. [20] in *G. domingensis*, *G. birdiae*, *L. filiformis* and *L. Intricate* (ranged 22.5-38.4%) and by Aguilera-Morales et al. [7] in *Enteromorpha* sp. (36.8%). The ash content varies between species, geography and seasons, but a general feature of macroalgae are their high ash contents [10]. The Barbate Estuary is an area that promotes accumulation fluvial sediments rich in minerals [37] and, therefore, their accumulation in algae. However, accumulation in our samples depended on the species because *Halymenia floresia* (50.2%) contained the highest amount of ash.

Caloric values of the studied macroalgae were calculated from protein, fat and carbohydrate contents following a previously reported method [28]. As shown in Table 1, caloric values ranged between 106.5 and 210.6 kcal/100 g of dry sample and were determined by the protein content. *Halymenia floresia* had the highest value (210.6 kcal/100 g dry sample) because of its high lipid and carbohydrate contents, followed by *Codium* spp. (161.4 Kcal/100 g), in which the caloric value was primarily determined by the protein content. *Ulva* spp. and *Saccorhiza polyschides* had the lowest caloric values because of their low protein contents, but they were rich in fiber.

Free amino acid composition

Free amino acids (mg/g protein) in the four species are shown in Table 2.

Amino acids	*Ulva spp.*	*Codium spp.*	*H. floresia*	*S. polyschides*
Aspartic acid	340.3 ± 1.5	28.5 ± 1.4	n.d.	n.d.
Threonine[b]	19.1 ± 0.8	7.5 ± 1.2	8.3 ± 0.2	n.d.
Serine	n.d.	105.1 ± 2.1	n.d.	2.8 ± 1.1
Glutamic acid	8.8 ± 0.3	15.2 ± 1.4	8.1 ± 0.1	n.d.
Lysine[b]	5.5 ± 0.5	0.2 ± 0.0	10.1 ± 0.3	0.7 ± 0.3
Arginine	210.2 ± 0.9	56.8 ± 2.7	42.4 ± 0.9	10.8 ± 0.7
Glycine	1.50 ± 1.0	0.5 ± 0.9	1.4 ± 0.0	0.2 ± 0.0
Phenylalanine[b]	18.4 ± 0.1	29.8 ± 1.6	4.9 ± 0.0	15.5 ± 0.8
Tryptophan[b]	89.8 ± 1.1	41.9 ± 2.6	17.6 ± 0.3	4.3 ± 1.2
Methionine[b]	0.1 ± 0.0	n.d.	6.1 ± 0.1	n.d.
Cysteine	29.1 ± 0.9	8.3 ± 2.1	6.6 ± 0.1	0.4 ± 0.0
Isoleucine[b]	23.6 ± 0.5	11.1 ± 1.9	3.4 ± 0.0	6.6 ± 0.8
Leucine[b]	26.3 ± 0.5	3.1 ± 1.6	5.3 ± 0.0	10.9 ± 1.4
Histidine	14.7 ± 0.1	8.4 ± 0.9	4.9 ± 0.2	17.6 ± 0.6
Valine[b]	1.1 ± 0.0	3.4 ± 1.1	0.1 ± 0.0	5.4 ± 0.7

TAA	788.5 ± 7.2	319.8 ± 5.4	119.2 ± 4.8	75.2 ± 6.1
TEAA	198.6 ± 2.3	105.4 ± 4.2	55.8 ± 6.1	61.0 ± 3.7
TEAA/TAA	0.25	0.33	0.47	0.81

Table 2: Amino acid contents of seaweeds studied (mg/g protein)[a]. TAA, total amino acids; TEAA, total essential amino acids; n.d., not detectable. [a] All values given are means of three determinations ($\bar{X}$ ± SD); SD: standard deviation. [b] Essential amino acid (EAA).

Seventeen amino acids were detected including essential amino acids such as threonine, lysine, phenylalanine, tryptophan, methionine, cysteine, isoleucine, leucine, histidine and valine. Alanine and tyrosine were not detected in any of the macroalga samples studied. Nevertheless in other studies if they were detected [8,33].

Levels of the different amino acids varied according to species and ranged from 0.1 ± 0.0 to 340.3 ± 1.5 (mg/g protein) in *Ulva* spp., from 0.2 ± 0.0 to 105.1 ± 2.1 (mg/g protein) in *Codium* spp., from 0.1 ± 0.0 to 42.4 ± 0.9 (mg/g protein) in *H. floresia*, and from 0.2 ± 0.0 to 17.6 ± 0.6 (mg/g protein) in *S. polyschides*.

Total free amino acids (TAA) were highest in *Ulva* spp. (788.5 mg/g protein), followed by *Codium* spp. (319.8 mg/g protein), *Halymenia floresia* (119.2 mg/g protein) and *Saccorhiza polyschides* (75.2 mg/g protein). The total free amino acids in *Ulva* spp. were significantly higher than those reported by Wong & Cheung [33] in other species. However, *Codium* spp., *Halymenia floresia* and *Saccorhiza polyschides* had lower values than those reported by Wong & Cheung [33] (range from 7.1-19.0% of protein), Ortiz et al. [8] (range from 10.4-12.6% of protein) and Dawczynski et al. [9] (range from 7.5-31.4% of protein).

The total protein and free amino acid contents of the different species are shown in Tables 1 and 2, respectively. The free amino acid contents in *Ulva* spp. and *Codium* spp. were 5.7 g/kg and 5 g/kg of dry weight sample, respectively, and nitrogen in both species principally consisted of free amino acids (78.9% and 32.0%, respectively). However, in *Saccorhiza polyschides* and *Halymenia floresia*, the contents of nitrogen present as free amino acids were 1.1 g/kg and 0.5 g/kg of dry weight sample, respectively (12.0% and 7.5%, respectively).

All macroalgae studied showed pronounced differences between the amino acid profiles and high levels of essential amino acids (TEAA), especially in *Ulva* spp. (382.5 mg/g protein), which stands out with its high level of tryptophan, followed of cysteine, isoleucine and leucine. *Codium* spp. contained high amounts of tryptophan (41.9 mg/g protein) and phenylalanine (29.8 mg/g protein), while *H. floresia* stood out for its lysine and methionine contents (17.6 mg/g and 6.1 mg/g protein, respectively). *S. polyschides* had the highest histidine and valine levels (17.6 mg/g and 5.4 mg/g protein, respectively).

Although the higher TEAA contents were observed in *Ulva* spp. and *Codium* spp., the relative TEAA/TAA value was higher in *Halymenia floresia* (0.8) because of its low amount of non-essential amino acids.

In terms of the non-essential free amino acids, *Ulva* spp. had the most complete amino acid profile and the highest levels of aspartic acid and arginine (340.3 mg/g and 210.2 mg/g, respectively). *Codium* spp. stood out for its serine, arginine and aspartic and glutamic acid contents (105.1, 56.8, 28.5 and 15.2 mg/g, respectively).

According to several authors [38,39], high levels of aspartic and glutamic acids may be responsible for organoleptic characteristics in macroalgae, including the intense flavor and taste. Therefore, the high level of aspartic acid in *Ulva* spp. (340.3 mg/g protein) could be related to its intense flavor and characteristic aroma.

Fatty acid composition

The fatty acid compositions of the macroalgae under study are given in Table 3.

Fatty acids	Methyl ester (%)			
	Ulva spp.	*Codium spp.*	*H. floresia*	*S. polyschides*
C 14:0	n.d.	n.d.	n.d.	n.d.
C 16:0	0.6 ± 1.0	0.3 ± 0.1	1.2 ± 0.5	0.5 ± 0.1
C 17:0	5.4 ± 1.8	0.9 ± 0.9	5.4 ± 0.7	1.9 ± 0.7
C 18:0	6.4 ± 0.2	3.9 ± 0.4	5.2 ± 0.3	8.7 ± 0.4
C 20:0	10.7 ± 2.3	11.5 ± 7.6	5.6 ± 3.7	7.3 ± 0.6
C 22:0	n.d.	n.d.	1.4 ± 0.4	n.d.
C 24:0	0.9 ± 1.7	0.4 ± 0.7	n.d.	2.1 ± 1.8
C $14:1_{\omega5}$	44.6 ± 2.6	48.4 ± 7.7	32.6 ± 2.9	35.8 ± 0.4
C $16:1_{\omega7}$	0.9 ± 1.4	n.d.	4.4 ± 2.9	1.8 ± 0.8
C $18:1_{\omega7}$	n.d.	n.d.	n.d.	n.d.
C $20:1_{\omega9}$	n.d.	n.d.	n.d.	n.d.
C $24:1_{\omega9}$	n.d.	0.3 ± 0.1	n.d.	n.d.
C $18:2_{\omega6}$	14.7 ± 1.8	19.3 ± 1.0	28.6 ± 2.3	21.8 ± 0.9
C $20:2_{\omega6}$	n.d.	n.d.	n.d.	n.d.
C $18:3_{\omega3}$	n.d.	n.d.	n.d.	0.6 ± 0.2
C $20:3_{\omega9}$	5.9 ± 3.7	1.1 ± 0.4	2.9 ± 0.1	3.9 ± 1.0
C $20:4_{\omega6}$	n.d.	n.d.	n.d.	n.d.
C $20:5_{\omega3}$	3.6 ± 1.7	6.6 ± 0.0	1.1 ± 0.7	1.4 ± 0.2
C $22:6_{\omega3}$	n.d.	0.2 ± 0.4	n.d.	n.d.
SFA	24.0 ± 1.1	17.0 ± 2.3	18.8 ± 0.8	20.5 ± 2.5
MUFA	45.5 ± 1.6	48.7 ± 2.6	37.0 ± 3.3	37.6 ± 0.6
PUFA	24.2 ± 3.6	27.2 ± 0.1	32.6 ± 1.7	27.7 ± 0.1
$\Sigma_{(MUFA+PUFA)}/\Sigma_{SFA}$	2.9 ± 0.3	4.5 ± 0.9	3.7 ± 0.1	3.2 ± 0.2
$PUFAs_{\omega6}$	14.7 ± 1.8	19.3 ± 1.0	28.6 ± 2.3	21.8 ± 0.9
$PUFAs_{\omega3}$	3.6 ± 1.8	6.8 ± 0.4	1.1 ± 0.7	2.0 ± 1.0
$Ratio_{\omega6/\omega3}$	4.1 ± 0.1	2.8 ± 0.3	26.0 ± 0.4	10.9 ± 0.9

Table 3: Fatty acid composition of seaweeds studied (g/100 g of total fatty acid)[a]. SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; nd, not detectable. [a]All values given are means of three determinations ($\bar{X}$ ± SD); SD: standard deviation.

The saturated fatty acid content (SFA) was between 17.0% and 24.0%, and monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) ranged from 37.0-48.7% and 24.2-32.6%, respectively. SFA levels in these macroalgae were lower than those found by Johns et al. [12] in green (23.9%), brown (27.9%) and red (33.8%) macroalgae or by Ortiz et al. [8] in *U. lactuca* and *D. Antarctica* (33.7% and 36.3%, respectively).

However, levels of MUFAs ranging 37.0-48.7% were higher than those observed in other species [8,12], while PUFA levels ranging 24.2-32.6% were similar to those found by other authors [8,9]. All macroalgae under study had higher total levels of polyunsaturated fatty acids (MUFA+PUFA) than saturated fatty acids (SFA) due to the high MUFA levels, and the unsaturated/saturated ratios ($\Sigma_{(MUFA+PUFA)}/\Sigma_{SFA}$) ranged from 2.9 in *Ulva* spp. to 4.5 in *Codium* spp. The fatty acid content and proportion of saturated and unsaturated fatty acids vary with seasons, geography [14], weather conditions and genetic variability [8,39]. According to Khairy and El-Shafay, MUFA levels in three macroalgae were highest in autumn. In the present study, seaweeds were collected in autumn, which could explain the high levels of fatty acids observed and the high 6/3 ratio, especially in *H. floresia* (26.0) and *S. polyschides* (10.9).

The four macroalgae studied contained C14:15 (myristoleic acid) and the essential fatty acid C18:26 (linoleic acid) as the major monounsaturated and polyunsaturated fatty acids, respectively. Arachidic acid (C20:0), followed by stearic acid (C18:0), were the main saturated fatty acids found in the studied species, ranging from 5.6-11.5% and 3.9-8.7%, respectively. These fatty acids levels were higher than those found in other macroalgae [8,9,30], in which the major fatty acids were C16:0 (palmitic acid), C18:1 (oleic acid) and C20:5 (eicosapentaenoic acid, EPA). However, C18:3 (linolenic acid) was only found in *Saccorhiza polyschides*, C22:0 (Behenic acid) only in *Halymenia floresia* and C22:6 (docosahexaenoic acid, DHA) and C24:1 (nervonic acid) only in *Codium* spp., indicating that some fatty acids seem to be species-specific in algae.

Overall, the algal species in the present study had low amounts of SFAs, high levels of MUFAs and a relationship with the ω6/ω3 fatty acids >1, which could be related to the species, conditions in collection area and season.

Mineral content

As shown in Table 4, the macroalga samples were rich in macrominerals (i.e., Na, K, Ca and Mg), with values ranging from 9690-14500 mg/100 g dry weight. These results are similar to those obtained by Rupérez (2002) in different species of brown (*Fucus vesiculosus*, *Laminaria digitata*, *Undaria pinnatifida*) and red (*Chondrus crispus*, *Porphyra tenera*) macroalgae, with values ranging from 8097-17885 mg/100 g dry weight.

Mineral	*Ulva spp.*	*Codium spp.*	*H. floresia*	*S. polyschides*
Na	3670 ± 216.4	3960 ± 106.8	5540 ± 763.5	2650 ± 176.9
K	3770 ± 114.6	4500 ± 182.4	5960 ± 861.3	10170 ± 112.4
Ca	1030 ± 321.5	820 ± 253.6	730 ± 283.4	1120 ± 94.2
Mg	2040 ± 34.7	410 ± 18.8	900 ± 162.7	560 ± 32.4
Σ macro elements	10510 ± 171.8	9690 ± 140.4	13130 ± 517.7	14500 ± 103.9
Fe	36.28 ± 0.49	45.14 ± 0.82	14.34 ± 0.39	25.12 ± 1.52
Zn	1.91 ± 0.65	0.78 ± 0.14	2.11 ± 0.01	8.57 ± 0.39
Cu	0.71 ± 0.37	0.45 ± 0.25	0.62 ± 0.23	0.94 ± 0.4
Σ microelements	38.9 ± 0.50	46.4 ± 0.40	17.1 ± 0.21	34.6 ± 0.19

Table 4: Mineral composition and trace elements of seaweeds studied (mg/100 g dry weight)[a]. [a]All values given are means of three determinations ($\bar{X}$ ± SD); SD: standard deviation.

However, the concentrations of Na, K, Ca and Mg varied slightly depending on the algal species. Na and K were the most abundant elements in all macroalgae, ranging from 2650 to 5540 and 3770-10170 mg/g dry weight, respectively, but Na/K ratios were below 1.0 in all samples (0.26-0.97). These ratios are similar to those found by Rupérez [10] in brown and red macroalgae. According to this author, a low ratio Na/K is interesting from a nutritional point of view. Since the instability of mineral salts of sodium and potassium is one of the most frequent causes of hypertension appears [40].

Sodium (5540 mg/100 g dry weight) was the most abundant element in *Halymenia floresia*, followed by potassium (10170 mg/100 g dry weight) in *Saccorhiza polyschides* and magnesium (2040 mg/100 g dry weight) in *Ulva* spp., whereas Ca contents were similar (0.7-1.1 mg/100 g dry weight) in the four species.

Microelement concentrations (i.e., Fe, Zn and Cu) also varied with the species. *Halymenia floresia* had the lowest levels (17.1 mg/100 g dry weight), and *Codium* spp. had the highest (46.4 mg/100 g dry weight). According to Ryan et al., these variations could be due to morphological and physiological differences in macroalge and their affinities for different metals [41].

Among the microelements analyzed, Fe was highest in all samples, ranging from 14.3 to 45.1 mg/100 g dry weight. This amount conforms to that reported by Yaich et al. [30] for *Ulva lactuca* and some of the species analyzed by Subba et al. [16].

Ulva spp. and *Codium* spp. contained significant amounts of Fe (36.3 and 45.1 mg/100 g dry weight, respectively), whereas *Saccorhiza polyschides* had the highest contents of Zn (8.57 mg/100 g dry weight) and Cu (0.9 mg/100 g dry weight). The ability of the four macroalga species to concentrate metals varied according to both species and type of metal accumulated. Similar results were obtained by Besada et al. [17] and Ryan et al., [41] that highlighted the complexity of the inter-relationships between metabolism, growth rate and metal uptake [17].

The high concentrations of macro and microminerals in *Ulva* spp., *Codium* spp., *Halymenia floresia* and *Saccorhiza polyschides* could be explained by the consumption and accumulation of nutritive elements in the collection medium. Studies by Martínez-Velásco et al. [36] showed that the mouth of the Barbate River is rich in minerals, and the presence of Zn and Cu in both water and sediments was demonstrated by Gutiérrez-Mas et al. [37]. There are wealth of trace elements associated with fluvial mineral accumulation in the Barbate River and existing wetlands in the area, likely resulting in the richness of minerals found in macroalgae sampled.

Conclusion

The moisture content of fresh macroalgae (84.3-98.6%) could be related to the morphology and structure of the species. Macroalgae with tubular structures such as *Codium* spp. had the highest moisture content, while the other seaweeds, which had laminar structures, had lower contents. Protein contents exhibited high variability (7.2-15.6%), which could be related to the species and their ability to assimilate nitrogen. Lipid levels were greater than 7% in all species studied, except *Ulva* spp. These high contents could be related to the particular conditions of the collection area (i.e., salinity, water temperature and nitrate content). Carbohydrate and fiber concentrations were lower than in samples reported in other studies because of the high nitrogen content in our collection area. This area is characterized by fluvial accumulation of sediments, and therefore, the ash content and macro and microelements were especially high. Na, K, Fe, Zn and Cu concentrations varied between species, which could be due to morphological and physiological differences in the macroalgae and their affinities for different metals [41-44].

All macroalgae studied contained high levels of essential amino acids (75.2-788.5 mg/g protein) and different amino acid profiles.

Levels of MUFAs in these macroalgae (37.0-48.7%) were higher than those observed in other species. The main saturated fatty acids were arachidic acid (5.6-11.5%) and stearic acid (3.9-8.7%).

These results highlight the nutritional potential of these macroalgae as food, either as fresh produce or as a processed food ingredient, and development of seaweed farming in this area should be evaluated.

Acknowledgment

We thank Innova Vegetalia del Mar S.L. for supplying the fresh macroalgae samples.

References

1. Skibola C (2004) The effect of Fucus vesiculosus, an edible brown seaweed, upon menstrual cycle length and hormonal status in three pre-menopausal women: a case report. Complement Altern Med 4: 10-17.
2. Teas J (1981) The consumption of seaweed as a protective factor in the etiology of breast cancer. Medical Hypotheses 7: 601-613.
3. (2014) FAO.
4. Gupta S, Abu-Ghannam N (2011) Recent development in the application of seaweeds or seaweed extracts as a means for enhancing the safety and quality attributes of foods. Innov Food Sci. & Emerg Technol 12: 600-609.
5. Kadam SU, Prabhasankar P (2010) Marine foods as functional ingredients in bakery and pasta products. Food Res Int 1975-1980.
6. Devi KP, Suganthy N, Kesika P, Pandian SK (2008) Bioprotective properties of seaweeds: In vitro evaluation of antioxidant activity and antimicrobial activity against food borne bacteria in relation to polyphenolic content. Complement Altern Med KP 8: 38.
7. Aguilera-Morales M, Casas-Valdez M, Carrillo-Domínguez S, González-Acosta B, Pérez-Gil F (2005) Chemical composition and microbiological assays of marine algae Enteromorpha spp. as a potential food source. J Food Compos Anal 18: 79-88.
8. Ortiz J, Romero N, Robert P, Aray J, Lopez-Hernández J, et al. (2006) Dietary fiber, amino acid, fatty acid and tocopherol contents of the edible seaweeds Ulva lactuca and Durvillaea antarctica. Food Chem 99: 98-104.
9. Dawczynski C, Schubert R, Jahreis G (2007) Amino acids, fatty acids and dietary fibre in edible seaweed products. Food Chem 103: 891-899.
10. Gómez-Ordóñez E, Jiménez-Escrig A, Rupérez P (2010) Dietary fibre and physicochemical properties of several edible seaweeds from the northwestern Spanish coast. Food Res Int 43: 2289-2294.
11. Norziah MH, Ching CY (2000) Nutritional composition of edible seaweed Gracilaria changgi. Food Chem 68: 69-76.
12. Johns RB, Nichols PD, Perry GJ (1979) Fatty acid composition of ten marine algae from Australian waters. Phytochem 18: 799-802.
13. Sánchez-Machado DI, López-Cervantes J, López-Hernández J, Paseiro-Losada P (2004) Fatty acids, total lipid, protein and ash contents of processed edible seaweeds. Food Chem 85: 439-444.
14. Denis C, Morançais M, Li M, Deniaud E, Gaudin P, et al. (2010) Study of the chemical composition of edible red macroalgae Grateloupia turuturu from Brittany (France). Food Chem 119: 913-917.
15. Rúperez P (2002) Mineral content of edible marine seaweeds. Food Chem 79: 23-26.
16. Subba PV, Mantri VA, Ganesan K (2007) Mineral composition of edible seaweed Porphyra vietnemensis. Food Chem 102: 215-218.
17. Besada V, Andrade JM, Schultze F, González JJ (2009) Heavy metals in edible seaweeds commercialised for human consumption. J Marine Syst 75: 305-313.
18. Lartigue L, Sherman T (2002) Field assays for measuring nitrate reductase activity in Enteromorpha sp. (Chloropphyceae), Ulva spp. (Chlorophyceae), and Gelidium sp. (Rhodophyceae). J Phycol 38: 971-982.
19. Marinho-Soriano E, Fonseca PC, Carneiro MA, Moreira WS (2006) Seasonal variation in the chemical composition of two tropical seaweeds. Bioresour Technol 97: 2402-2406.
20. Gressler V, Yokoya NS, Toyota MT, Colepicolo P, Mancini J, et al. (2010) Lipid, fatty acid, protein, amino acid and ash contents in four Brazilian red algae species. Food Chem 120: 585-590.
21. Sobrino JA, Li Z, Stoll MP, Becker F (1994) Improvements in the split-window technique for land surface temperature determination. IEEE Trans Geosci Remote Sens 32: 243-253.
22. Sobrino I, Silva L, Bellido JM, Ramos F (2002) Rainfall, river discharges and sea temperature as factors affecting abundance of two coastal benthic cephalopod species in the gulf of Cadiz (SW Spain). Bull Mar Sci 71: 851-865.
23. Torres MA, Coll M, Heymans JJ, Christensen V, Sobrino I (2013) Food-web structure of and fishing impacts on the Gulf of Cadiz ecosystem (South-western Spain). Ecological Modelling 265: 26-44.
24. Anfuso E, Ponce R, González-Castro C, Forja JM (2010) Coupling between the thermohaline, chemical and biological fields during summer 2006 in the northeast continental shelf on the Gulf of Cadiz (SW Iberian Peninsula). Sci Mar 74: 47-56.
25. AOAC (2000) Official methods of analysis of AOAC international, (17th edn.) Gaithersburg, USA.
26. International Union of Pure and Applied Chemistry (IUPAC) (1992) In: Paquot C, Hautfenne A (eds) Standard method for the analysis of oils, fats and derivatives (7th edn.) London: Blackwell Scientific Publications.
27. AFNOR (1996) Dosage des minéraux-méthodes par spectrométrie d'émission de flamme. NF-T-90-019. Association Française de Normalisation (AFNOR), La Défense, France.
28. Livesey G, Buss D, Coussement P, Edwards DG, Howlett J, et al. (2000) Suitability of traditional energy values for novel foods and food ingredients. Food Control 11: 249-289.
29. Pereira L, Armado AM, Critchley AT, Van de Velde F, Ribeiro-Claro PJA (2009) Identification of selected seaweed polysaccharides (phycocolloids) by vibrational spectroscopy (FTIR-ATR and FT-Raman). Food Hydrocoll 23: 1903-1909.
30. Yaich H, Garna H, Besbes S, Paquot M, Blecker C, et al. (2011) Chemical composition and functional properties of Ulva lactuca seaweed collected in Tunisia. Food Chem 128: 895-901.
31. McHugh DJ (2003) A guide to the seaweed industry. FAO Fish Tech Pap 441: 105.
32. Navarro-Angulo L, Robledo D (1999) Effects of nitrogen source, N: P ratio and N-pulse concentration and frequency on the growth of Gracilaria cornea (Gracilariales, Rhodophyta) in culture. Hydrobiologia 399: 315-320.

33. Wong KH, Cheung PCK (2000) Nutritional evaluation of some subtropical red and green seaweed. Part I- proximate composition, amino acid profiles and some physico-chemical properties. Food Chem 71: 475-482.
34. Floreto EAT, Teshima S, Ishikawa M (1996) Effects of nitrogen and phosphorus on the growth and fatty acid composition of Ulva pertusa Kjellman (Chlorophyta). Bot Mar 39: 69-74.
35. Floreto EAT, Teshima S (1998) The fatty acid composition of seaweeds exposed to different levels of light intensity and salinity. Bot Mar 41: 467-481.
36. Martínez-Velasco C, Forja JM, Gómez-Parra A (1999) Hydrochemistry characterisation of protected marine ecosystems in Cadiz. Bol Inst Esp Oceanogr 15: 451-456.
37. Gutiérrez-Mas JM, Moral JP, Sánchez A, Domínguez S, Muñoz-Pérez JJ (2003) Multicycle sediments on the continental shelf of Cadiz (SW Spain). Estuar. Coast. Shelf Sci 57: 667-677.
38. Mabeau S, Fleurence J (1993) Seaweed in food products: Biochemical and nutritional aspects. Trends Food Sci Technol 4: 103-107.
39. Macler BA (1986) Regulation of carbon flow by nitrogen and light in the red alga Gelidium coulteri. Plant Physiol 82: 136-141.
40. Geleijnse, Kok (2003) Grobbee, Blood pressure response to changes in sodium and potassium intake: a metaregression analysis of randomised trials. J Hum Hypertens 17: 471-480.
41. Ryan S, McLoughlin P, O'Donovan O (2012) A comprehensive study of metal distribution in three main classes of seaweed. Environ Pollut 167: 171-177.
42. Peinado I, Girón J, Koutsidis G, Ames JM (2014) Chemical composition, antioxidant activity and sensory evaluation of five different species of Brown edible seaweeds. Food Res Int 66: 36-44.
43. Fleurence J (1990) "Seaweeds proteins: Biochemical nutritional aspects and potential uses". Trends Food Sci & Tech 10: 25-28.
44. Pereira V, Pontes M, Câmara JS, Marques JC (2008) Simultaneous analysis of free amino acids and biogenic amines in honey and wine samples using in loop orthophthalaldeyde derivatization procedure. J Chromatogr A 1189: 435-443.