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RESEARCH ARTICLE

Chemical composition, nutritional and antioxidant properties of the red edible seaweed *Porphyra columbina*

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Abstract

Proximate composition, fatty acids and amino acid profiles and nutritional (chemical score, protein digestibility, PDCAAS and mineral dialyzability) and antioxidant properties (TEAC, DPPH and power reduction) from *Porphyra columbina* were evaluated. Total dietary fiber (48.02 ± 1.13 g/100 g dry weight) and protein (24.61 ± 0.21 g/100 g dry weight) were the two most abundant components in this seaweed. The main saturated and unsaturated fatty acids were C16:0 and C20:5 ($n-3$), respectively. The limiting amino acid was tryptophan with a chemical score of 57%. Protein digestibility was 74.33 ± 3.0%. *Porphyra columbina* has high mineral content with good Na/K relationship and medium value of potential mineral accessibility (P, Ca and Zn dializability: 18.75 ± 0.01, 17.62 ± 0.16 and 16.70 ± 0.44, respectively). The highest antioxidant properties were obtained with an acetone/water extraction system. This work provides important information about chemical composition and nutraceutical new properties of *P. columbina*.

Keywords

Mineral bioaccessibility, nutraceutical food, protein quality, radical scavenging, red seaweeds

History

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Introduction

The use of seaweeds as animal and human food is a very old and widespread practice, especially in Japan and South Eastern Asia, where macroalgae represent an important economic resource and are largely used in human nutrition (Pérez et al., 2007). Seaweeds can contain high amounts of carbohydrates, proteins and minerals (Rupérez, 2002). Although they have a low lipid caloric, seaweeds can be a source of essential fatty acids such as eicosapentaenoic acid, $C_{20:5}$ (n−3). On the other hand, as seaweed polysaccharides cannot be entirely digested by human intestinal enzymes, they are considered to be a source of dietary fiber. However, seaweed dietary fiber differs in composition, chemical structure, physicochemical properties and biological effects from those of land plants (Urbano & Goñi, 2002). Also, they are a rich source of minerals, especially macro and micronutrients necessary for human nutrition. The mineral fraction of some seaweeds accounts for up to 40% of dry matter and in some cases even higher percentages than that of land plants and animal products are recorded. Evaluation of minerals in any edible seaweed is important from both the nutritional and the toxicological point of view (Rao et al., 2007). However, very little is known about mineral dialyzability as an estimation of potential *in vitro* bioaccessibility.

The genus *Porphyra*, traditionally known as nori in Japan, kim in Korea and zicai in China, is a popular food due to its rich content of protein, vitamins, minerals and dietary fiber (Rao et al., 2007). *Porphyra* cultivation in Japan is a great aquaculture industry with an average production of 400 000 tons (wet wt) per year. The genus is represented by nearly 133 species from all over the world. Among the red algae found on hard substratum in Patagonia Argentina coasts, *Porphyra columbina* has significant economic interest (Pérez et al., 2007). However, information on the chemical composition of *P. columbina* is very scarce. Moreover, the nutritional properties of *P. columbina* are not completely known yet, and there are no available data about chemical score, protein digestibility and potential mineral availability. Finally, the antioxidant activity has been studied only in fractions of this alga, but never in the raw seaweed. The aim of this study was to evaluate proximate composition, fatty acids and amino acid profiles, as well as nutritional (chemical score, protein digestibility, PDCAAS and mineral dialyzability) and antioxidant properties (TEAC, DPPH and power reduction) from *P. columbina.*

Materials and methods

Sampling site

Phorphyra columbina was collected from the hard substratum in Punta Maqueda (46°00′S, 67°34′W) within San Jorge Gulf. Punta Maqueda, a pristine beach far away from anthropogenic activities, is located 30 km to the south of Comodoro Rivadavia, Argentina.

Sampling and sample preparation

One kilogram of *P. columbina* specimen was hand-picked at Punta Maqueda. In order to obtain homogeneous samples, representative of the entire algal population living in the zone under study.

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The collection was carried out from August to October 2010. Samples were taken to the laboratory at 4 °C inside plastic bags. To remove adherent seawater, sediment, organic debris, macro fauna and epibiota, they were scraped and rinsed with distilled water. The samples were dried at environment temperature (22–25 °C), ground to obtain a powder with a particle size lower than 1 mm, using a laboratory hammer mill (Retsch, Haan – Germany). Then samples were passed through a 0.85 mm mesh sieve and stored at 4 °C in plastic bags until analysis.

Proximate composition

Protein, ash, crude lipid, phytic acid and moisture of *P. columbina* were determined using A.O.A.C. (1995) procedures. Total, soluble and insoluble dietary fiber were determined according to Lahaye (1991) method modified by Prosky et al. (1992).

Fatty acid profile

Fatty acids were determined by gas chromatographic quantification of their methyl esters prepared according to Cert et al. (2000), with slight modification. Algae were dispersed in water (5%, w/w) and homogenized. The mixture was extracted three times with 1 mL of chloroform:methanol (2:1). The lower phase was concentrated to dryness and dissolved in 2 mL of heptane and treated with 0.2 mL methanolic KOH (2 N). Then, the mixture was stirred for 30 min at room temperature. The heptane phase was concentrated to 0.1 mL and analyzed by gas chromatography in a Hewlett Packard 5890 Series II with a HP-23 column (60 m × 0.25 mm × 0.25 microm). Oven temperature: 180 °C Injector temperature: 225 °C Detector temperature: 250 °C.

Amino acid profile and protein quality

Amino acid analysis

Samples (2 mg) were hydrolyzed with 4 ml of 6 N HCl. The solutions were sealed in tubes under nitrogen and incubated in an oven at 110 °C for 24 h. Amino acids were determined after derivatization with diethyl ethoxymethylenemalonate by high-performance liquid chromatography (HPLC), according to the method of Alaiz et al. (1992), using D,L-α-aminobutyric acid as internal standard. The HPLC system consisted of a Model 600E multi-system with a 484 UV–vis detector (Waters, Milford, MA) equipped with a 300 × 3.9 mm i.d. reversed-phase column (Novapack C18, 4 m; Waters). A binary gradient was used for elution with a flow of 0.9 ml/min. The solvents used were (A) sodium acetate (25 mM) containing sodium azide (0.02% w/v) pH 6.0 and (B) acetonitrile. Elution was as follows: time 0.0–3.0 min, linear gradient from A/B (91:9) to A/B (86/14); 3.0–13.0 min, elution with A/B (86/14); 13.0–30.0 min, linear gradient from A/B (86:14) to A/B (69/31); 30.0–35.0 min, elution with A/B (69:31). Eluted amino acids are detected at 280 nm. The column was maintained at 18 °C.

Tryptophan was determined by HPLC-RP chromatography after basic hydrolysis according to Yust et al. (2004).

Chemical score (CS) was calculated by the method of FAO/WHO as shown below:

$$\mathrm{CS} = [(\text{mg of EAA/g protein})/(\text{mg of EAA/g FAO requirement protein pattern})]$$

where *EAA* is each essential amino acid and *FAO requirement protein pattern* is amino acid scoring pattern for use in preschool children (FAO/WHO/UNU, 1985).

Protein digestibility corrected amino acid score (PDCAAS) (FAO/WHO/UNU, 1985) was calculated as:

$$\mathrm{PDCAAS} = \mathrm{CS} \times \text{protein digestibility (PD)}.$$

Determination of mineral dialyzability

The method developed by Miller et al. (1981), modified by Drago et al. (2005), was used. This method measures mineral dialyzability under controlled pH conditions after a digestion-simulating physiological process. In order to adjust the pH during the digestion and dialysis stage, and to obtain an uniform final pH in digest/dialysate systems, a 0.5 mol/L PIPES buffer with pH varying according to the matrix was used. Dialyzability of mineral was calculated as the amount of dialysate mineral expressed as a percentage of total mineral content in the sample:

$$\mathrm{MD}(\%) = (\text{mg } M_D/\text{mg } M_S) \times 100.$$

where, MD (%) is the percentage of mineral dialysated, mg M_D is milligrams of mineral dialysated, and mg M_S is milligrams of mineral of the sample. Dialyzability was used as an indicator of potential bioaccessibility of phosphorous, calcium, iron and zinc and was named as follows: PD (%), CaD (%), FeD (%) and ZnD (%), respectively.

Total mineral content in the sample was measured by atomic absorption spectroscopy after dry mineralization. Ash was removed with 20% HCl (v/v). An atomic absorption spectrometer (IL 551 device; Instrumentation Laboratory, Norwood, MA) was used.

In vitro protein digestibility

The *in vitro* protein digestibility of algae was measured using the method described by Rudloff & Lönnerdal (1992). The protein digestibility was calculated as:

$$\mathrm{PD}(\%) = [(\mathrm{NPN} - \mathrm{NPNi})/(\mathrm{N} - \mathrm{NPNi})] \times 100.$$

where, PD (%) is the digestibility of protein samples, NPN is non-protein nitrogen soluble in 20% of trichloroacetic acid after digestion with pepsin and pancreatin enzymes, N is total nitrogen of sample and NPNi is initial non-protein nitrogen soluble in 20% of trichloroacetic acid from sample. The nitrogen content (NPN, N and NPNi) was determinate using Kjeldhal method.

Total phenolic content (TPC)

Total phenolic content (TPC) from *P. columbina* was determined according to Cian et al. (2013). For this, three different extraction systems were used: methanol (100), acetone:water (80:20) and acetone:water:acetic acid (80:15:5). The samples were dispersed at 10% (w/w) in each system. The extracts were stirred for 30 min and then centrifuged at 8000 × *g*. TPC from supernatant was quantified using Folin–Ciocalteu reagent. A standard curve with serial gallic acid solutions was used for calibration. Results were expressed as mg gallic acid g^{-1} of dry seaweed.

Antioxidant properties

Antioxidant properties were determined from the supernatant of different extraction systems (methanol, acetone:water and acetone:water:acetic acid).

Trolox equivalent antioxidant capacity (TEAC)

To estimate the antioxidant capacity, $ABTS^{\bullet}+$ radical cation decolorization assay according to Cian et al. (2012) was used. To estimate the TEAC, a concentration–response curve for the absorbance at 734 nm for $ABTS^{\bullet}+$ as a function of concentration

of standard Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) solution (0–2.5 mmol L^{-1}) in 0.01 mmol L^{-1} (PBS, pH 7.4) was performed. The absorbance reading was taken at 6 min after initial mixing.

DPPH radical scavenging activity assay

DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity was measured according to the method of Blois (1958). An aliquot of 25 μL of extract was mixed with 175 μL of methanol solution containing 0.08 mmol L^{-1} DPPH radical. The mixture was allowed to stand for 30 min in the dark, and the absorbance was monitored at 517 nm. The difference between the blank and the sample was used for calculating the scavenging activity as percentage of inhibition.

Reducing power activity assay

The reducing power activity was determined according to the method of Oyaizu (1986). The extracts were mixed with 0.2 mL of 0.2 mol L^{-1} phosphate buffers (pH 6.6) and 0.2 mL of 1% (w/v) potassium ferricyanide. The mixture was incubated at 50 °C for 20 min. Then, 0.5 mL of 10% (w/v) trichloroacetic acid was added and 0.2 mL of the mixture was mixed with 0.2 mL of distilled water and 40 μL of 0.1% (w/v) ferric chloride. After standing at room temperature for 10 min, the absorbance was measured at 700 nm. An equivalent volume of distilled water instead of the sample was used for the blank.

Statistical analysis

All analyses were performed in triplicate from each sample ($n = 7$) and results are expressed as mean ± SD. The data were analyzed by one-way analysis of variance, using the software Statgraphics Plus 3.0 (Warrentong, VA). Least significant difference test was used to determine statistical differences between samples. The significance was established at $p < 0.05$.

Results and discussion

Table 1 shows proximate composition of red edible seaweeds *P. columbina*. It is well known that red seaweeds have high protein level (Galland-Irmouli et al., 1999). In this sense, the crude protein content of genus *Porphyra* is comparable with that of high-protein plant foods such as soybean (Norziah & Ching, 2000). *Porphyra columbina* protein content was similar to that obtained by Sánchez-Machado et al. (2004) for *Genus Porphyra.* It is important to note that *P. columbina* was collected in the southern hemisphere spring (2010) and the protein content of seaweeds varies not only between species (Fleurence, 1999) but also between seasonal periods (Mishra et al., 1993).

Table 2 shows amino acids profile of *P. columbina*. For most seaweed, aspartic and glutamic acids together constitute a large part of the amino acid fraction (Fleurence, 1999). In this work, this figure amounted to 22.7 g/100 g of proteins, thus constituting the most abundant amino acids. Similar results were obtained for other red seaweeds such as *Porphyra acanthophora* (27 g/100 g of proteins), *Hypnea charoides* (20.8 g/100 g of proteins), *Palmaria palmata* (24.8 g/100 g of proteins; Galland-Irmouli et al., 1999) and *Laurencia* species (15.5–27.4 g/100 g of proteins; Lewis, 1974). On the other hand, Munda (1977) reported that these two amino acids can represent between 22 and 44 g/100 g of proteins. The predominance of acidic amino acids over basic amino acids is typical of red seaweeds (Galland-Irmouli et al., 1999), their high levels being responsible for seaweed special flavor and taste (Mabeau et al., 1992).

The essential amino acids leucine, lysine, threonine, valine, phenylalanine + tyrosine and methionine + cysteine were present in relatively high levels. However, isoleucine, histidine and tryptophan showed low amounts. The chemical score (CS) was 57%, according to FAO/WHO/UNU (1985) requirement pattern, and the limiting amino acid was tryptophan. A relatively good ratio of essential to non-essential amino acids (0.65 g essential amino acids/g non-essential amino acids) was observed. This result was similar to that found by Galland-Irmouli et al. (1999) for *Palmaria palmata* (0.66 g essential amino acids/g non-essential amino acids) and higher than the value reported by Fleurence (1999) for *Porphyra tenera* (0.57 g essential amino acids/g non-essential amino acids). On the other hand, *P. columbina* also had free amino acids, alanine being the most abundant (Table 2).

In vitro protein digestibility (PD) for *P. columbina* was 74.33 ± 3.0%. This value agrees with that reported by Mišurcová et al. (2010) for *Palmaria palmata* and is similar to the protein digestibility of plant proteins, but is lower than that of animal proteins. This may be due to a high fiber content of *P. columbina* (Table 1), which could block the access of digestive enzymes and also decrease the activity of proteolytic enzymes, thus producing a decrease in protein digestibility (Urbano & Goñi, 2002).

Porphyra columbina presented a PDCAAS value of 0.43 ± 0.03, which was equal or higher than that of various legumes (Henley & Kuster, 1994). The PDCAAS, which is based on FAO recommendations of amino acids requirements (FAO/WHO/UNU, 1985) and PD, is nowadays the most recommended

Table 1. Proximate composition of *P. columbina*.

Composition	*P. columbina* (g/100 g dw)
Crude protein ($N \times 6.25$)	24.61 ± 0.21
Ash	6.46 ± 0.09
Total dietary fiber	48.02 ± 1.13
Insoluble dietary fiber	26.60 ± 2.20
Soluble dietary fiber	21.42 ± 5.12
Crude lipid	0.25 ± 0.06
Moisture	12.79 ± 0.07

Means ± SD ($n = 7$), dw (dry weight of seaweed).

Table 2. Amino acid profile of *P. columbina*.

Amino acids	Total amino acids (g 100 g^{-1} protein)	Free amino acids (g 100 g^{-1} protein)	FAO/WHO/UNU (1985) requirement pattern[a]
Asp	12.22 ± 0.20	0.29 ± 0.01	
Glu	10.50 ± 0.56	0.19 ± 0.01	
Ser	6.16 ± 0.09		
His	1.26 ± 0.08		1.90
Gly	8.87 ± 0.14		
Thr	5.91 ± 0.13	0.02 ± 0.00	3.40
Arg	6.19 ± 0.16		
Ala	12.54 ± 0.29	0.47 ± 0.02	
Pro	3.96 ± 0.41		
Tyr	2.55 ± 0.05		6.30
Phe	3.70 ± 0.06		
Val	5.85 ± 0.11		3.50
Met	1.68 ± 0.07		2.50
Cys	1.89 ± 0.03		
Ile	2.71 ± 0.05		2.80
Trp	0.63 ± 0.01		1.10
Leu	7.38 ± 0.11		6.60
Lys	6.01 ± 0.10		5.80

[a]*FAO requirement protein pattern* is amino acid scoring pattern for use in children ≥ 5 year of age (FAO/WHO/UNU, 1985).
Means ± SD ($n = 7$).

Table 3. Relative fatty acid content of *P. columbina*.

Fatty acids	(%)
C12:0	0.42 ± 0.03
C14:0	1.27 ± 0.09
C16:0	21.55 ± 0.70
C18:0	3.55 ± 0.15
Sum C16:1	3.13 ± 0.27
Sum C18:1	8.50 ± 0.54
C20:1	2.13 ± 0.01
C18:2 n−6	3.41 ± 0.09
C20:2 n−6	0.79 ± 0.06
Sum C18:3	11.40 ± 1.15
C20:4 n−6	15.52 ± 0.08
C20:5 n−3	28.36 ± 0.33

Means ± SD ($n = 7$).

Table 4. Major minerals, trace elements and phosphorus, calcium, iron and zinc dializability from *P. columbina*.

Mineral	Mineral content (mg 100 g^{-1} dw)	Dializability (%)
Na	414.22 ± 8.96	ND
K	1444.17 ± 56.30	ND
P	379.90 ± 7.90	18.75 ± 0.01
Ca	443.70 ± 6.64	17.62 ± 0.16
Mg	491.53 ± 3.44	ND
Fe	22.00 ± 0.40	2.63 ± 0.02
Zn	1.46 ± 0.09	16.70 ± 0.44
Cu	0.51 ± 0.05	ND

ND, not determined. Means ± SD ($n = 7$), dw (dry weight of seaweed).

theoretical parameter for evaluating the nutritional quality of food protein. It is better than other methods since it determines the quality of a protein based on the amino acid requirements of a 2- to 5-year-old child, adjusted for digestibility. The highest PDCAAS value for a given protein is 1.0. This protein will provide 100% of the indispensable amino acids recommended by FAO (FAO/WHO/UNU, 1985).

According to previous research, the total lipid content of seaweeds is always less than 4 g/100 g dry weight of seaweed (Herbreteau et al., 1997) and this is the case for *P. columbina* (Table 1). Its lipid content was lower than that reported by Sánchez-Machado et al. (2004) for *Genus Porphyra* (1.03 ± 0.4 g/100 g dry weight of seaweed), which may be due to differences in capability of accumulating lipids and type of seaweed species (Gressler et al., 2010).

Table 3 shows relative fatty acid contents of *P. columbina.* The main fatty acids in the studied *P. columbina* ranged from C14:0 to C20:0 with a predominance of unsaturated fatty acids of the series C20 (C20:4 (*n*–6) and C20:5 (*n*–3)). Among saturated fatty acids, C16:0 was the most abundant. This result agrees with that reported by Gressler et al. (2010) for four red seaweeds (*Laurencia filiformis*, *L. intricata*, *Gracilaria domingensis* and *G. birdiae*). According to the literature, the genus *Porphyra* has the highest amount of saturated fatty acid as palmitic acid (Colombo et al., 2006; Sánchez-Machado et al., 2004), though these authors found higher values as compared with our results. C18:0 was the second most abundant saturated fatty acid, although it was in a much smaller proportion compared to C16:0. Small quantities of C12:0 and C14:0 were observed.

Among unsaturated fatty acids, the most abundant in decreasing order were C20:5 (*n*–3), C20:4 (*n*–6), Sum of C18:3 and Sum of C18:1. Similar results were obtained by Colombo et al. (2006) for *Porphyra perforata*. In this sense, for genus *Porphyra* the eicosapentaenoic acid content was very similar to that found in our work (24.05%; Sánchez-Machado et al., 2004). It is important to point out that eicosapentaenoic acid derived from C18:3 has antithrombotic, hypolipidemic, and antiinflammatory effects, being therefore considered beneficial to health (Norziah & Ching, 2000).

Total dietary fiber was the most abundant component in this seaweed (Table 1). This value was higher than those found in most land vegetables. This observation was in accordance with previous results found for red seaweeds (Wong & Cheung, 2000). Furthermore, total dietary fiber of *P. columbina* was comparable to that of *Phorphyra tenera* (33.6–50 g/100 g dry weight of seaweed) (Rupérez & Saura-Calixto, 2001). Seaweeds contain large amounts of dietary fiber which are particularly rich in the soluble fraction (Darcy-Vrillon, 1993). In fact, the ratio insoluble/soluble fiber was 1.2, lower than in most vegetable foods. The importance of both types of fiber was reviewed by Brownlee (2011). The chemical nature and physico-chemical properties of some common seaweed dietary fibers such as alginates, carrageenans and agars are quite well -known (Al-Alawi et al., 2011), but most seaweed dietary fibers have still not received much attention particularly for their effect on protein digestibility and mineral accessibility.

Ash content of *P. columbina* (Table 1) was considerably lower than the value reported by most authors for red algae. Gressler et al. (2010) found for four Brazilian red seaweeds (*Laurencia filiformis*, *Laurencia intricata*, *Gracilaria domingensis* and *birdiae*) an ash content ranging from 22.5 to 38.4 g/100 g dry weight of seaweed. Rupérez (2002) also reported an ash value of 20.59 ± 0.16 g/100 g dry weight of seaweed for *Phorphyra tenera*. This reduction in ash content may be explained considering that in this work the seaweed was washed with distilled water prior to the experiences. It is appropriate to note that the value found for *P. columbina* is similar to that reported for land vegetables with an average value of 5–10 g/100 g dry weight of seaweed (USDA, 2001).

Table 4 shows mineral content and P, Ca, Fe and Zn dialyzability from *P. columbina.* Regarding minerals, most algae show a high content of Na. In our work, however, the sodium content of *P. columbina* was almost 10 times lower than that reported in the literature (Table 4). For example, Na content for *P. tenera* was 3627 ± 115 mg/100 g dry weight of seaweed (Rupérez, 2002). This reduction in Na content in our work may be attributed to having washed *P. columbina* with distilled water prior to the analysis, as was explained before. K content was higher than Na content and Na/K ration was less than 0.3 (0.29), which is interesting from the nutritional point of view, since the intake of sodium chloride and diets with a high Na/K ratio have been related to the incidence of hypertension. Na/K ratios in common salt foods such as olives and sausages, for instance, are 43.63 and 4.89, respectively (Ortega-Calvo et al., 1993).

P, Ca and Mg contents were similar to those reported by Pérez et al. (2007) for the same seaweed (Table 4). The Ca/P ratio was 1.17, this value being slightly lower than that found by Pérez et al. (2007) for *P. columbina* (1.27). Also, Ca and Mg contents were similar to those reported earlier by Rupérez (2002) for *P. tenera* and followed the same pattern reported for red seaweeds (Mg > Ca) (McDermid & Stuercke, 2003).

Fe content obtained in this study is similar to that reported by Pérez et al. (2007) for *P. columbina* (21.3 mg/100 g dry weight of seaweed) (Table 4). Our value was higher than that found by Rupérez (2002) for *P. tenera* (10.3 mg/100 g dry weight of seaweed), but less than the one reported by Rao et al. (2007) for *Porphyra vietnamensis* (33 mg/100 g dry weight of seaweed). Zn and Cu content was similar to that found by Muse et al. (1999) for this same seaweed. Additionally, Rao et al. (2007) reported a

Cu content very similar to that found in our work (0.54 mg/100 g dry weight of seaweed) for *P. vietnamensis*. In our work, Zn content was below the maximum amount allowed in macroalgae for human consumption in Japan and France: 1.5 and 10 mg/100 g, respectively (Indegaard & Minsaas, 1991).

The P, Ca, Fe and Zn dialyzability from *P. columbina,* the results are similar to those reported for other foods. In this sense, FeD was 4.59% for wheat pasta (Dyner et al., 2007) and 2% for lettuce (Yang & Tsou, 2006); ZnD was 12.72% for wheat pasta (Dyner et al., 2007) and 30% for corn flakes (Cagnasso et al., 2010); CaD was 41.31% for wheat pasta (Dyner et al., 2007) and 11.1% for soybeans (Kamchan et al., 2004).

Algae are known to be rich in a wide variety of minerals, but they are also rich in other components, such as dietary fiber and polyphenolyc compounds, which could affect mineral bioavailability (Urbano & Goñi, 2002). It should be noted that phytic acid is also considered an anti-nutritional factor related to the decrease in the availability of minerals (Szkudelski, 1997). However, algae have very low content of this compound (Garcíaa-Casal et al., 2007). In this sense, the value found in *P. columbina* was 0.386 ± 0.04 g $100\,g^{-1}$ dry weight of seaweed.

Figure 1(A) shows TPC of different extracts from *P. columbina*. The lowest value of TPC was obtained with methanolic extraction system and the highest value with the acetone/water and acetone/water/acetic acid extraction system. These results were similar to that found by Uma et al. (2010) for a terrestrial plant *Lawsonia inermis*. In this case, free phenolic content for acetone/water (80:20) extraction system was higher than that obtained for acetone alone. In this sense, Chew et al. (2008) found for two seaweeds (*Caulerpa racemosa* and *Kappaphycus alvarezii*) higher values of polyphenol content with methanol/water extraction system (50:50) than methanol alone (100%). The addition of small quantity of water to organic solvent usually creates a more polar medium, which facilitate the extraction of polyphenols. It has been postulated that acetone has the ability to inhibit protein–polyphenol complex formation during extraction or even break down hydrogen bonds formed between phenolic group and protein carboxyl group (Wang et al., 2009), resulting in a higher content of polyphenols extracted from *P. columbina*.

TEAC, DPPH inhibition and reducing power activity of *P. columbina* are shown in Figure 1(B), (C) and (D), respectively. The highest values of TEAC and DPPH inhibition were obtained with acetone/water extraction system. The increase in antioxidant properties respect to methanol extraction system may be due to increases of phenolic compounds in the extracts (Figure 1A). In this way, several studies have shown that polyphenolic compounds have a potent radical scavenger activity (Cian et al., 2012). Good correlation was evidenced between total polyphenolic content and DPPH radical scavenging activity from *P. columbina* ($r = 0.9686$). Similar results were obtained by Wang et al. (2009) who reported a high correlation between total polyphenolic content and DPPH radical scavenging activity from Icelandic seaweed extracts, indicating an important role of algal polyphenols as chain-breaking antioxidants. On the other hand, the highest values of reducing power were obtained with methanol and acetone/water extraction systems (Figure 1D). Similar results were obtained by Ganesan et al. (2008) for three selected Indian red seaweeds (*Eucheuma kappaphycus, Gracilaria edulis* and *Acanthophora spicifera*). For *P. columbina*, all extracts exhibited the Abs value <1.0. Similar findings were also reported by Kuda et al. (2005). The reducing power could be associated with the presence of reductones that are reported to be terminators of free radical chain reaction (Duh, 1998).

Red seaweeds also contain phycobiliproteins and mycosporine-like amino acids (MAAs), which could act as antioxidants. In this way, Bermejo et al. (2008) demonstrated antioxidant properties of phycocyanin isolate obtained from a protein extract

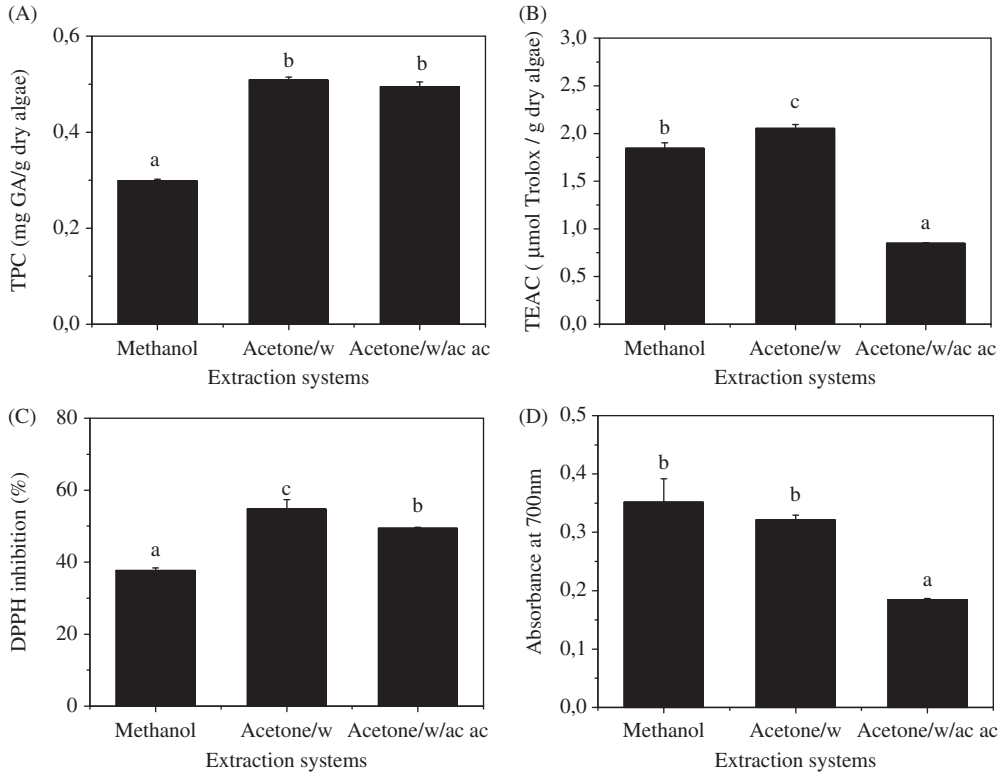

Figure 1. Total phenolic contents (TPC) (A), Trolox equivalent antioxidant capacity (TEAC) (B), DPPH inhibition (C) and reducing power activity (D) of *P. columbina* with different extraction systems (100% methanol; 80% acetone/20% water; and 80% acetone/15% water/5% acetic acid). Data are expressed as mean ± SD. All samples were analyzed in triplicate; different letters mean significant differences between samples ($p < 0.05$).

of the blue green bacterium *Spirulina platensis*. In this sense, De la Coba et al. (2009) reported the antioxidant properties of three MAAs isolated from the red algae *Porphyra rosengurttii*, *Gelidium corneum* and *Ahnfeltiopsis devoniensis*. These results indicated that *P. columbina* has bioactive compounds, which are good electron donors and could react with free radicals to terminate the radical chain reaction.

Conclusions

Although other seaweeds have been extensively studied for the past few decades, with the focus on proximate composition there has been no research focused on the chemical composition, nutritional and antioxidant properties of *P. columbina*. The red edible seaweed examined in this work has a considerable protein content, better chemical score than cereals and protein digestibility similar to that of plant foods. *P. columbina* has a high mineral content with medium value of potential mineral accessibility (except for iron), low phytic acid content and good Na/K relationship. Also, *P. columbina* has bioactive compounds, which are good electron donors and could act as antioxidant. This fact and the high dietary fiber level make *P. columbina* a healthy low-fat food.

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Declaration of interest

The authors report no conflicts of interests. The authors alone are responsible for the content and writing of the article.

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