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Bio-accessibility of bioactive compounds (ACE inhibitors and antioxidants) from extruded maize products added with a red seaweed *Porphyra columbina*

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ABSTRACT

An expanded maize product added with red seaweeds *Porphyra columbina* (3.5 g 100 g^{-1}) was developed and bio-accessibility of minerals and bioactive compounds such as ACE inhibitors and antioxidants provide by algae were evaluated using a pepsin/pancreatin digestion and equilibrium dialysis method. Extruded maize added with red seaweed showed higher dialyzability of ACE inhibitor compounds (41.0% ACE inhibition), total phenolic content (0.83 mg gallic acid/g dialysate) and antioxidant capacity (36.6% DPPH inhibition, 2.4 mM TEAC, power reduction and 99.4% copper-chelating activity) than extruded maize. Results about bio-accessibility of bioactive compounds provided by red edible seaweeds may help food technologists to tailor new bio-functional foods, such as functional snacks.

1. Introduction

Seaweeds are a part of staple diet from time immemorial in the orient as they are nutritionally rich materials; but to a much lesser extent in the rest of the world (Prabhasankar et al., 2009b). They are excellent source of biologically active phytochemicals, which include carotenoids, phycobilins, fatty acids, polysaccharides, vitamins, sterols, tocopherol, phycocyanins among others. Many of these compounds have been recognized to possess biological activity and hence beneficial for use in human and animal healthcare. Some of the potential benefits include control of hyperlipidemia, thrombosis, tumor, and obesity (Kadam & Prabhasankar, 2010).

Prabhasankar, Ganesan, and Bhaskar (2009a) and Prabhasankar et al. (2009b) developed pasta with two Indians brown seaweed (*Sargassum marginatum* and *Undaria pinnatifida*) as an ingredient to improve the bio-functional and nutritional qualities of product. Different levels of seaweeds were substituted to obtain seaweed-incorporated pasta and pasta without seaweed was used as control. However, as far as we know, there are no published papers related to the production of other cereal products added with seaweeds such as expanded maize products. Even less, expanded maize products added with a red seaweed *Porphyra columbina*.

The genus *Porphyra*, traditionally known as nori in Japan, kim in Korea and zicai in China, is a popular red seaweed food, due to its rich content of protein, vitamins, minerals and dietary fiber. *P. columbina* is a red seaweed found on hard substratum in Patagonia Argentina coasts with a high total dietary fiber and protein contents (~45 g 100 g^{-1} and ~30 g 100 g^{-1} dry weight respectively) (Cian, López-Posadas, Drago, Sánchez de Medina, & Matínez-Augustin, 2012). Among red algae proteins, phycobiliproteins have drawn attention because of their immunomodulatory, ACE inhibition and antioxidant properties (Cian, Martínez-Augustin, & Drago, 2012). Also, many researchers have attributed the antioxidant capacity mainly to phenolic compounds present in the seaweed (Wang, Jónsdóttir, & Ólafsdóttir, 2009).

Extrusion cooking is one of the most important food processing technologies which have been used since the mid 1930s for the production of breakfast cereals, ready to eat snack foods, and other textured foods (Brennan, Brennan, Derbyshire, & Tiwari, 2011). It is a high temperature and shear process that is characterized by forming a melt from the starchy ingredient, at high temperature (140–180 °C) and low moisture content (12–17 g 100 g^{-1}). In a short residence time (15–30 s) non cohesive material (flour and

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grits) is converted in a new textured structure. Starch is the main constituent of the extruded snacks and is responsible for most of their structural attributes. Thus, these snacks are dense in energy but are nutritionally poor and it is possible to add beneficial nutrients to them (Dehghan-Shoar, Hardacre, & Brennan, 2010).

The aims of this work were to develop extruded maize products added with a red seaweed *P. columbina* as functional food and evaluate the bio-accessibility of bioactive compounds (ACE inhibitors and antioxidants).

2. Materials and methods

2.1. Reagents

All reagents were obtained from Sigma Chemical Co. (St. Louis, MO, USA).

2.2. Raw materials

One kilogram of different specimens of *P. columbina* was hand-picked in Punta Maqueda (Comodoro Rivadavia, Argentina). The seaweed was 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 sieve and stored at 4 °C in plastic bags until analysis. *P. columbina* composition in dry base was: protein: 24.61 g 100 g^{-1}, fat: 0.25 g 100 g^{-1}, ash: 6.46 g 100 g^{-1}, total dietary fiber: 48.09 g 100 g^{-1} and moisture 12.79 g 100 g^{-1}. Maize grits composition in dry base was: protein: 8.92 g 100 g^{-1}, fat: 0.27 g 100 g^{-1}, ash: 0.28 g 100 g^{-1}, carbohydrates: 83.14 g 100 g^{-1} and moisture 7.39 g 100 g^{-1}.

2.3. Preparation of extruded maize products added with red seaweed P. columbina

Commercial maize grits and red seaweed *P. columbina* were blended in the following ratios: 100:0 (M); 98.3:1.7 (MPc_1); 96.5:3.5 (MPc_2) and 94.8:5.2 (MPc_3) (maize grits: *P. columbina*; g:g). Extrusion was carried out with a 20 DN Brabender single screw extruder using 4:1 compression ratio screw, 150 rpm, 16.5 g 100 g^{-1} grits moisture, 175 °C barrel temperature and 160 °C die temperature. The feeding rate of the extruder was at full capacity. The composition of extruded products was determined using AOAC procedures (1995).

2.4. Physical characterization of extruded products

All extruded products were air-dried in an oven at 50 °C until a moisture content of 6 g 100 g^{-1} was reached, this moisture level being considered adequate for texture evaluation. Axial expansion, specific volume (SV), specific mechanical energy consumption (SMEC), in J g^{-1}, was determined according to González, Torres, and De Greef (2002).

Sample was milled using Ciclotec mill (UD Corp Boulder Colorado-USA) using a 1 mm sieve. Water solubility was determined according to González et al. (2002). Water absorption (WA) was determined using Bauman method and expressed as ml of water/g of sample.

2.5. Color and sensory characteristics of extruded products

Extruded product color was determined with a Konica Minolta Chroma Meter CR-400 (Konica Minolta Chroma Co., Osaka, Japan) set to C illuminant/2° observer. A CIE-Lab color scale was used to measure the degree of lightness (L^*), redness ($+a^*$) or greenness ($-a^*$), and yellowness ($+b^*$) or blueness ($-b^*$) of the films. Extruded products color was measured on the surface of this standard plate and total color difference (ΔE^*) was calculated as follow:

$$\Delta E^* = \left[(L^*\text{film} - L^*\text{standard})^2 + (a^*\text{film} - a^*\text{standard})^2 + (b^*\text{film} - b^*\text{standard})^2\right]^{0.5} \quad (1)$$

The following equations were used to convert $L^*a^*b^*$ coordinates to $L^*C^*h^*$ coordinates.

$$C* = \left(a*^2 + b*^2\right)^{0.5} \quad (2a)$$

$$h* = \arctan(b*/a*) \quad (2b)$$

Trained panelists ($n = 8$) from Instituto de Tecnología de Alimentos – FIQ – UNL performed the sensory evaluation of extruded samples. Selected attributes were: color, smell, taste and flavor, and mouth texture (the most important attributes for an expanded product) evaluating crispness and adherence. The methodology used was Quality Descriptive Analysis (QDA) according to Murray, Delahunty, and Baxter (2001). Non structured scales were used to assess attributes and preliminary assays were done in order to establish the anchor extremes (1 and 9) of a 10 scale. During tasting, the panel scored in each unstructured scale, the perceived intensity of each attribute. Then, the intensities of each attribute were measured in each scale, in order to assign a value for statistical analysis.

2.6. Amino acid analysis of M and MPc_2

Amino acids were determined after derivatization with diethyl ethoxymethylenemalonate by high-performance liquid chromatography (HPLC), according to the method of Alaiz, Navarro, Giron, and Vioque (1992), using D,L-α-aminobutyric acid as internal standard. Tryptophan was determined by HPLC-RP chromatography after basic hydrolysis according to Yust et al. (2004).

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

$$\text{CS} = (\text{mg of EAA/g protein}) / (\text{mg of EAA/g FAO requirement protein pattern}) \quad (3)$$

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

2.6.2. Protein efficiency ratio values (PER) and predicted biological value (BV)

Protein efficiency ratio values (PER) and predicted biological value (BV) were calculated according to Pastor-Cavada, Juan, Pastor, Alaiz, and Vioque (2011).

2.6.3. Protein digestibility corrected amino acid score (PDCAAS) (FAO/WHO, 1988) was calculated as

$$\text{PDCAAS} = \text{CS} \times \text{protein digestibility (PD)} \quad (4)$$

2.7. In vitro protein digestibility

The *in vitro* protein digestibility of M and MPc_2 extruded products was measured using the method described by Rudloff and Lönnerdal (1992). The protein digestibility was calculated as:

$$PD(\%) = [(NPN - NPNi)/(N - NPNi)] \times 100 \quad (5)$$

where: PD (%) is the digestibility of proteins samples, NPN is non-protein nitrogen soluble in 20 g 100 mL^{-1} 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 g 100 mL^{-1} of trichloroacetic acid from sample. The nitrogen content (NPN, N and NPNi) was determinate using Kjeldhal method.

2.8. Total phenolic content (TPC)

Total phenolic content of M and MPc_2 extruded products was determined according to Cian, Alaiz, Vioque, and Drago (2013) using different extraction systems (100 mL methanol; 80 mL acetone/20 mL water; and 80 mL acetone/15 mL water/5 mL acetic acid) were used. A standard curve with serial gallic acid solutions was used for calibration. Results were expressed as mg gallic acid g^{-1} of dry extruded products.

2.9. Antioxidant properties of M and MPc2

Antioxidant properties of M and MPc_2 extruded products were determined from supernatant of different extraction systems (methanol, acetone:water and acetone:water:acetic acid).

2.9.1. Trolox equivalent antioxidant capacity (TEAC)

To estimate the TEAC, a concentration-response curve for the absorbance at 734 nm for ABTS+ 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 according to Cian, Martínez-Augustin, et al. (2012). The absorbance reading was taken at 6 min after initial mixing.

2.9.2. DPPH radical scavenging activity assay

DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity was measured according to the method of Cian, Martínez-Augustin, et al. (2012). The absorbance at 517 nm was taken at 30 min after initial mixing. The difference between the blank and the sample was used for calculating the percentage of inhibition.

2.9.3. Reducing power activity assay

The reducing power activities of M and MPc_2 were determined according to the method of Ahmadi, Kadivar, and Shahedi (2007). The absorbance was measured at 700 nm. An equivalent volume of distilled water instead of the sample was used for the blank.

2.10. Bio-accessibility of minerals and bioactive compounds from extruded products

2.10.1. Determination of mineral dializability

The method developed by Miller, Schricker, Rasmussen, and Van Campen (1981), modified by Drago, Binaghi, and Valencia (2005), was used. This method measures mineral dialyzability under controlled pH conditions after a digestion-simulating physiological process. Dialyzability of mineral was calculated as the amount of dialyzate mineral expressed as a percentage of total mineral content in the sample:

$$MD(\%) = (mgM_D/mgM_S) \times 100 \quad (6)$$

where: MD (%) is the percentage of mineral dialyzated, mgM_D is milligrams of mineral dialyzated, and mgM_S is milligrams of mineral of the sample. Dialyzability was used as an indicator of potential bioaccessibility of magnesium, calcium, iron and zinc and was named as follows: MgD (%), CaD (%), FeD (%) and ZnD (%), respectively.

Total mineral content in the sample was measured by atomic absorption spectroscopy after dry mineralization. Ash was removed with 10 mL HCl 100 mL^{-1}. An atomic absorption spectrometer (Atomic Absorption spectrophotometer Analyst 300 Perkin–Elmer, Norwalk, CT, USA) was used.

2.10.2. Determination of bioactive compounds dializability

Bioactive compound dializability from M and MPc_2 extruded products was assayed using pepsin and pancreatin digestion. The extruded products were ground previously and then prepared to 10 g solid 100 g^{-1} dispersion using deionized water. Aliquots (25 g) of homogenized samples were adjusted to pH 2.0 with 4 mol L^{-1} HCl and after addition of 0.8 mL pepsin digestion mixture (16 g 100 mL^{-1} pepsin solution in 0.1 mol L^{-1} HCl), were incubated at 37 °C for 2 h in a shaking water bath. At the end of pepsin digestion, dialysis bags (cut off: 8–6 kDa) containing 20 mL 0.15 mol L^{-1} PIPES buffer were placed in each flask and were incubated for 50 min in a shaking water bath at 37 °C. Then, 6.25 mL of pancreatin solution (0.4 g 100 mL^{-1} pancreatin in 0.1 mol L^{-1} $NaHCO_3$) was added to each flask and the incubation continued for another 2 h. Then, bag contents dialyzates from M and MPc_2 extruded products were weighed and named M_D and MPc_{2D}, respectively.

TPC, TEAC, DPPH inhibition and reducing power activity of dialyzates were evaluated as described above.

Angiotensin-converting enzyme activity inhibition was determined according to Hayakari, Kondo, and Izumi (1978) and antihypertensive activity was expressed as ACE inhibition (%). To determine the concentration causing an inhibition of 50% (IC50%) serial dilutions of M_D and MPc_{2D} from 0 to 2.0 g L^{-1} protein were made.

Copper-chelating activity was determined by the assay of β-carotene oxidation according to Megías et al. (2008). The degradation of β-carotene was monitored by recording the decrease in absorbance. Copper-chelating activity at 470 nm was calculated as follow:

$$CCA(\%) = [(Abs\ control(-) - Abs\ sample)/(Abs\ control(-) - Abs\ control(+))] \times 100 \quad (7)$$

2.11. Statistical analysis

All results were expressed as mean ± SD. The data were analyzed by one-way analysis of variance, using the software Statgraphics Plus 3.0. The statistical differences between samples were determined using the LSD (least significant difference) test. The significance was established at $P < 0.05$.

3. Results and discussion

3.1. Physical characterization of extruded products

Table 1 shows the values obtained from the physical evaluation of the extruded samples corresponding to M, MPc_1, MPc_2 and MPc_3. As *P. columbina* proportion increase in extruded maize products, torque, feed caudal (Qa), specific mechanical energy consumption (SMEC), expansion and specific volume (SV) decreased ($p < 0.05$). It is also observed that torque decreased at higher rates than Qa, indicating a reduction in the level of friction. As friction level decreases there is a reduction in the degree of cooking estimated by SV. These results are in agreement with other authors working with mixtures of cereals and other vegetables and legumes (Dehghan-

Table 1
Physical characterization of extruded products.

Sample	Torque (UB)	Qa (g min^{-1})	SMEC (J g^{-1})	Expansion	SV (cm^3 g^{-1})	Solubility (%)	WA (ml g^{-1})
M	600.1 ± 1.4^d	98.9 ± 3.6^b	56.1 ± 2.0^d	3.5 ± 0.0^d	7.3 ± 0.0^d	32.9 ± 0.2^a	7.9 ± 0.3^a
MPc_1	475.2 ± 0.7^c	91.7 ± 0.4^a	47.9 ± 0.2^c	3.4 ± 0.0^c	6.9 ± 0.0^c	32.2 ± 2.0^a	8.0 ± 0.1^a
MPc_2	375.0 ± 0.6^b	88.7 ± 1.6^a	39.1 ± 0.7^b	3.2 ± 0.0^b	6.1 ± 0.0^b	31.4 ± 3.0^a	8.0 ± 0.2^a
MPc_3	325.1 ± 0.4^a	87.7 ± 0.1^a	34.2 ± 0.1^a	3.2 ± 0.0^a	5.8 ± 0.0^a	33.2 ± 0.7^a	8.1 ± 0.1^a

Qa, feed caudal; SMEC, specific mechanical energy consumption; SV, specific volume and WA, water absorption. Mean ± SD ($n = 3$). Different letters in the same row mean significant differences between samples ($p < 0.05$). Commercial maize grits and *P. columbina* blends: 100:0 (M); 98.3:1.7 (MPc_1); 96.5:3.5 (MPc_2) and 94.8:5.2 (MPc_3).

Shoar et al., 2010; Pastor-Cavada, Drago, et al., 2011). The variations in the responses can be due to the differences in chemical composition i.e. total dietary fiber, protein and ash content of different blends. Veronica, Olusola, and Adebowale (2006) observed that as fiber and protein-rich materials were added to starchy materials, SV of expanded product was decreased. This behavior can be due to the fact that during the extrusion process fiber reduced the degree of cooking and consequently the expansion ratio.

Solubility and water absorption (WA) were not affected by the addition of *P. columbina* to maize. Probably due to these methods are less sensitive than those mentioned above to assess the degree of cooking.

3.2. Color and sensory characteristics of extruded products

CIE-Lab color parameters (L^*, a^* and b^*), total color difference (ΔE), chroma (C^*), hue (h) of extruded products are shown in Table 2. These results give an idea of the visual aspect of the developed extruded products. As *P. columbina* proportion increased in extruded products, L^* and b^* parameters gradually decreased ($p < 0.05$) while a^* and h values increase ($p < 0.05$), indicating that expanded products changed from light yellow to dark green. MPc_3 having the lowest L^*, b^*, ΔE and C^* values ($p < 0.05$). Inverse relationships between L^* or b^* and *P. columbina* content in extruded products were obtained. The Pearson correlation coefficients were $r^2 = 0.9489$ and $r^2 = 0.9164$ for L^* and b^*, respectively. These results could be due to red seaweeds have pigments like phycobiliproteins or chlorophyll (Cian, López-Posadas, et al., 2012).

Color score from sensory evaluation was increased with the addition of *P. columbina* ($p < 0.05$). Color values of M, MPc_1, MPc_2 and MPc_3 were 1.4, 3.7, 6.0 and 7.1, respectively. These results are in agreement with CIE-Lab color parameters and inverse relationships between L^*, b^* or C^* and color from extruded products were obtained ($r^2 = 0.9625$, $r^2 = 0.9622$ and $r^2 = 0.9904$, respectively). MPc_3 color exceeded the limit value given by the panel (6) and could be considered as not adequate. There was no significant difference for the smell and taste between expanded products added with *P. columbina*. However, the flavor of M was lower than that found for MPc_1, MPc_2 and MPc_3 ($p < 0.05$), but all samples were within of acceptable limit. Mouth texture for all extruded products was highly crispy with an average value of 8.3, but there were no significant differences between samples. Adherence was increased with the addition of *P. columbina* and M, MPc_1, MPc_2 and MPc_3 values were 1.2, 1.7, 2.0 and 2.5, respectively. However, only adherence from MPc_3 was higher than that found for M ($p < 0.05$).

Sensory analysis of the different extruded products revealed that the most suitable addition rate was 3.5 g 100 g^{-1} (MPc_2), since it allow good sensory characteristics in terms of color, flavor and mouth texture. Therefore, MPc_2 and control (M) were selected for further analysis.

3.3. Proximate composition and amino acid analysis of M and MPc_2

Table 3 shows proximate composition of M and MPc_2. Replacing maize grits with *P. columbina* powder considerably improved the protein and total dietary fiber contents. This can be due to red seaweeds have high protein and fiber level (Fleurence, 1999). Also, ash corresponding to MPc_2 was higher than that obtained for M. This increase in ash content may be explained considering that red seaweed are rich in mineral content ranging from 22.5 to 38.4 g 100 g^{-1} dry weight (Gressler et al., 2010).

Table 4 shows amino acids profile of M and MPc_2. Aspartic and glutamic acid, leucine and alanine were the most abundant amino acids in M and MPc_2. The increase of aspartic acid level in MPc_2 was due to the acid amino acids constitute a large part of the amino acid fraction in most seaweed (Fleurence, 1999). The ratio of MPc_2 to M for the content of each amino acid was approximately 1.0. Therefore, for each amino acid, its content in MPc_2 was similar to that obtained for M. However, the contents of Met and Pro in MPc_2 were higher than M (1.98 and 1.36, respectively), while the Arg content in MPc_2 was lower (0.83). Therefore, the incorporation of *P. columbina* to maize extruded products improved Met and Pro maize content. It is important to note that both amino acids play an important role in bioactive properties, such as antioxidant and antihypertensive activity.

The chemical score (CS) was 37.8% and 35.9% for M and MPc_2, respectively; according to FAO/WHO (1985) requirement pattern. The limiting amino acid for both extruded products was lysine. This result was similar to that found by Prabhasankar et al. (2009b) for pasta prepared with *U. pinnatifida*.

Theoretical PER values for M and MPc_2 were above 2 (PER_1: 6.26 ± 0.22 and 6.12 ± 0.02, PER_2: 6.21 ± 0.24 and 6.19 ± 0.01, PER_3: 11.09 ± 0.43 and 11.24 ± 0.03; for M and MPc_2 respectively). PER

Table 2
CIE-Lab color parameters (L^*, a^* and b^*), total color difference (ΔE), chroma (C^*) and hue (h) from extruded products.

Sample	CIE-$L^*a^*b^*$ color parameters					
	L^*	a^*	b^*	ΔE	C^*	h
M	87.1 ± 0.5^d	−1.5 ± 0.1^b	39.9 ± 0.4^d	39.6 ± 0.4^d	39.9 ± 0.3^d	92.1 ± 0.1^a
MPc_1	79.5 ± 0.8^c	−2.2 ± 0.0^a	32.1 ± 0.6^c	36.3 ± 0.2^c	32.0 ± 0.1^c	94.1 ± 0.0^b
MPc_2	76.7 ± 0.7^b	−2.2 ± 0.1^a	28.6 ± 0.3^b	35.7 ± 0.1^b	28.3 ± 0.2^b	94.7 ± 0.0^b
MPc_3	72.8 ± 0.5^a	−2.3 ± 0.1^a	26.4 ± 0.4^a	34.5 ± 0.1^a	25.9 ± 0.2^a	94.7 ± 0.0^b

Mean ± SD ($n = 3$). Different letters mean significant differences between samples ($p < 0.05$). Commercial maize grits and *P. columbina* blends: 100:0 (M); 98.3:1.7 (MPc_1); 96.5:3.5 (MPc_2) and 94.8:5.2 (MPc_3).

Table 3
Proximate composition of maize expanded products (M) and maize extruded products added with *P. columbina* (MPc_2).

Composition	M (g/100 g dw)	MPc_2 (g/100 g dw)
Crude protein ($N \times 6.25$)	8.92 ± 0.04^a	9.60 ± 0.01^b
Ash	0.28 ± 0.01^a	0.57 ± 0.05^b
Total dietary fiber	6.84 ± 0.01^a	7.05 ± 0.03^b
Insoluble dietary fiber	5.29 ± 0.05^a	5.79 ± 0.01^b
Soluble dietary fiber	1.55 ± 0.01^b	1.27 ± 0.03^a
Crude lipid	0.27 ± 0.05^a	0.31 ± 0.05^a
Moisture	7.39 ± 0.10^a	7.06 ± 0.20^b

Means ± SD ($n = 3$), dw (dry weight). Different letters in the same row mean significant differences between samples ($p < 0.05$). MPc_2: commercial maize grits and *P. columbina* blend (96.5:3.5).

has been recognized as a valuable method for the calculation of protein quality and theoretical PER values bear a good relationship with real PER. Low quality proteins have PER values below 1.5, and above 2 they are considered high quality proteins (Pastor-Cavada, Juan, et al., 2011).

On the other hand, theoretical BV found for M and MPc_2 was 21.8 ± 1.7 and 22.2 ± 0.5, respectively. These values were similar to that reported by Pastor-Cavada, Juan, et al. (2011) for a wild legume *Lathyrus hirsutus* (24.5%).

No significant differences for *in vitro* protein digestibility (PD) were found between M and MPc_2. PD values for M and MPc_2 were 89.02 ± 3.47% and 91.21 ± 0.26, respectively. These values are similar to PD value of plant proteins but are lower than those of animal proteins (Balandrán-Quintana, Barbosa-Cánovas, Zazueta-Morales, Anzaldúa-Morales, & Quintero-Ramos, 1998).

M and MPc_2 presented a PDCAAS value of 32.7 and 33.7%, respectively; which was equal or higher than that of various legumes. The PDCAAS, which is based on FAO recommendations of amino acids requirements (FAO/WHO/UNU, 1985) and PD, is nowadays the most recommended theoretical parameter for evaluating the nutritional quality of food proteins. It is better than other methods since it determines the quality of a protein based on the amino acid requirements of a 2–5 year old child, adjusted for digestibility. The highest PDCAAS value for a given protein is 100. This protein will provide 100% of the indispensable amino acids recommended by FAO (FAO/WHO/UNU, 1985).

Table 4
Amino acids (AA) profile of maize extruded products (M) and maize extruded products added with *P. columbina* (MPc_2); and the ratio of MPc_2 to M amino acids.

Amino acids	Total AA of M (g 100 g^{-1} protein)	Total AA of MPc_2 (g 100 g^{-1} protein)	Ratio MPc_2/M
Asp	7.40 ± 0.25^a	8.32 ± 0.09^b	1.12
Glu	22.49 ± 0.32^a	21.63 ± 0.50^a	0.96
Ser	6.19 ± 0.07^b	5.74 ± 0.02^a	0.93
His	2.84 ± 0.08^a	2.68 ± 0.08^a	0.94
Gly	4.20 ± 0.06^a	4.13 ± 0.02^a	0.98
Thr	3.97 ± 0.03^b	3.85 ± 0.01^a	0.97
Arg	4.57 ± 0.04^b	3.79 ± 0.07^a	0.83
Ala	9.28 ± 0.07^a	10.03 ± 0.09^b	1.08
Pro	2.57 ± 0.08^a	3.50 ± 0.09^b	1.36
Tyr	3.41 ± 0.04^a	3.40 ± 0.01^a	1.00
Val	3.97 ± 0.05^a	4.08 ± 0.07^a	1.03
Met	0.51 ± 0.00^a	1.01 ± 0.06^b	1.98
Cys	2.16 ± 0.12^a	2.01 ± 0.09^a	0.93
Ile	2.92 ± 0.00^b	2.69 ± 0.01^a	0.92
Trp	0.44 ± 0.00^a	0.52 ± 0.02^a	1.19
Leu	15.50 ± 0.53^a	15.46 ± 0.03^a	1.00
Phe	5.66 ± 0.06^a	5.37 ± 0.19^a	0.95
Lys	1.93 ± 0.01^b	1.83 ± 0.01^a	0.95

Means ± SD ($n = 3$). Different letters in the same row mean significant differences between samples ($p < 0.05$). MPc_2: commercial maize grits and *P. columbina* blend (96.5:3.5).

3.4. Total phenolic content (TPC)

Fig. 1A shows total phenolic contents (TPC) of M and MPc_2. For both samples, the lowest value of phenolic content was obtained with methanolic extraction system and the highest value with the acetone/water extraction system. Similar results have been observed in others foods; Turkmen, Velioglu, Sari, and Polat (2007), obtained higher yields of polyphenol extraction for black tea with acetone/water, ethanol/water and methanol/water extraction system than pure solvents. 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 M and MPc_2.

As shown in Fig. 1A, TPC extracted with acetone/water system was higher for MPc_2 than M. This increase is due to phenolic compounds provided by *P. columbina* (Cian, López-Posadas, et al., 2012). As it is known, seaweeds are a rich source of various natural bioactive compounds such as polyphenols (Wang et al., 2009).

3.5. Antioxidant properties

Trolox equivalent antioxidant capacity (TEAC), DPPH inhibition and reducing power activity of M and MPc_2 are shown in Fig. 1B–D, respectively. For all extraction systems, the highest values of TEAC, DPPH inhibition and reducing power were obtained with MPc_2. The increase in antioxidant properties respect to control (M) may be due to phenolic compounds provided by red seaweeds and release during extraction process. In this way, several studies have shown that polyphenolic compounds have a potent radical scavenger activity (Wang et al., 2009). Similar results were obtained by Prabhasankar et al. (2009a) who worked with pasta and *S. marginatum* blends. Also, the antioxidant properties of acetone/water extracts which have the highest TPC were higher than those obtained with methanol or acetone/water/acetic acid. In this sense, Wang et al. (2009) reported a high correlation between total polyphenolic content and DPPH radical scavenging activity from Icelandic seaweeds extracts, indicating an important role of algal polyphenols as chain-breaking antioxidants.

On the other hand, red seaweeds contain phycobiliproteins, which could act as antioxidants. In this way, Bermejo, Pinero, and Villar (2008) demonstrated antioxidant properties of phycocyanin isolate obtained from a protein extract of the green alga *Spirulina platensis*. These results indicated that the addition of *P. columbina* to maize grits provide bioactive compounds to extruded product, which are good electron donors and could react with free radicals to terminate the radical chain reaction.

3.6. Bio-accessibility of mineral and bioactive compounds

3.6.1. Mineral dializability

Table 5 shows mineral content and Ca, Mg, Fe and Zn dializability from M and MPc_2. Regarding minerals, no significant differences of K, Fe and Zn contents between M and MPc_2 were found. However, Na, P, Ca and Mg contents were higher for MPc_2 than for M. This result may be attributed to the addition of red seaweed, which is a good source of these minerals. Also, Ca and Mg contents of MPc_2 followed the same pattern reported for red seaweeds (Mg > Ca) (Pérez et al., 2007).

As regards Mg dializability, MPc_2 had higher values than M. However, the addition of *P. columbina* decreases significantly Ca, Fe and Zn dializability. In this sense, algae are known to be rich in dietary fiber, polyphenolyc compounds and phytic acid, which

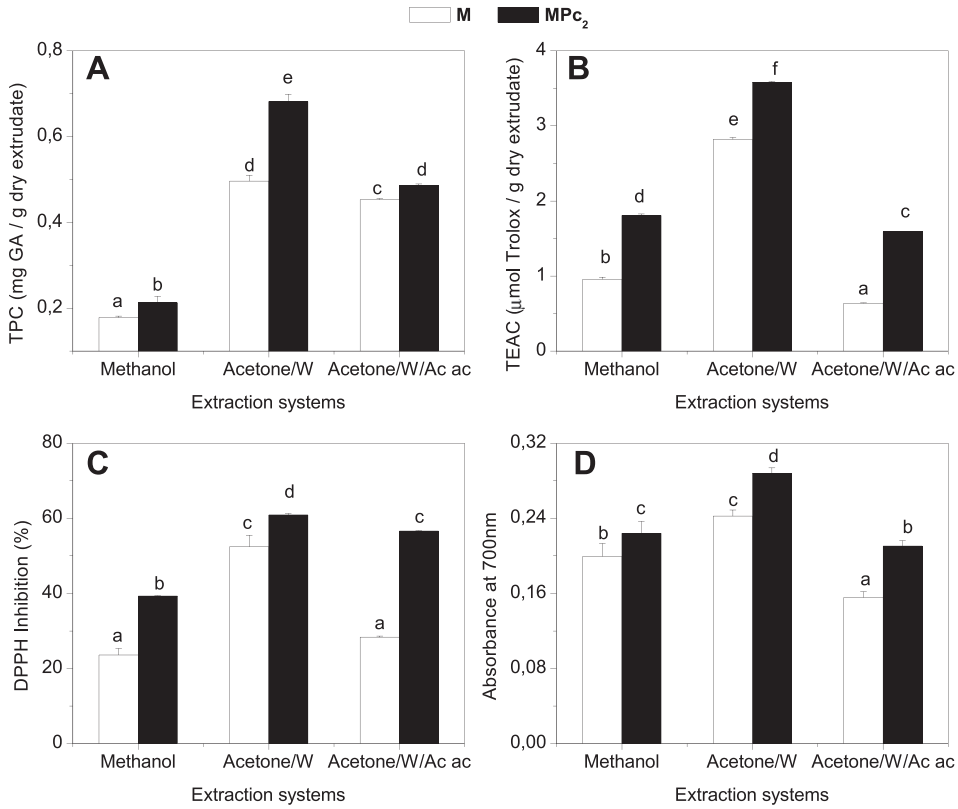

Fig. 1. Total phenolic contents (TPC) (A), Trolox equivalent antioxidant capacity (TEAC) (B), DPPH inhibition (C) and reducing power activity (D) of maize extruded products (M) and commercial maize grits/*P. columbina* blend (96.5:3.5; MPc_2) with different extraction systems (100 mL methanol; 80 mL acetone/20 mL water; and 80 mL acetone/15 mL water/5 mL acetic acid). Data are expressed as mean ± SD. All samples were analyzed in triplicate; letters with $P < 0.05$ means significant differences.

could affect mineral bioavailability (Nilka de Oliveira et al., 2009). Fiber components may form insoluble complexes with minerals, and thus reduce their bioavailability. On the other way, phytic acid is considered an antinutritional factor because it might interfere with bioavailability and/or digestibility of some nutrients, such as proteins and trace minerals (Rehman & Shah, 2004). It occurs in the form of mixed salts, as phytates and has a strong ability to form complexes with multivalent metal ions, especially iron, calcium, and zinc. This binding can result in very insoluble salts (varying with the pH) with poor minerals bioavailability.

3.6.2. Bioactive compounds dializability

Results showed that the antioxidant activity of M and MPc_2 could be preserved after treatment with gastrointestinal enzymes and those bioactive compounds from M and MPc_2 were bio-accessible. The highest values of TEAC, DPPH inhibition and reducing power were obtained with MPc_{2D} (Fig. 2A–C). Also, MPc_{2D} showed a high inhibition of β-carotene oxidation in the presence of copper and copper-chelating activity and it was significantly higher than that obtained for M_D (Fig. 2D). The increase in antioxidant properties and copper-chelating activity respect to the control (M_D) may be due to phenolic compounds provided by red seaweeds released during proteolysis process (Wang et al., 2010). In this sense, TPC of MPc_{2D} was higher than that found for M_D (0.83 ± 0.00 and 0.77 ± 0.00 mg GA/g dialyzate, respectively), suggesting that the phenolic compounds dialyzated through membrane from red seaweed were mainly responsible of increased in antioxidant properties of extruded products.

M_D showed some degree of ACE inhibition, but it was lower than MPc_{2D} ACE (35.5% and 41.0%, respectively). Also, the IC50 value for MPc_{2D} was lower than that found for M_D (1.20 ± 0.01 and 1.43 ± 0.01 g L^{-1} of protein, respectively). This confirms that addition of red seaweed to maize grits provides compounds which improve antihypertensive properties of extruded product and they are bio-accessible. In this way, phenolic compounds also inhibit ACE activity through sequestration of the enzyme metal factor, Zn^{2+} ion (Wijesekara & Kim, 2010). Hence, phenolic compounds provided by *P. columbina* and released during digestion might inhibit ACE activity.

It is appropriate to note that pepsin and pancreatin digestion not only released phenolics linked to proteins but also bioactive peptides. Bioactive peptides consist of chains of 2–30 amino acid residues which are released from the parent protein to exert a bioactive effect. In this sense, Cian, Martínez-Augustin, et al. (2012) found that *P. columbina* was source of immunosuppressive,

Table 5
Major minerals, trace elements and magnesium, calcium, iron and zinc dializability of maize expanded products (M) and maize extruded products added with *P. columbina* (MPc_2).

Mineral	Mineral content (mg 100 g^{-1} dw)		Dializability (%)	
	M	MPc_2	M	MPc_2
Na	37.56 ± 0.02[a]	125.91 ± 0.12[b]	ND	
K	72.09 ± 1.93[a]	71.24 ± 0.32[a]	ND	
P	74.92 ± 1.22[a]	93.11 ± 1.15[b]	ND	
Ca	2.89 ± 0.03[a]	12.93 ± 0.32[b]	100.02 ± 2.65[b]	60.05 ± 0.39[a]
Mg	30.18 ± 0.66[a]	47.66 ± 1.72[b]	38.87 ± 0.11[a]	59.31 ± 0.55[b]
Fe	0.89 ± 0.01[a]	0.94 ± 0.05[a]	20.03 ± 0.34[b]	16.51 ± 0.23[a]
Zn	0.48 ± 0.00[a]	0.44 ± 0.04[a]	60.94 ± 2.17[b]	39.73 ± 0.18[a]

ND: Not determined. Means ± SD ($n = 3$), dw (dry weight). Different letters in the same row mean significant differences between samples ($p < 0.05$). MPc_2: commercial maize grits and *P. columbina* blend (96.5:3.5).

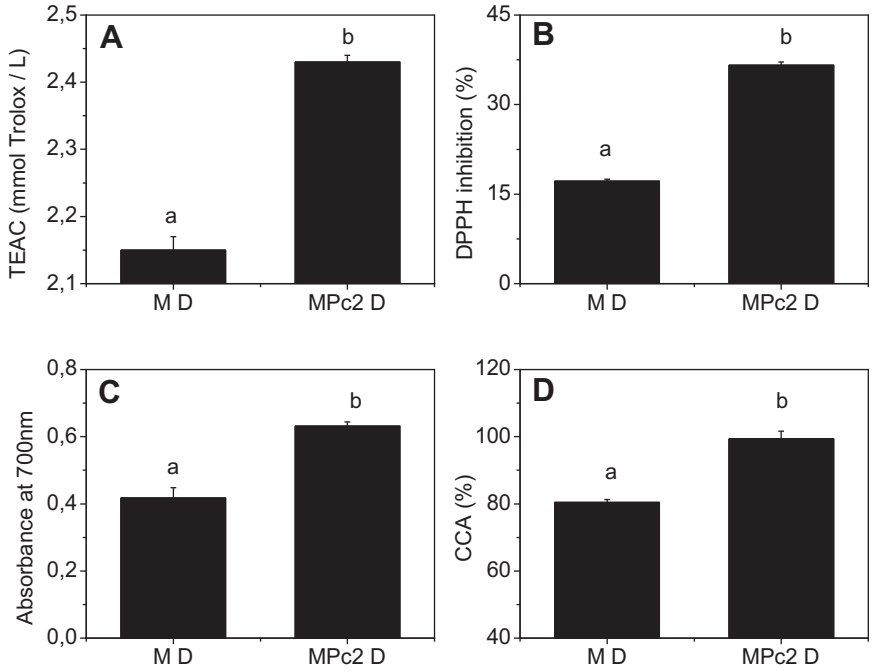

Fig. 2. Trolox equivalent antioxidant capacity (TEAC) (A), DPPH radical inhibition (B), reducing power (C) and copper-chelating activity by assay of β-carotene oxidation at 120 min (D) of maize extruded products dialyzate (M_D) and commercial maize grits/*P. columbina* blend dialysate (96.5:3.5; MPc_{2D}). Data are expressed as mean ± SD. All samples were analyzed in triplicate; letters with $P < 0.05$ means significant differences.

antioxidant and ACE inhibitor peptides. Therefore, the addition of red seaweed to maize grits provides other proteins than maize, like phycobiliproteins which could act as an important source of bioactive peptides. It is noteworthy that bioactive peptides generated from MPc_2 not only were more active than those generated from M but also, they were bio-accessible.

Finally, antioxidante and ACE inhibitory activity observed in MPc_{2D} may be due to the sum of the activites of polyphenols and peptides provide by *P. columbina.*

4. Conclusions

There are some works that add edible seaweed to improve the bio-functional and nutritional qualities of cereal product like pasta, but so far, there has been no research focused on the production of expanded maize products added with red seaweeds and the study of bio-accessibility of bioactive compounds such as ACE inhibitors and antioxidants provide by algae. Extruded maize added with red seaweed showed higher dialyzability of ACE inhibitor compounds, total phenolic content and antioxidant capacity (DPPH, TEAC, power reduction and Copper-chelating activity) than extruded maize, which imply that snack added with algae supply bio-accessible and bioactive compounds. Results about bio-accessibility of bioactive compounds provided by red edible seaweeds may help food technologists to tailor new bio-functional foods, such as functional snacks and to add value to the traditional maize extrusion process.

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