Pak. J. Bot., 49(1): 161-168, 2017.

NUTRITIONAL AND PHENOLIC CHARACTERIZATION OF *MORINGA OLEIFERA* LEAVES GROWN IN SINALOA, MÉXICO

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Abstract

The present study shows the results of a research done on the chemical composition, minerals, fatty acid (FA) and phenolic compounds contents, and antioxidant capacity in two variants of *Moringaoleifera* leaves grown in Sinaloa, Mexico. The leaves of the two variants of *Moringa oleifera* revealed high protein content (31.69-36.83%, db). The mineral analysis of dried leaves showed the following minerals content: calcium=15.08-15.58 g•Kg-1, magnesium=3.55-3.62 g•Kg-1, sodium=1.42-1.54 g•Kg-1, potassium=2.69-3.01 g•Kg-1, iron=120.19-105.31 ppm, manganese=54.5-59.77 ppm, zinc=56.48-46.89 ppm and copper=10.92-7.96 ppm. The main fatty acids were linolenic acid (62.72-66.19%) having the highest value, followed by palmitic (17.13-17.26%), linoleic (9.65-7.64%), oleic (3.24- 2.14%)**,** and stearic acid (2.71-2.66%). The total dietary fiber (TDF) content was found at levels of 29.16-29.11% (db). On average, the three most abundant amino acids in both variants were tyrosine, glutamate, aspartate, histidine, phenylalanineand leucine acid. The two Moringa variants showed total phenolic and flavonoids contents of 71.08-76.63 mg EAG•g-1 (db) and 55.7-60.3 mg QE•g-1 (db), respectively. The antioxidant capacity of the ORAC assay was 154.71-182.31 μmol ET•g-1 (db), while the DPPH assay value was 86.82-87.92%. Finally, gallic, chlorogenic, caffeic, coumaric and ferulic acids were found in a concentration range of 1.238-1.193, 0.585-0.568, 0.503-0.485, 1.090-1.114 and 0.536-0.518 mg•g-1, respectively. Our results show that *Moringa oleifera* leaves are an important source of protein, fatty acids, minerals and phenolic compounds that could be used in food, nutraceutical and pharmaceutical industry.

Key words: Moringa, Nutrients, Nutraceuticals, Antioxidants, Phenolics

Abbreviations

LPM, Moringa Long Pod; SPM, Moringa Short Pod; DPPH, 1, 1-difenil-2-picrilhidrazil; ORAC, Oxygen radical absorbance capacity.

Introduction

Moringa oleifera is the most widely known and studied species of the genus Moringa, belongs to the *Moringaceae* family. The genus Moringa includes 13 species: *M. arborea*, *M. concanensis*, *M. drocanensis*, *M. drouhardii*, *M. hildebrandtii*, *M. pygmeaeM. pilgrim*, *M. rospoliana*,*M. ovalifolia*, *M. stenopetala*, *M. rivae*, *M. oleifera***,** and *M. borziana* (Olson, 2002). This tree is native to the Himalayas, India northeast, Bangladesh, Afghanistan**,** and Pakistan. It is spread out around the planet such as tropical and subtropical weather in Central America. It is known by various common names: Benzolivo, Mlonge, Mulangay, Stick, Kelor, Moringa, Reseda, Nébéday, and Sajna Saijhan, etc. (Pérez *et al.*, 2010). Moringa is quickly gaining attention because it is a nutritional and caloric source and has a low cost of production. It has been used as Food Supplement because of its proven nutritional and nutraceutical wealth. Recently, there has beena lot of interest in this plant due to the results of plenty research and relevant publications on the topic that highlights its nutritional and medicinal properties (Olson & Fahey, 2011). Furthermore, it has been evaluated as a useful food ingredient and as a product such as wood, charcoal, lubricating oil**,** and water clarifier (Folkard & Sutherland, 1996).

Moringa tree has a high potential for cultivation in Mexico and many parts of tropical America because of its unique combination of characteristics (Pérez *et al.*, 2010). One of the most attractive features of Moringa is the high protein content in their leaves (Yang *et al.*, 2006). Moyo *et al.* (2011) highlighted the protein content of 30.3% in dry sheets; most of this seems to be directly assimilated into the human body. Furthermore, the amino acid content demonstrates a desirable nutritional balance. These results show that the leaves contain a substantial amount of nutrients and can be included in diets as a supplement to our daily nutritional requirements (Oduro *et al.*, 2008). Although Moringa is characterized by its high content of protein and vitamins, it also has light levels of antinutritional substances (Makkar & Becker, 1996). Nevertheless, such substances are not found in lethal doses; therefore, they cannot cause negative side effects (Olson & Fahey, 2011). Makkar & Becker (1996) showed that Moringa leaves do not contain significant levels of antinutritional substances; in this sense, the leaves can be considered free of lethal doses or adverse effects on human health. However, the content of phenolic compounds is affected by environmental conditions, the variety of the plant,andthe ripening stage of the leaves. In Sinaloa, Moringa exists in the wild and as low technology crops with possible agronomic potential (Pérez *et al.*, 2010).

Two variants have been identified: plants whose fruit ranges in size from 15 to 25 cm recognized as Moringa short pod (SPM); and another one, whose fruit varies from 30 to 80 cm called Moringa long pod (LPM). However, nutritional composition, nutraceutical and antioxidant capacity of these materials are unknown. Therefore, additional analysis is neededto identify significant genotypes in the production and quality of the leaves in order to reach further growth of these plants by clonal reproduction and genetic improvement of species (Steinitz *et al.*, 2009). The identification, propagation, selection and domestication of materials with features of interest are necessary to determine the agronomic potential of Moringa (Pérez *et al.*, 2010). Thus**,** the objective of this study was to characterize the nutraceutical and nutritional properties of two variants of Moringa grown in Sinaloa: The long pod (LPM) and the short pod (SPM).

Materials and Methods

Plant material: Leaves from long pod and short pod trees of two variants of *Moringa oleifera* were collected in November 2013 in Imala, Culiacan, Mexico (24 ° 51' 23" N, 107 ° 12' 56" W, at 160 m ASL). Plant material was washed in a 150 ppm chlorine solution and then dried in an electric oven at 55-60ºC for 6 h to a constant weight for moisture determination. Finally**,** pulverized in a fine mill to get Moringa leaves flour.

Preparation of methanolic extract: It was made using 1g of a powdered sample of dried leaves mixed with 10 mL of methanol. The mixture was homogenized in a tissue homogenizer Ultra-Turrax for one minute and incubated at 200 rpm for 2 h, centrifuged at 8000 g for 20 min and 4°C. The supernatant (extract) was recovered and stored at 8°C for their subsequent analysis.

Chemical composition: The methods used for Analysis of total crude protein, moisture, fat, carbohydrates, ash, and crude fiber followed the recommendations of The Official Methods of Association of Official Analytical Chemists (AOAC) (Anon., 1998). The total carbohydrates were determined by the difference method [100 − (proteins + fats + moisture + ash in percentage)] (Valdez-Solana *et al.*, 2015).

Mineral analysis: Minerals content was quantified according to the official AOAC method No. 955.06 (Anon., 2005). After acid digestion of the ash, the sample was filtered and reached to 100 mL with deionized water. Using an atomic absorption spectrophotometer, the absorbance for each mineral was measured at specific wavelengths: Ca (422.7nm), Na (589.6nm), K (769.9nm), Mg (285.2nm), Mn (279.5nm), Fe (248.3nm), Cu (324.7nm) and Zn (213.9nm). A calibration curve of reference standards of known concentration was used for each mineral. The concentration of each of the minerals was calculated in ppm.

Determination of amino acids composition: The composition of amino acids was determined by HPLC according to Vázquez *et al.* (1995) with minimal variations. Sample Preparation: Hydrolysis**;** 3 mg of Moringa flour defatted were weighed into hydrolysis tubes and 3 mL of 6M HCl were added. Then the tubes were sealed under vacuum for 3 minutes. Subsequently, the tubes were placed in a dry bath for hydrolysis at 120°C for 24 h. Extraction**;** The hydrolysate was evaporated at 65°C and washed using 3 mL of distilled water to remove HCl; then amino acids were recovered using 1 mL of sodium citrate buffer pH 2.2 and stored at 0°C until derivatization and chromatographic quantification.

Derivatization of the sample: Aliquots of 100 µL of hydrolysate, 40 µL of internal standard 100 mM were mixed and filled to a volume of 1 mL with sodium citrate buffer pH. Subsequently, 250 µL of these dilution were withdrawn and mixed with 250 µL of OPA (O-phthalaldehyde) in a syringe for liquid chromatography, followed by filtration (nylon 0.2 um). A 10 µL of the derivative was injected into chromatograph (HPLC) model 9012 (Varian, Palo Alto, CA). Timing from derivatization to sampling for injection should not exceed 2 min.

Amino acids profile analysis by RP-HPLC: The amino acid profile was performed by liquid chromatography high-resolution reversed phase model 9012 (Varian, Palo Alto, CA); Varian fluorescence detector, injector capacity 10 µL and column (Restek Pinnacle II, C18, 5 um 150 mm x 4.6 mm). The mobile phase was solvent A: sodium acetate buffer (0.1 M, pH 7.2); methanol and tetrahydrofuran were used as an organic modifier (900: 95: 5 v/v/v) (Sigma Chemical Co.), solvent B: methanol (Sigma Chemical Co.). The gradient flow was 1.5 mL min-1 (min/A%: B%): 0/100: 0, 0.5/80: 20, 7.5/80: 20, 10/50: 50, 15/50: 50, 18/20: 80, 20/20: 80, 23/0: 100, 25/100: 0, 30/100: 0. The detection was by fluorescence using the wavelengths of emission 455 nm and excitation 340 nm. The column heater was maintained at 30°C. The identification and quantification of amino acids were performed by comparing the retention time of the sample against amino acid standards of known concentration using the computer program (version 4.0 Chromatography Varian Star).

Determination of fatty acids composition: Fatty acids were determined by standard method with some modifications as reported by Folch *et al.* (1957) and the AOAC 963.22 (Anon., 1998). Fat removal. 10 g of sample were weighed, placed in a 250 mL Erlenmeyer flask, mixed with 60 mL of Folch reagent (1 volume of methanol plus two volumes of chloroform) and homogenized. Subsequently, vacuum filtered on a Buchner funnel, the residue was mixed with 50 mL of Folch reagent and homogenized again. The residue was washed with 50 mL of Folch reagent, the flask was cleaned and vacuum filtered again. The filtrates (60 + 50 + 50 mL) were mixed in a dropping funnel and added **to** 40 mL of 0.73% sodium chloride, stirred vigorously and allowed to settle overnight. After 24 hours**,** the lower phase (organic) (F1) was decanted and filtered through anhydrous sodium sulfate. The filtrate was recovered in a round flat bottom flask. The upper phase (F2) was washed with 50 mL of a mixture of 20% NaCl (0.58%) and 80% of Folch reagent. It waved and left to stand for 2 hours. Then it was decanted and filtered through anhydrous sodium sulfate to get F3. F1 and F3 were mixed, evaporated and dried in the

rotary evaporator. Methylation: after evaporating the chloroform, 0.5 g of sodium hydroxide and three glass beads were added to methanol. The flask was placed in a cooling rosary and refluxed for 10 min. Subsequently, Boron trifluoride (BF3) was added to the top of the condenser and refluxed for another 5 min. Then, 4 ml of heptane were added underwent reflux for 2 min. The ball flask and the contents were added in a test tube, and a saturated NaCl was added (stir gently) until it changed its milky white color. After that, a pinch of sodium sulfate was added to remove the fatty acids. The upper phase was taken and filtered through a Pasteur pipette previously packed with fiberglass**,** and the filtrate was recovered in a vial of 2 ml. The vial was kept in a nitrogen atmosphere and was later placed in the freezer. The organic phase (1 mL) was filtered through a 0.45 µm membrane. A sample (1 µL) was injected into the gas chromatography system. All samples were analyzed in triplicate. The equipment used was a gas chromatograph (Varian CP-3800, USA) with a flame ionization detector (FID) equipped with a 30 m x 0.32 mm ID, 0.25 mm Omegawax 320 column (Supelco, USA). Helium was used as carrier gas at a flow rate of 3 mL•min-1. The oven temperature was kept at 140°C for 5 min, preset at a maximum temperature of 240°C at a rate of 4°C for 1.5 min. Both the injector and detector temperature were set at 260°C. For identification and quantification of fatty acids, the retention times of the sample were compared with those of a standard mixture consisting of 37 fatty acid methyl esters (Supelco, Bellefonte, USA). The results were expressed in percentage of fatty acid contained in the sample.

Total soluble phenolics: The method developed by Folin & Ciocalteu (Swain & Hillis, 1959) was used. The extract was oxidized with Folin-Ciocalteu reagent and the reaction was quenched after 3 minutes with sodium carbonate. The absorbance of the resulting blue was measured at 725 nm after 120 min incubation protected from light. Gallic acid was used as standard at concentrations of 0 to 0.4 mg•mL-1 to calculate the results.

Total flavonoids: The method of aluminum chloride was used to determine the total flavonoid content of the extracts, as reported by Ebrahimzadeh *et al.* (2009), with slight adjustments. An aliquot of 20 µL of the prepared extract was taken and placed in a 96-well plate. Subsequently added 112 µL of distilled water plus 60 µL of methanol. Next, 4 µL of 10% aluminum chloride were added plus 4 µL of 1 M potassium acetate. Incubation of the sample was performed in the dark during 30 min. After that, absorbance was read at 415 nm using a Synergy HT Microplate Reader (BioTek, Inc., USA). A standard calibration curve was generated using known concentrations 415 nm quercetin. The concentration of flavonoids in the test samples was calculated using a standard curve and expressed as mg equivalent•g-1 quercetin sample.

Antioxidant capacity by DPPH method: The antioxidant capacity was measured using the percentage of inhibition of the DPPH radical determined by the DPPH (1,1-diphenyl-2-picrylhydrazyl) method developed by Brand-Williams *et al.* (1995), using a microplate reader Synergy HT (BioTek, Inc. EEU), measuring absorbance at 515 nm. The results were expressed as a percentage of DPPH radical inhibition.

Antioxidant capacity by ORAC method: 96-well dark-microplate (Costar, USA), which was added to 25 µL of extracts dilutions (in phosphate buffer) of *Moringa oleifera* leaf, 25 µL of a target were used, and 25 µL of the Trolox standard curve. The plate was placed in a microplate reader model Synergy HT (BioTek, Inc., USA) which was at 37°C in the time of incubation. Microplate reader dispensed in each of the plate 200 µL of 0.96 mM fluorescein and 75 µL of 2,2'-azobis, 2-amidino-propane dihydrochloride (AAPH) 95.8 mM. The reaction started after the last reagent was added measuring the fluorescence at 70 sec intervals for 70 min at a wavelength of 485 nm for excitation and 580 nm emission. The calculations were performed using the linear regression equation of the standard curve and the area under the curve fluorescence loss. Results are expressed as µmol Trolox equivalent (mol TE)•g-1 (Huang *et al.*, 2002).

Phenolics UPLC profile: Moringa components based on different types of free or conjugated chemical interactions by UPLC chromatography with diode array detector (DAD) were separated by liquid chromatography (Corral-Aguayo *et al.*, 2008). Samples (1 mL) of the extract were homogenized in sodium phosphate buffer 50 mM (10 mL) with an ULTRA-TURRAX® T 25 digital (IKA Works, North Carolina, USA). The homogenate was centrifuged at 10000 rpm for 10 min at 4°C on a Thermo Scientific 120 centrifuge (Thermo Fisher Scientific, Inc., Waltham, MA, USA) and the supernatant to a separatory funnel Kimax® No. 22 was transferred. The residue was resuspended in 10 mL of ethyl acetate, homogenized, and centrifuged again under the same conditions and the supernatant was transferred to the funnel. This procedure was repeated two more times until the residue has no coloration. The various supernatants were mixed and the time needed to phase separate in the funnel, and then the upper phase was collected and expected, the little flow solvent evaporated nitrogen and stored at -20°C until analysis. For analysis of UPLC-DAD**,** a sample of the extract obtained above was added to 4 ml of ethyl acetate. The supernatant was filtered through a Sep-Pak C18 cartridge. An aliquot of 20 uL was injected into a liquid chromatograph (Acquity-UPLC) (Water Inc, USA) with a diode array detector (PDA). An Acquity UPLC BEH C18 column of 100 mm length x 2.1 mm in diameter with a particle size of 1.7 microns and a pore size of 100 Å was used. The mobile phase "A" used was a mixture of 95% Water, Methanol 2% and 3% formic acid, and phase "B" was a mix of 95% Methanol, Water 2% and 3% formic acid , using a gradient: 0 min, 90% A, 10% B (0.3 mL • min); 3 min, 75% A, 25% B (0.3 mL • min); 5 min, 70% A, 30% B (0.25 mL • min); 9 min, 60% A, 40% B (0.3 mL • min); 11 min, 50% A, 50% B (0.3 mL • min); 12 min, 0% A, 100% B (0.3 mL • min); 13 min, 0% A, 100% B (0.3 mL • min); 15 min, 90% A, 10% B (0.3 mL • min); 16 min, 90% A, 10% B (0.3 mL • min) with a flow of 0.3 mL•min-1. The reading was performed at 190 and 420

nm. Quantification of phenol was conducted using standard calibration curves of chlorogenic acids, gallic, ferulic, coumaric, and t-cinnamic acid (Sigma Chemical Co., USA) using concentrations from 5 to 50 µg • mL-1.

Statistical analysis: A completely two-factor randomized experiment with ten replicates, and three replicates for each variable, was used. Data were analyzed in Minitab 16.

Results and Discussion

Chemical composition: Table 1 shows the values of the proximate composition (moisture, lipid, total ash, protein, crude fiber and carbohydrates) for both long and short pod Moringa. Highlights its protein content and ash, being considerably higher in LPM regarding SPM. These values are similar to those reported by Alfaro (2008) in leaves of Moringa, 33.50 ± 1.10% protein and 8.78% ash. These results support the potential of Moringa as a source of dietary protein described by other authors. Although crude protein levels found in this study are higher than those reported by other authors Moringa plants from other places in Mexico (the states of Sonora, Michoacan, and Coahuila) (Sánchez-Machado *et al.*, 2010; Valdez-Solana *et al.*, 2015). These variations can be caused by weather variations, crop management, if they are cultivated or wild, the state of maturity of the plant at the time of collection, and the type of post-collection processing. Therefore, Moringa leaves of both variants are a good potential for additional protein source in the human diet.

Amino Acids Profile: The amino acids profile **in** both variants of *Moringa oleifera* (Table 2) shows that the total amino acid concentrations are in the range of 0.98% to 3.95%. SPM and LPM had similar levels of amino acid profile and no significant differences. The total amino acid content revealed that the essential amino acids represented 40% of LPM and 51 % of SPM. The amino acids that were concentrated in a higher proportion in both variants are glutamic acid, aspartic acid, histidine, tyrosine, leucine, and arginine; while the lowest concentrations are methionine serine and lysine. The amino acid profile in *Moringa oleifera* leaves were tested in earlier studies (Makkar & Becker 1996; Sánchez-Machado *et al.*, 2010; El-Massry *et al.*, 2013). The composition of amino acids as aspartic acid, glutamic acid, histidine, glycine, arginine, alanine, tyrosine, methionine, valine, phenylalanine, isoleucine, and leucine, except lysine, show a variation of the published data by at least one of the mentioned researchers. Both variants of *Moringa oleifera* contain high percentages of essential amino acids except for methionine, commonly deficient in green leaves. It could be possible that the variations in the amino acid composition of the leaves are influenced by the quality of the protein and the origin of the plant (cultivated or wild).

Lysine content of Moringa leaves of both variants contains an acceptable level of lysine amino acid that is frequently found in low concentrations in vegetables, legumes and cereals exception.

In general, the content of essential amino acids of the evaluated materials cover the requirements recommended by WHO and FAO to a child (3-10 years), vulnerable population group daily intake when it comes to availability of quality protein. Therefore, Moringa could be incorporated into the human diet, particularly for children to prevent or cut malnutrition.

Mineral Composition: Eight essential minerals are identified i.e. iron (LPM: 120.19 ± 8.41 ppm and SPM: 105.31 ± 8.89 ppm), zinc (LPM: 56.48 ± 5.13 ppm and SPM: 46.89 ± 4.88 ppm), and copper (LPM: 10.92 ± 0.91 ppm and SPM: 7.96 ± 0.34 ppm) in Moringa leaves (Table 3). Potassium, calcium, magnesium, and sodium that are nutritionally important, levels of daily intake requirements of the general population. LPM and SPM showed significant differences in micronutrients (i.e., Cu, Zn and Fe) but not in macronutrients. Meanwhile, SPM is higher in Mn concentration than LPM. From these results, Moringa could help to prevent diseases related to malnutrition.

Table 1. Proximal content of *Moringa oleifera* leaf.

Determination (%)	Moringa variants	
	Long pod	Short pod
Protein	36.83 ± 2.16^{b}	31.69 ± 2.25^{a}
Fat	8.16 ± 0.50^{a}	7.57 ± 0.96^{a}
Crude Fiber	3.37 ± 1.36^{a}	4.03 ± 2.14^{a}
Ash	6.56 ± 0.57^{b}	8.03 ± 0.48^{a}
Moisture	3.79 ±0 .48^{a}	3.88 ± 0.18^{a}
Total solids	96.21 ± 0.48^{a}	96.12 ± 0.18^{a}
Carbohydrates	41.29 ± 0.54^{a}	44.79 ± 4.10^{a}

Different letter in the same row indicates significant difference (p>0.05)

Table 2. Amino acids content of *Moringa oleifera* (g AAS•100g^{-1}).

Amino acid (%)	Moringa variants	
	Long pod	Short pod
Aspartate	2.04 ± 0.79^{a}	3.12 ± 0.67^{a}
Glutamate	2.64 ± 0.71^{a}	3.53 ± 0.72^{a}
Serine	1.88 ± 1.48^{a}	0.98 ± 0.23^{a}
Histidine *	2.59 ± 1.14^{a}	2.94 ± 0.47^{a}
Glycine + Treonina	1.64 ± 0.49^{a}	2.30 ± 0.20^{a}
Arginine	1.53 ± 0.54^{a}	0.91 ± 0.13^{a}
Alanine	0.90 ± 0.25^{a}	1.58 ± 0.37^{a}
Tyrosine *	3.95 ± 0.99^{a}	3.21 ± 1.09^{a}
Methionine *	1.45 ± 0.89^{a}	1.05 ± 0.39^{a}
Valine *	1.79 ± 0.56^{a}	1.42 ± 0.24^{a}
Phenylalanine *	2.29 ± 0.30^{a}	1.69 ± 0.04^{a}
Isoleucine *	1.48 ± 0.81^{a}	1.09 ± 0.20^{a}
Leucine *	2.06 ± 0.68^{a}	2.18 ± 0.51^{a}
Lysine *	1.37 ± 1.14^{a}	0.98 ± 0.41^{a}

Different letter in the same row indicates significant difference (α<0.05)
* Essential amino acid

Table 3. Mineral contents of dried *Moringa oleifera* leaves.

Mineral	Moringa variants	
	Long pod	Short pod
Na*	1.42 ± 0.12^{a}	1.54 ± 0.15^{a}
K*	2.69 ± 0.29^{b}	3.01 ± 0.30^{a}
Mg*	3.55 ± 0.31^{a}	3.62 ± 0.34^{a}
Ca*	15.08 ± 1.71^{a}	15.58 ± 1.16^{a}
Cu**	10.92 ± 0.91^{a}	7.96 ± 0.34^{b}
Mn**	54.50 ± 5.52^{b}	59.77 ± 4.42^{a}
Zn**	56.48 ± 5.13^{a}	46.89 ± 4.88^{b}
Fe**	120.19 ± 8.41^{a}	105.31 ± 8.89^{b}

Different letter in the same row indicates significant difference (p>0.05).
*Macro-elements (g•Kg^{-1}), **Micro-elements (ppm)

Table 4. Fatty acid composition (% of total fatty acids).

Fatty acid (%)	Moringa variants	
	Long pod	Short pod
Lauric (C12:0)	0.16 ± 0.03[a]	0.18 ± 0.09[a]
Myristic (C14:0)	0.78 ± 0.07[a]	1.05 ± 0.41[a]
Palmatic (C16:0)	17.13 ± 0.77[a]	17.26 ± 0.11[a]
Palmitoleic (C16:1)	0.27 ± 0.03[a]	0.23 ± 0.01[a]
Heptadecanoic (C17:0)	0.18 ± 0.00[a]	0.19 ± 0.01[a]
Stearic (C18:0)	2.71 ± 0.34[a]	2.66 ± 0.16[a]
Oleic (C18:1 c+t)	3.24 ± 0.99[a]	2.14 ± 0.50[a]
Linoleic (C18:2 c+t)	9.65 ± 1.51[a]	7.64 ± 1.06[a]
Linolenic (C18:3 n3)	62.72 ± 3.31[a]	66.19 ± 1.23[a]
Arachidic (C20:0)	0.32 ± 0.05[a]	0.37 ± 0.11[a]
Arachidonic (C20:4)	0.18 ± 0.04[a]	0.22 ± 0.05[a]
Behenic (C22:0)	0.48 ± 0.07[a]	0.57 ± 0.21[a]
Tricosanoic(C23:0)	0.20 ± 0.04[a]	0.25 ± 0.06[a]
Lignoceric (C24:0)	0.74 ± 0.06[a]	0.80 ± 0.21[a]
Satured	22.70 ± 0.44[a]	23.59 ± 1.42[a]
Monounsaturated	3.51 ± 1.01[a]	2.37 ± 0.50[a]
Polyunsaturated	72.55 ± 1.78[a]	74.05 ± 1.42[a]

Different letter in the same row indicates significant difference ($\alpha<0.05$)

Table 5. Phenolic compounds and antioxidant activity of methanolic leaf extracts of *Moringa oleifera*.

Determination	Moringa variants	
	Long pod	Short pod
Flavonoids total (mg EQ•g^{-1})	60.26 ± 7.21[a]	55.703 ± 7.00[a]
Total phenolic (mg GAE•g^{-1})	76.63 ± 10.63[a]	71.08 ± 12.05[a]
ORAC (µmol ET•g^{-1})	154.71 ± 36.95[a]	182.31 ± 32.68[a]
DPPH (µmol TE•g^{-1})	981.22 ± 7.58[a]	968.97 ± 23.87[a]
DPPH (%)	87.92 ± 2.15[a]	86.82 ± 0.68[a]

Different letter in the same row indicates significant difference ($p>0.05$)

mg EQ•g^{-1}	mg Quercetin equivalents per g dry weight
mg GAE•g^{-1}	mg gallic acid equivalents per g dry weight
µmol ET•g^{-1}	µmol Trolox equivalents per g dry weight
% Inhibition	Inhibition of DPPH radical

Table 6. Phenolic acids profile (mg•g^{-1}).

Phenolic acid	Moringa variants	
	Long pod	Short pod
Gallic	1.238 ± 0.011[a]	1.193 ± 0.042[b]
Chlorogenic	0.585 ± 0.006[a]	0.568 ± 0.019[b]
Caffeic	0.503 ± 0.005[a]	0.485 ± 0.017[b]
Coumaric	1.090 ± 0.019[a]	1.114 ± 0.035[a]
Ferulic	0.536 ± 0.006[a]	0.518 ± 0.016[b]

Different letter in the same row indicates significant difference ($\alpha<0.05$)

Fatty acids content: 14 fatty acids were identified in both variants (Table 4). Linolenic acid was found in high amount followed by palmitic acid; both represent 80% of total fatty acids, similar to that presented by Sánchez-Machado *et al.* (2010). The material showed the presence of linoleic acid and linolenic acid as essential fatty acids. The occurrence of polyunsaturated fatty acids was increased by 96% as compared to monounsaturated fatty acids. The consumption of polyunsaturated fatty acids caused decreased levels of total and LDL cholesterol, having a cardioprotective role of these compounds. That effect is because they are antiarrhythmic agents that improve vascular endothelial function and descend blood pressure, which inhibits platelet aggregation. That is associated with an impediment to the formation of plaques on the inside of blood vessels and adherence to endothelium. It has been observed that people whose diets are rich in polyunsaturated fatty acids show a low incidence of cardiovascular disease.

Total soluble phenolics and flavonoids: SPM and LPM had similar levels of total phenols and flavonoids (Table 5), and there were no significant differences ($P>0.05$) in SPM and LPM. The phenol content was higher than previously reported for Moringa (45.21 mg GAE•g-1) (Adisakwattana & Chanathong, 2011). In the case of total flavonoids, results were greater than those reported in 37.0 mg GAE•g-1 (Saikia & Upadhyaya, 2011). The values indicate that 78% of total phenolic compounds corresponded to flavonoids. Phenolic compounds or polyphenols are derived from the secondary metabolism of plants. These compounds are commonly found in plants and have been extensively exploited because of its multiple biological activities, including antioxidant effects. Flavonoids and phenolic acids are receiving increased attention as potential antioxidants, mainly due to its strong presence in a significant number of consumer foods (García-Cruz *et al.*, 2012). In phenolics and flavonoids, at least, one hydroxyl ion is substituted with an aromatic ring forming chelate complexes with metal ions thus are readily oxidized. They, therefore, serve as great units to donate electrons. The antioxidant activity of the phenolic compounds in the above reports is shown to be mainly due to its redox properties, allowing them to act as reducing agents, hydrogen donors, or singlet oxygen quenchers (Sankhalkar, 2014). It turns out that most researches done conclude there is a correspondence between phenolic compounds and antioxidant activity in plants. This study confirms the antioxidant potential in vitro of crude methanolic extracts, whose activity is likely to be due to phenolic compounds and flavonoids sample**;** therefore, *Moringa oleifera* leaves can be considered as a source of antioxidant compounds with activity sufficient to reduce the activity of free radicals and reactive oxygen species.

Antioxidant activity: The results of DPPH and ORAC antioxidant activity were similar between LPM and SLM with no significant ($P<0.05$) differences observed. As seen in Table 5, extracts of both variants showed ORAC antioxidant capacity similar to the 121 µmol TE•g-1 reported by Yang *et al.* (2006). Also, the DPPH free radical protocol was used to evaluate the ability of the extracts of the leaves of the two Moringa variants to eliminate free radicals, forming stable diamagnetic molecules (Table 5) (Singh *et al.*, 2009). The DPPH assay was expressed in terms of antiradical power and values ranged from 86.82% for LPM to 87.92% for SPM, finding in both methanol extracts of *Moringa oleifera* a good scavenger of free radicals similar to the 86.77% DPPH reported by Singh *et al.* (2009). It was observedthe relationship between antioxidant activity and phenolic compounds in both variants. One of the most important factors that determine the antioxidant activity of the polyphenols is the degree of hydroxylation and the position of the hydroxyls in the molecule. The flavonoids due to oxygen heterocycle are more active than non-flavonoid molecules. In turn, solubility and steric effects of each molecule may be affected by the type of structure of such molecule**s**, as glycosylated derivatives of other adducts which can increase or decrease of antioxidant activity (Jahan *et al.*, 2015). The flavonoid compounds commonly found in plants as glycosides, but the action of

enzymes or some processes can release the corresponding aglycone. The activity of phenolic acids is also based on the hydroxyl groups of the aromatic ring and the binding of these compounds to organic acids and sugars to form esters. The mechanisms by which these compounds act vary depending on the concentration and types of compounds present in foods (Zapata, 2007; Jahan *et al.*, 2015). Our results suggest the potential of Moringa as a functional ingredient in foods that may also aid in the prevention of illnesses related to oxidative stress.

Phenolic acids UPLC profile: The Phenolic acids profile **in** both variants of the *Moringa oleifera* (Table 6) shows that the total phenolic acid concentrations are in the range of 0.485 mg•g-1 to 1.238 mg•g-1. The presence of phenolic compounds is shown in (Fig. 1): gallic, chlorogenic, caffeic, coumaric**,** and ferulic, which peaks occurred in 1,099, 3,074, 3,492, 4,905 and 5,726 min for LPM, respectively. For SPM, these compounds**,** whose peaks presented at 1.099, 3.064, 3.484, 4.896, and 5.717 min respectively**,** were also confirmed (Fig. 2). The concentrations of phenolic acids are within the ranges as reported by Prakash *et al.* (2007), except ferulic acid. The difference can be explained since chelates plant produces a variety of secondary metabolites, defense mechanisms against pests, predators and different levels of water stress. Moreover, Leone *et al.* (2015) report the presence of ferulic acid at levels of 0.0661 to 0.0969 mg•g-1 lower concentration tan that found in this study. Similarly, the amount of ferulic acid found in the leaves of *Moringa oleifera* was comparable with the amount found in some whole grains like brown rice and cornmeal, but far below the amounts found in various grains, fruit, and vegetables such as peanuts, orange, eggplant, and spinach (Zhao and Moghadasian, 2008). Therefore, Moringa can be considered a product with possible application in the food, nutraceutical and pharmaceutical industries.

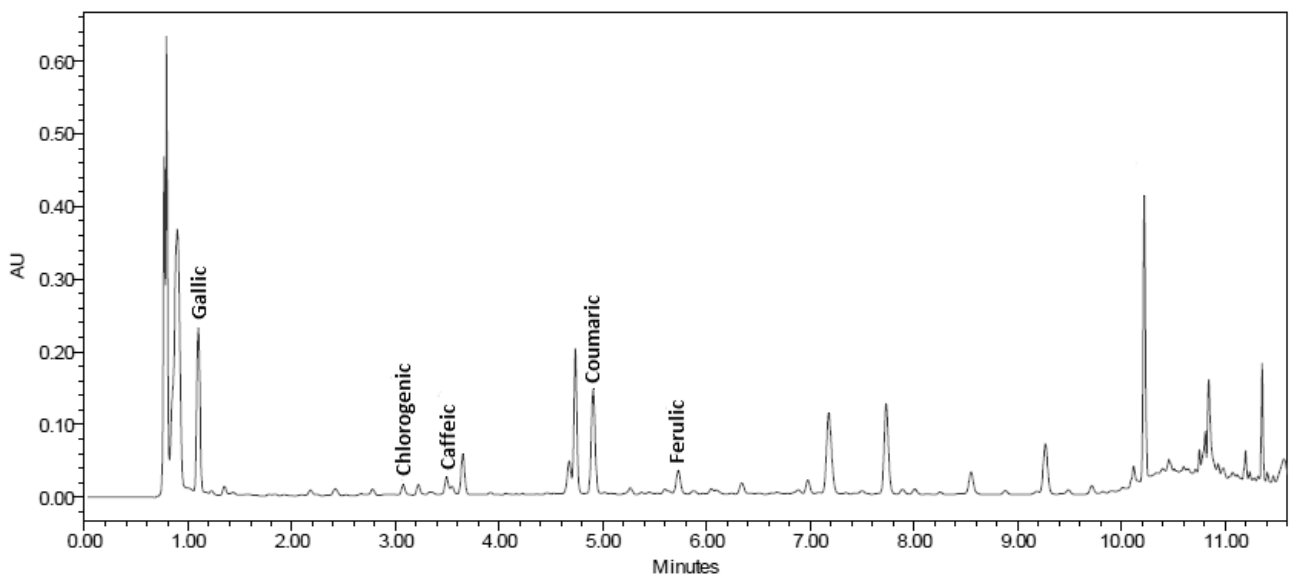

Fig. 1. UPLC-DAD chromatogram of methanolic extracts of *Moringa oleifera* leaves (LPM).

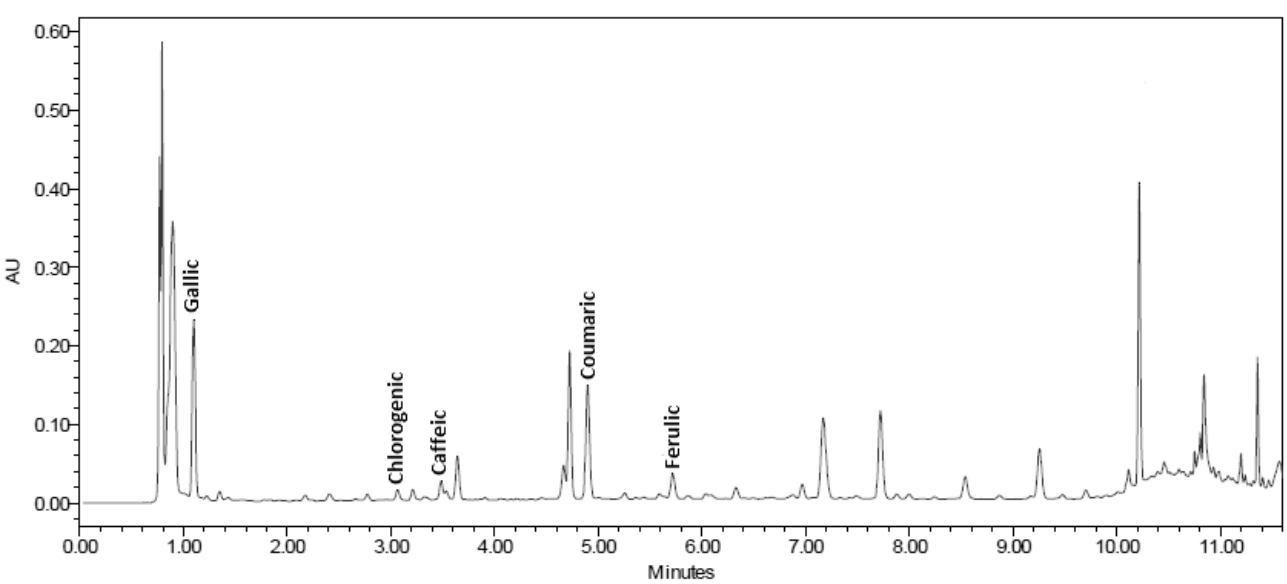

Fig. 2. UPLC-DAD chromatogram of methanolic extracts of *Moringa oleifera* leaves (SPM).

Conclusion

The *Moringa oleifera* leaves variants show potential to be used as functional ingredients for human food; this is shown by its protein content and amino acids profile, also both variants have a high content of dietary fiber and low lipid content. Unsaturated fatty acids are present in both variants. The study showed that the concentration of phenolic compounds in the extracts *Moringa oleifera* is enough to be considered as a potential antioxidant supplement source. Therefore, Moringa leaves can be considered a product with potential application in the food, nutraceutical and pharmaceutical industries, which can have positive financial and social benefits to the population.

Acknowledgment

We gratefully acknowledge the Laboratory of Functional Foods and Nutraceuticals, Centro de Investigación en Alimentación y Desarrollo, AC (CIAD-Unidad Culiacán), Miss Laura Contreras-Angulo, Mr. Alexis Emus-Medina and Miss Briseida Pérez-Meza, for their help and excellent technical support. Authors would also like to thank CONACYT-México for the scholarship granted.

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(Received for publication 28 December 2015)