Morphological characteristics, chemical composition and antioxidant activity of seeds by four wild *Opuntia* species from North of Mexico

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ABSTRACT

Morphological characteristics and chemical composition of seeds of four species of *Opuntia* genus endemic to the Samalayuca Valley were evaluated in this study. Morphology and chemical composition of the four species were clearly different. Seeds of *O. polyacanta var. arenaria* presented the highest values of area, length and width. However, *O. phaeacantha* showed the highest values in palmitoleic acid (1.34 ± 0.12%), stearic acid (5.60 ± 0.47%), oleic acid (19.42 ± 1.04%), alpha linolenic acid (2.42 ± 0.33%), arachidic acid (0.61 ± 0.11%), and eicosenoic acid (1.74 ± 0.36%); but, this species showed the lowest values in linoleic acid (54.35 ± 2.91 %) content. In conclusion, seeds of *O. phaeacantha* could be an important new source of health-promoting polyunsaturated fatty acid, and its use in arid and semi-arid regions should be encouraged.

***Keywords*:** Morphological seed, Chemical composition, Opuntioideae, fatty acids, antioxidant capacity.

INTRODUCTION

The *Opuntia* genus of plants is native to the American continent. Species of this genus are found to grow naturally at different altitudes, climates, and level of annual precipitation in this continent (Rebman and Pinkava, 2001; Reyes-Aguero *et al*., 2006). In Mexico, 84 *Opuntia* species have been reported to this date, but little is known about these weakly protected species despite their significant commercial potential and environmental impact (Illoldi-Rangel *et al*., 2012). *Opuntia* genus are dominant components of natural vegetation of the Chihuahuan Desert and these species contribute to the diet of insects, birds and mammal species (Mandujano *et al*., 1997; Montiel and Montaña, 2000; Soberon *et al*., 2001).

Furthermore, they are also used as source of forage, raw and food material by local communities. Morphology and chemistry composition of seeds has been well characterized in the case of the commercial *Opuntia ficus-indica* specie (Rebman and Pinkava, 2001; Özcan and Juhaimi, 2011 Chougui *et al*., 2013). However, the nutritional value of seeds from other *Opuntia* species growing under natural conditions has been only partially investigated. Previous reports have specified that cladodes, seed and pulp of *Opuntia* genus were rich in oleic, palmitic and linolenic acids, while the fruits constitute an important source of functional compounds, including pigments (carotenoids and betalains), vitamins and minerals (Kuti, 2004; Guevara-Figueroa *et al*., 2010). These compounds have been further examined for their contribution to a healthy human diet and also as ingredients for the design and development of new foods (Saenz *et al*. 2004). Recently, fruits of these species have also been examined for their reported medicinal properties, showing several benefits as hepatoprotective, preventing or treating anemia, as well as antinflammatory, antihyperglycemic and hypocholesterolemic effects (Sanyal *et al*. 2015; González-Ponce *et al*., 2016; Jung-Woo *et al*., 2016: Ondarza, 2016).

The morphological study of *Opuntia* seeds contribute to the taxonomic information of the different species. However, in the consumption of the fruits of *Opuntia*, as well as in the preparation of some juices, the seeds constitute a waste. Therefore, local *Opuntia* species seeds could constitute an important new source of fatty acids, nutritional vitamins, polyphenols, flavonoids, tannins, natural antioxidants and oil. Yet to date, no proximal composition, antioxidant activity, phenolic content and fatty acids of seed of Opuntioideae for Northern Chihuahuan Desert has been published. In this study, the morphometric and chemical characterization of the seeds of some species of *Opuntia* located in Samalayuca area of the state of Chihuahua, Mexico, have been analyzed.

MATERIAL AND METHODS

Reagents

Methylene blue, Folin-Ciocalteu reagent, Na_2CO_3, K_2CO_3, gallic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), TROLOX, Na_2SO_4, and were acquired from Sigma (St. Louis, MI, USA). Ethyl ether, toluene, and methanol were purchased from J. T. Baker S. A. de C. V. (Estado de México, México). Fatty acids standards FAME MIX C8-C22 were purchased from Supelco (Bellefonte, PA, USA). HCl was obtained from Monterrey Chemistry Products (Monterrey, Nuevo León, México).

Plant Material

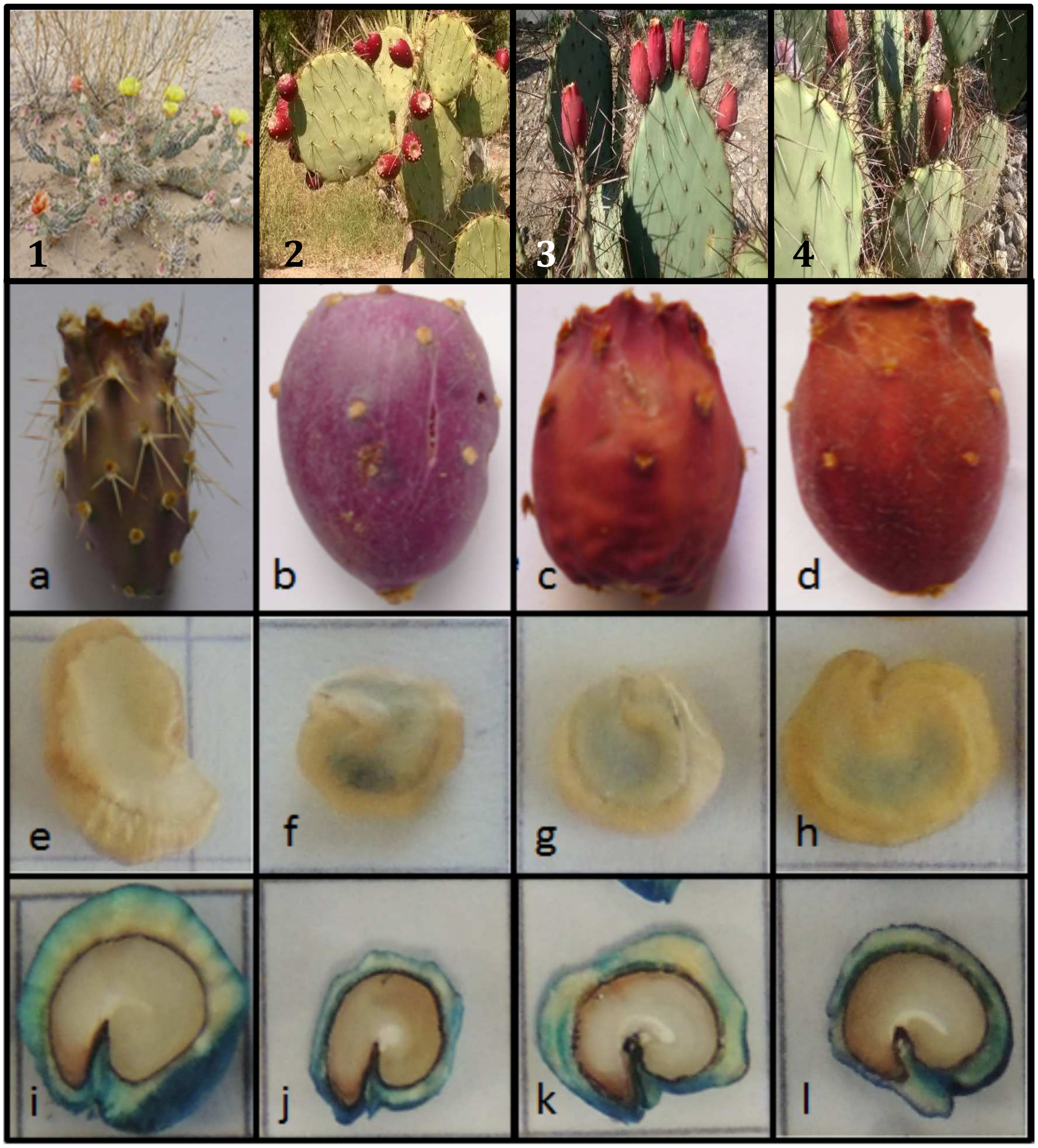
Opuntia fruits were sampled in Samalayuca Medanos Valley, area situated at 50 km south of Juarez City, Chihuahua State, Mexico (31°39’36’’ - 29°25’12’’ North Latitude and 109°02’24’’ - 107°14’24’’ West Longitude). The area has been described as a very dry ecosystem with an annual average temperature of 12–18°C, and at present, an annual precipitation is 200–400 mm. Plants of *O. engelmannii*, *O. polyacantha* var. arenaria, *O. macrocentra*, and *O. phaeacantha* were randomly collected, with 20 m apart from each other. Twenty undamaged and homogeneous fruits from individual plants per specie were collected, based on their presentation of maximum fruit maturation. Fruits were stored in an airtight polyethylene bag and immediately transported to the laboratory of the Universidad Autónoma de Ciudad Juárez. All samples were lyophilized (Labconco freeze dry/shell freeze system, Labconco Corp., Kansas City, MO), milled in a Nutribullet household mixer (Nutribullet, LLC, USA), and stored at −80°C.

Morphological Analyses

Ten fruits per specie were used for weight and size determination (Photograph 1). To examine the seed characteristics, seeds were separated from the juicy pulp, washed thoroughly with distilled water, and then dried and stored in paper bags at ambient temperature until further analysis. Seeds (20 units per specie) were used for size and weight determinations. Seed weight was determined in an analytical balance (Mettler Toledo AJ150, Ciudad de México, México). Then, seed were digitally photographed under a stereomicroscope, and the images were studied using image analysis software (ImageJ, Bethesda, MD, USA) to obtain qualitative and quantitative data. External properties such as area, length and maximum diameter of seed were measured according to the procedures proposed by Guerrero-Muñoz *et al.* (2006). Internal structures of seeds were characterized under the microscope to determine the seed coat and the funicular envelope composition of hard seeds of these species. Ten healthy seed were immersed for 4 days in methylene blue. Seeds were scarified with a knife by making a longitudinal cuts and internal structures such as embryo plus cotyledon and coat areas were measured according to the procedures proposed by Guerrero-Muñoz *et al.* (2006).

Chemical Analyses

All the proximate analyses were determined on three analytical replicates and were determined in concordance to the AOAC (2006). Moisture percentage content was measured by evaporation at 130°C for 2 h (AOAC, 930.15). The total protein content was obtained by the total nitrogen quantified by the Kjeldahl method (AOAC, 992.15). The ash content was calculated by the incineration of the samples at 525°C to constant weight (AOAC, 923.03). Total lipids were extracted in Soxhlet apparatus for 6 h using ethyl ether as solvent extractor (AOAC, 923.05). Total carbohydrates were calculated by difference (100 – (Σ protein + total lipids + total minerals)).



Photograph 1. Plant images of four Opuntioideae species growing at the Samalayuca area: (1) *O. polyacantha* var. arenaria, (2) *O. engelmannii*, (3) *O. macrocentra*, and (4) *O. phaeacantha*. Fruits and morphological analysis of seeds are shown in the same column for each species.

Total Phenolics

Phenolic compounds were extracted with 80% (v/v) methanol solution according to the protocol of Alvarez-Parrilla *et al*. (2010a). Briefly, 0.1 g of each sample was weighed into screw–cap tubes. Then 5 mL of the solvent was added, sonicated for 15 min, and centrifuged at 4,000 rpm for 15 min. This process was performed twice, and the extracts were placed quantitatively in

10 mL volumetric flask. One hundred microliters of the above extracts were taken and 500 µL of the Folin-Ciocalteu reagent was added allowed to stand for 2 min. Thereafter, 400 µL of Na_2CO_3 were added and incubated at 50°C for 15 min. Finally, the mixture was cooled in an ice bath, 250 µL were collected and placed in a microplate well. Absorbance was measured at 740 nm in a BioRad x Mark Plus (Hercules, CA, USA), and the data were obtained with the Microplate Manager 6.0 (Tokyo, Japan) computer software. A calibration curve was performed using gallic acid as a standard and the results were expressed as milligrams gallic acid equivalents (GAE) by 100 g of dry sample.

Determination of Antioxidant Activity by DPPH Method

Antioxidant capacity was analyzed following the method of Alvarez-Parrilla *et al*. (2010b). The solutions were made in 80% methanol. DPPH solution (60 mM) was prepared in methanol; 5 µL of the extract were added to 195 µL of the DPPH solution and the absorbance read at 517 nm every min for 1 h. TROLOX was used as standard and results were expressed as mmol TROLOX equivalents per 100 g dry seed (TE/100 g).

Fatty Acids Profile by Gas Chromatography

The fatty acid profile was determined by gas chromatography in accordance with the procedure of Núñez-Gastélum *et al*. (2011). Briefly, 0.5 g dry sample was weighed in a tube with a screw cap and treated with 2 mL of toluene and 3 mL of HCl methanolic 5% (v/v). The mixtures were vortexed and placed into a water bath for 2 h. After the samples had cooled to room temperature, 3 mL K_2CO_3 6% and 2 mL of toluene was added to the sample, and followed by agitation in the vortex. The samples were then centrifuged for 5 min at 2,400 rpm (Compact II Centrifuge, Clay Adams, Sparks, MD). Once the organic phase was separated and dried with Na_2SO_4 anhydride, 1 mL of the organic phase was filtered with a 0.45 mm membrane. All samples were analyzed in triplicate. The equipment consisted of a gas chromatograph 3800 with a flame ionization detector, with a capillary column CP-Sil 88 (60 m x 0.25 mm i.d., thickness of 0.25 mm) and a CP-8410 auto-injector, all from Varian Inc. (Palo Alto, CA).

The injection volume was 1 mL (at 220°C), the carrier gas was helium (1 mL min^{-1}), and the detector temperature was held constant at 235°C. The column temperature was held at 120°C for 1 min, then increased to 170°C at a rate of 3°C min^{-1}, held 1 min, and finally augmented to 235°C, which was maintained for 5 min. Peak identifications were based on comparing retention times with the standards. The area of the peaks was quantified using the software Galaxie Workstation (Varian Inc., Palo Alto, CA). The relative amount of each fatty acid (percentage of a fatty acid in total fatty acids) was quantified by integrating the area under the peak and dividing the result by the total area for all fatty acids.

Statistical analysis

Normality of frequency distributions was tested by the Kolmogorov–Smirnov test. A one–way ANOVA was performed to test for differences in fruit and seed morphometry data. Pearson's correlation was carried out to test for correlation between fruit and seed morphometry. Statistical analysis was conducted using SPSS v.8.0 software (SPSS Inc. Chicago, IL, USA).

RESULTS AND DISCUSSION

The ecological and economic importance of Opuntioideae species in the Desert of Chihuahua region, and the practical consequences of large scale surveys with the purpose of such as identifying source of plantations by the conservation purposes would be enough to justify the present work. Until now, few studies of Opuntioideae variability based in morphological and composition of their seeds have been conducted in Samalayuca area. In the present study, *Opuntia* natural variability has been assessed by making a comparison of morphological traits and chemical composition of the seed from different native species. Results revealed statistically significant differences in seed traits (Table 1). *O. polyacanta var. arenaria* presented the highest values in seed weight, seed area, seed length, seed width, embryo plus cotyledon, and coat areas; however, *O. engelmannii* had the lowest ones.

Table 1. Seed morphology of four *Opuntia* species collected from Samalayuca area. The descriptive statistics are presented in terms of the mean ± SD. Mean values with the same letters indicate homogeneous subsets for P≤0.05 according to Duncan test.

Morphological characteristics	*O. polyacantha*	*O. engelmannii*	*O. phaeacantha*	*O. macrocentra*
Seed (g)	0.028 ± 0.00^{c}	0.009 ± 0.00^{a}	0.026 ± 0.00^{c}	0.017 ± 0.00^{b}
Seed area (mm)	19.69 ± 2.18^{c}	8.38 ± 1.41^{a}	14.04 ± 1.60^{b}	13.15 ± 1.56^{b}
Seed length (mm)	5.42 ± 0.58^{c}	3.54 ± 0.32^{a}	4.55 ± 0.23^{b}	4.33 ± 0.16^{b}
Seed width (mm)	4.58 ± 0.44^{c}	3.07 ± 0.36^{a}	3.77 ± 0.42^{b}	3.72 ± 0.19^{b}
Embryo plus cotyledon area (mm)	6.83 ± 1.04^{c}	3.65 ± 0.58^{a}	4.99 ± 0.47^{b}	4.84 ± 0.66^{b}
Coat area (mm)	10.23 ± 1.4^{c}	4.00 ± 0.84^{a}	7.56 ± 1.12^{b}	7.23 ± 1.13^{b}

Chemical composition

The protein content, total phenolics, and DPPH assay were shown differed significantly among the species. Protein content measurement varied from 10.45 ± 1.17% (*O. phaeacantha*) to 14.74 ± 0.62% (*O. engelmannii*) (Table 2). In comparison with other species of the genus *Opuntia*, the protein content was similar that previously reported values (11.8 ± 1.17%) (El *et al*., 1998). Regarding the content of total lipids, in this study were obtained values of 3 to 4 fold compared to those observed for the species *O. joconostle* and *O. matudae* (Morales *et al*., 2012), and only 1.5 times more than *O. ficus-indica* (El *et al*., 1998). The total mineral content was higher for *O. joconostle* and *O. matudae* species (Morales *et al*., 2012), but 3 times lower than that reported for *O. ficus-indica* (El *et al*., 1998). On the other hand, total carbohydrates were around 70% of the sample constitution, which are very similar value to that observed for *O. heliabravoana* (72.97%), *O. joconostle* (72.62%), and *O. ficus-indica* (74.68%) (Prieto-García *et al*., 2006).

Total phenolics

The results of the analysis for total phenolic contents demonstrated that seed of *Opuntia* species of Samalayuca area are good source of phenolic compounds, varying from 10.78 ± 1.03 (*O. polyacanta var. arenaria*) to 12.88 ± 0.42 (*O. macrocentra*) mg GAEm 100 g^{-1} (Table 2). There are few reports on the phenolic content and antioxidant properties in seeds of *Opuntia* genus. Morales *et al*. (2012) found higher values, (in excess of 5-fold) for the content of phenolic compounds for *O. matudae* and *O. joconostle* seeds, compared with the present study. DPPH assay varied from 2.25 ± 0.09 (*O. polyacanta var. arenaria*) to 4.29 ± 0.35 (*O. phaeacantha*) mmol TE 100 g^{-1}. Results expressed as mmol TE 100^{-1} g were not found for other *Opuntia* genus, but the correlation with the phenolic content in the samples was adequate.

Fatty acids content

Currently, the essential fatty acids are of great interest because it of their potential role preventing human cardiovascular diseases. Some investigations have shown that *Opuntia* seed oil present higher content of linoleic acid compared to other plant oil seed, and these characteristics confirm the suggestion that *Opuntia* seed may be an interesting natural source of edible oil containing high amount of healthy fatty acids. In the present study, linoleic acid (C18:2n6) was the dominating fatty acid in seed ranging from 66.47 ± 0.85% (*O. polyacanta var. arenaria*) to 54.35 ± 2.91% (*O. phaeacantha*) (Table 2). These values presented a similar proportion to those registered for different cultivar of *O. ficus-indica* seeds (Chougui *et al*., 2013).

However, other authors have found values in the range of 70 and 80% in seed from *O. matudae* and *O. joconostle* species (Guzman-Maldonado *et al*., 2010; Morales *et al*., 2012). Oleic acid (C18:1n9) varied from 15.47 ± 0.24% (*O. polyacanta var. arenaria*) to 19.42 ± 1.04% (*O. phaeacantha*). The percentages obtained in this study were similar to those presented for *O. ficus-indica* species (Chougui *et al*., 2013). Palmitic acid (C16:0) content varied from 11.01 ± 2.06% (*O. engelmannii*) to 13.72 ± 1.51% (*O. macrocentra*). These values were in the range reported for *O. ficus-indica* cultivars (Morales *et al*., 2012), but, higher levels of palmitic acid content have previously been determined for *O. ficus-indica* species (Osorio-Esquivel *et al*., 2011). These authors concluded that the differences in oil content could be related to the environmental condition and the state of maturation of the fruit.

Stearic acid (C18:0) content varied from 3.60 ± 0.43% (*O. polyacanta var. arenaria*) to 5.60 ± 0.47 (*O. phaeacantha*), values which were within the range of 3.3% to 4.2% reported by others researchers (Morales *et al*., 2012). Palmitoleic acid (C16:1n7) content varied from 0.19 ± 0.06% (*O. polyacanta var. arenaria*) to 1.34 ± 0.12% (*O. phaeacantha*). Alpha linolenic acid (C18:3n3) varied from 0.60 ± 0.05% (*O. polyacanta var. arenaria*) to 2.42 ± 0.33% (*O. phaeacantha*). Arachidonic acid (C20:0) varied from 0.26 ± 0.03% (*O. polyacanta var. arenaria*) to 0.61 ± 0.11% (*O. phaeacantha*). And eicosenoic acid (C20:1n9) varied from 0.23 ± 0.01% (*O. engelmannii*) to 1.74 ± 0.36% (*O. phaeacantha*). These percentages of oil content were in the same proportion as those reported in others works (Ramadan and Mórsel, 2003; Mannoubi *et al*., 2009; Guzman-Maldonado *et al*., 2010; Morales *et al*., 2012). Nonetheless, the contents of lipids could depend on degree of ripeness, fruit processing, fruit cultivar and storage conditions (Osorio-Esquivel *et al*., 2011).

Table 2. Chemical composition, total phenolic, antioxidant activity and fatty acid profile of seeds of four *Opuntia*. The descriptive statistics are presented in terms of the mean ± SD. Mean values with the same letters indicate homogeneous subsets for P≤0.05 according to Duncan test.

Chemical composition	*O. polyacantha*	*O. engelmannii*	*O. phaeacantha*	*O. macrocentra*
Water content (%)	4.23 ± 0.38^{a}	4.79 ± 0.54^{a}	5.09 ± 0.79^{a}	4.88 ± 0.69^{a}
Total minerals (%)	2.55 ± 0.38^{a}	2.57 ± 0.05^{a}	2.51 ± 0.07^{a}	2.71 ± 0.09^{a}
Protein (%)	11.47 ± 1.40^{a}	14.75 ± 0.63^{b}	10.45 ± 1.18^{a}	11.72 ± 1.23^{a}
Lipids (%)	9.97 ± 2.38^{a}	10.45 ± 1.38^{a}	9.23 ± 2.57^{a}	9.61 ± 0.163^{a}
Carbohydrates (%)*	71.78	67.44	72.72	71.08
Total phenolics**	10.78 ± 1.03^{a}	12.55 ± 0.23^{b}	12.87 ± 0.23^{b}	12.89 ± 0.43^{b}
Antioxidant activity***	2.25 ± 0.09^{a}	3.41 ± 0.20^{b}	4.30 ± 0.36^{c}	3.81 ± 0.56^{b}
C16:0	12.70 ± 0.57^{a}	11.01 ± 2.06^{a}	13.47 ± 0.18^{a}	13.72 ± 1.51^{a}
C16:1n7	0.19 ± 0.06^{a}	0.80 ± 0.25^{b}	1.34 ± 0.12^{c}	1.05 ± 0.31^{b}
C18:0	3.60 ± 0.43^{a}	3.89 ± 0.45^{a}	5.60 ± 0.47^{b}	3.77 ± 0.32^{a}
C18:1n9	15.47 ± 0.24^{a}	16.47 ± 0.65^{a}	19.42 ± 1.04^{b}	15.70 ± 0.14^{a}
C18:2n6	66.47 ± 0.85^{b}	66.27 ± 3.82^{b}	54.35 ± 2.91^{a}	63.99 ± 3.03^{b}
C18:3n3	0.60 ± 0.05^{a}	0.62 ± 0.49^{a}	2.42 ± 0.33^{b}	0.77 ± 0.22^{a}
C20:0	0.26 ± 0.03^{a}	0.26 ± 0.03^{a}	0.61 ± 0.11^{b}	ND
C20:1n9	0.62 ± 0.06^{b}	0.23 ± 0.01^{a}	1.74 ± 0.36^{d}	0.82 ± 0.08^{c}

*Calculated by difference; **mg GAE by 100 g sample in dry weight; ***mmol TE by 100 g dry seed; ND: No detected

In order to determine if external and internal seed morphological traits, and chemical composition among the distinct *Opuntia* species could be related, a Person's correlation was made. The results showed that the protein content was inversely correlated with seed weight (r = −0.672) and seed area (r = −0.578). Notably, total phenolic content was inversely correlated

($p< 0.01$) with seed area (r= −0.671), seed length (r= −0.687). DPPH assay was inversely correlated with seed weight (r = −0.693).

CONCLUSION

This study discusses for first time the major morphological and nutritional characteristics of seeds of common *Opuntia*, collected in Samalayuca area of Chihuahuan Desert. The results could encourage further application of these seeds of *Opuntia* as a novel and non-conventional source of functional food, oils and nutraceuticals, mainly due his abundance. Seed of *O. phaeacantha* could be an important new source of health-promoting polyunsaturated fatty acid and its use in arid and semi-arid regions should be encouraged.

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