CHEMICAL AND POTENTIALLY FUNCTIONAL COMPOUNDS OF SELECTED PRICKLY PEAR FRUIT SEEDS (*OPUNTIA* SPP.)

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Fatty acid profile and antioxidant activity of oil seed were determined through proximate analyses on seed of eleven elite genotypes of Mexican prickly pear. Eleven Opuntia genotypes from the Opuntia National Repository (Mexico) were selected due to their high production. These analyses showed best results as follows: 8.69 % protein for 'Amarilla Montesa'; 16.8 % fats for 'Cardona'; 10.5 % carbohydrates for 'Xoconostle Cuaresmeño', and 1.81 % ashes for 'San Juanera'. Five fatty acids were the most common: linoleic (73.1 % in 'Xoconostle Cuaresmeño'); oleic (16.8 % in 'Cardona'); palmitic (14.3 % in 'Rosa de Castilla'); linolenic (5.6 % in 'Rojo Vigor'), and stearic (3.8 % in 'Tapón Aguanoso'). Highest content of phenolics was for 'Xoconostle Cuaresmeño' (141.81 mg GAE/100 g), while highest antioxidant capacity was for 'San Juanera' (51.68 mMol TE/l). The previous data support the idea that prickly pear seed has good protein quality and an excellent ratio of oleic:linoleic oil (ranging from 1:1 to 3:1 v/v), which are considered beneficial to human health.
Keywords: antioxidant, ratio of unsaturated fatty acid, *Opuntia*, seeds

Chemische und potentiell funktionelle Verbindungen von Samen ausgewählter Feigenkaktus-Genotypen (*Opuntia* spp.). Das Fettsäureprofil und die antioxidative Aktivität von Ölsaaten wurden durch Kurzanalyse der Samen von elf Elite-Genotypen mexikanischer Feigenkakteen bestimmt. Elf *Opuntia*-Genotypen aus dem Opuntia National Repository (Mexico) wurden aufgrund ihres hohen Ertrags ausgewählt. Die besten Resultate waren wie folgt: 8,69 % Protein für 'Amarilla Montesa'; 16,8 % Fette für 'Cardona'; 10,5 % Kohlenhydrate für 'Xoconostle Cuaresmeño'; 1,81 % Asche für 'San Juanera'. Am häufigsten waren fünf Fettsäuren: Linolsäure (73,1 % in 'Xoconostle Cuaresmeño'); Ölsäure (16,8 % in 'Cardona'); Palmitinsäure (14,3 % in 'Rosa de Castilla'); Linolensäure (5,6 % in 'Rojo Vigor'); Stearinsäure (3,8 % in 'Tapón Aguanoso'). Der höchste Gehalt an Phenolen wurde bei 'Xoconostle Cuaresmeño' (141,81 mg GAE/100 g) gefunden, die höchste antioxidative Kapazität bei 'San Juanera' (51,68 mMol TE/l). Diese Daten stützen die Annahme, dass Feigenkakteensamen eine gute Proteinqualität und ein ausgezeichnetes Ölsäure:Linolsäure-Verhältnis (im Bereich von 1:1 bis 3:1 v/v) aufweisen, die als vorteilhaft für die menschliche Gesundheit angesehen werden.
Schlagwörter: Antioxidantien, Verhältnis ungesättigter Fettsäuren, *Opuntia*, Samen

Opuntia spp., the most important genus of *Cactaceae* with regard to food products, includes a huge number of species and varieties. To date, the most common and heavily consumed cactus fruits are those from *Opuntia ficus-indica* (Moussa-Ayoub et al., 2016). Xoconostles or acidic cactus pears, as described by their composite name in Náhuatl (xoco = sour or acid, nōxtle = 'tuna' or prickly pear), are produced by *Opuntia joconostle* that bear fruit prized for their fleshy acidic mesocarp (Bravo Hollis and Sánchez Mejorada, 1978). They are morphologically different from their cousins, the prickly pears, which are recognized by their sweet, juicy, and seedy endocarp.

Xoconostle fruit are characterized by a thin peel, highly acidic pulp, both are edible, and have a long shelf life (Gallegos-Vázquez et al., 2012). They are sour, with a pH that ranges between 2.71 and 3.42 (Scheinvar et al., 2009). Their color ranges from green-to-yellow and red, and the fruits are used by small factories that produce jellies, beverages, and candied xoconostle (Zavaleta Beckler et al., 2001). Betalains, water-soluble nitrogenous pigments, are found in cactus fruit and comprise purple-to-red betacyanins and yellow-to-orange betaxanthins, and these have antioxidant activity and a preventive potential against selected degenerative diseases (Moussa-Ayoub et al., 2011). According to Bensadón et al. (2010) fruits and cladodes are sources of high-quality fiber and natural antioxidants as well.

Nearly all of the nutrients required by humans can be found in cultivated plants, as well as a great diversity of compounds that promote a healthy life (Grusak and Dellapenna, 1999). For an example, fatty acids bound to triacylglycerol are accumulated in the seeds; these types of compounds represent main energy storage in seeds, and are the most abundant carbon chains available in nature that can have many uses, beginning with food to industrial staples (Thelen and Ohlrogge, 2002). During the past 100 to 150 years, n-6 fatty acids from corn, sunflower, safflower, cotton, and soybean have exhibited an increased consumption in food; nevertheless, there is a belief that these fatty acids induce blood viscosity and vasospasm related to vasoconstriction (Simopoulos, 1999). In order to address these problems, seeds from wild plants were analyzed in order to know their nutritional value and their n-3 fatty acids (polyunsaturated fatty acids), since these are acknowledged as beneficial, having anti-inflammatory, antithrombotic, antiarrhythmic, and vasodilation properties in humans when present at an appropriate proportion (1-2:1 of n-6 and n-3, respectively). Luo et al. (2010) obtained from cladodes an extract (mainly phytosterol, polyunsaturated fatty acids, phytol, palmitic acid, palmitate, and vitamin E) with anti-glycemic activity from Opuntia Milpa Alta, while Zhong et al. (2010) found antioxidant activity in polysaccharides such as rhamnose, arabinose, and glucose (1.00, 2.98, and 2.57 in a molar ratio, respectively) in *Opuntia ficus* indica cladodes. Ramadan and Mörsel (2003) found in Opuntia seeds and pulp linolenic, palmitic, and stearic acid in the mature fruit of prickly pear. Finally, Tlili et al. (2011) and Yeddes et al. (2012), utilizing SC-CO_2 (supercritical carbon dioxide) and hexane, obtained 57.6 % linoleic acid, 22.3 % oleic acid, and 14.3 % palmitic acid from Tunisian *Opuntia* seeds. The previous data provide a clue that seeds from the Mexican prickly pear may be a source of bioactive compounds and around 10 % of oil content (Gallegos-Vázquez et al., 2012; López, 1995). The objective of this work was to study the content of fatty acids in seeds from Mexican wild Opuntias that can be employed for therapeutic uses.

MATERIALS AND METHODS

PLANT MATERIAL

Eleven *Opuntia* genotypes from the *Opuntia* National Repository (CRUCEN-Universidad Autónoma de Chapingo) located in El Orito, Zacatecas, Mexico (22° 44.7' north latitude and 102° 36.4' west longitude) were selected from a 6-acre area of the repository where the yields were the highest. These genotypes included six commercial varieties, 'Pico chulo' (*Opuntia megacantha* Salm-Dick); 'Cristalina' (*Opuntia albicarpa* Scheinvar); 'Rojo Pelón' (*Opuntia ficus-indica* (L.) Mill.); 'Rojo Vigor' (*Opuntia ficus-indica* (L.) Mill.); 'Amarilla Montesa' (*Opuntia megacantha* Salm-Dick), and 'Xoconostle Cuaresmeño' (*Opuntia joconostle* F.A.C. Weber), three varieties cultivated on a small scale and their distribution is restricted to solar orchards 'San Juanera' (*Opuntia lassiacantha* Pfeiff), 'Rosa de Castilla' (*Opuntia megacantha* Salm-Dick), and 'Selección Hidalgo', and two wild species, 'Tapón Aguanoso' (*Opuntia robusta* H.L. Wendl) and 'Cardona' (*Opuntia streptacatha* Lem.). Sample collection of

ten fruit of each genotype was selected from a 6-acre area of the repository in the period of maturation of most of the genotypes, which includes the months of August to September. *Opuntia* fruit were peeled and the pulp was macerated in a blender until the seeds were easily collected; then, the seeds were washed several times and dried at room temperature. From each genotype, 150 g of seeds were ground to powder and the particles were passed through Tyler No. 20 mesh (0.84 mm).

CHEMICAL ANALYSIS

ETHER EXTRACTS (SOXHLET)

The ether extract protocol was carried out according to the Association of Official Analytical Chemists (AOAC, 1990). Briefly, 2 g of ground seed were placed in Goldfish cartridges (Whatman, Kent, England), after recording of the total weight; then, 50 ml hexane were added. After 2 h, the cartridges were dried at 130 °C for 2 h, and, when these were cool, they were weighed again. The ether extract was determined by the difference in weight. Extractions were made in triplicate.

CRUDE PROTEIN

The Micro-Kjeldahl protocol described by Villegas and Mertz (1970) was followed: 0.1 g of each dry seed sample was placed in a digestion tube with 4 ml of the sulfuric-salicylic acid mix (25 g of salicylic acid diluted in 1 L in concentrated sulfuric acid (98 %)). The tubes were sealed with parafilm and incubated during 12 h; afterward, 0.6 g of a catalytic mix of selenium (cat. 8030, Merck, Darmstadt, Germany) and glass beads were added. Samples were heated at 180 °C during 60 min until they turned blue-green in color. After cooling, the following were added to the samples: 29 ml of distilled water; 5 ml of boric acid solution (40 g/l) used as an indicator; 20 ml of bromocresol green solution (Sigma-Aldrich, St. Louis, USA) and 20 ml 10N of NaOH (Merck, Darmstadt, Germany). Samples were placed in the distillation equipment (Rapid Still II, Labconco, Kansas City, USA), and the process was stopped when 75 ml of the distilled product were collected. This distilled volume was titrated with 0.01 N of H_2SO_4. Crude protein (%) was calculated as % N*6.25 (AOAC, 1980). The determinations were made in triplicate.

ASHES

Following the AOAC procedure for ashes #(AOAC, 1990), a porcelain refractory crucible was heated to 130 °C for 2 h. After cooling, 1 g of dried sample was placed in the crucible, and then the latter was placed back into the furnace in order to reach 500 °C, until only white ashes were evident. The sample was allowed to cool and, after weighing, the ash content was determined by the difference. Analysis was performed in three independent determinations.

TOTAL DIETARY FIBER

For this determination, the gravimetric method of Prosky et al. (1988) was utilized after slight modifications. Briefly, 1 g of ground and defatted seed (AOAC, 1990) was added to 50 ml of phosphate-buffered (PBS) containing 1.4 g of Na_2HPO_4 + 8.4 g Na H_2PO_4/l (Sigma-Aldrich, St. Louis, USA), 0.08 M, pH 6.0, and 0.1 ml of thermostable α-amylase. The mixture was stirred in a 250 ml beaker, covered with aluminum foil, and placed in a heating bath set at 95 °C for 15 min, stirring every 5 min. After cooling to room temperature, the pH was adjusted to 7.5 ± 0.2 with NaOH and 0.1 ml of fresh protease solution (50 mg protease/ml of PBS). The beakers were covered with aluminum foil again and placed in the heating bath set at 60 °C under continuous stirring for 30 min. After cooling, the pH was set between 4.0 and 4.6 using 10 ml 0.325 M HCl. Again, 0.1 ml of amylase was added to the sample and placed back into the heating bath set at 60 °C under continuous stirring for 30 min. After that, four 95 % ethanol volumes were added and the samples were covered at room temperature for 12 h. Samples were centrifuged at 5000 rpm for 10 min in weighed tubes; the supernatant was discarded and the pellet was frozen and lyophilized. Total dietary fiber was estimated as TDF (%) = (average R_1, R_2 (mg) – Protein (mg) – Ash (mg)) × 100. Three repetitions were made.

CARBOHYDRATES

Carbohydrates were determined by differences (Bainy et al., 2008) as follows: CH = 100 (%) - Protein (%) - Ashes (%) - TDF (%).

MINERALS

For determination of the minerals, wet digestion was performed as described by Jones et al. (1973). First, 0.5 g of dry ground samples was placed into test tubes (25 mm in width and 350 mm in height) adding 4 ml of nitric acid at 65 % (v/v) (Suprapur grade, Merck, Darmstadt, Germany); the tubes were sealed with parafilm and left standing for 30 min. The tubes were placed on a hot digestion plate set between 120 and 150 °C during 1 h until the brown fumes disappeared. The tubes were removed from the plate to allow them to cool to room temperature for 15 min. After that, 2 ml of perchloric acid were added to the samples and they were placed back on the hot digestion plate set between 170-210 °C. Digestion was stopped once white smoke was evident and the digested samples became translucent. The digested samples were removed from the plate to cool them. Then, deionized water was poured into the tubes in order to reach a 50 ml final volume. Only Ca required a further step: 1/10 dilution. From these dilutions, minerals were determined through atomic absorption with the following wavelengths: Fe = 248.3 nm, Zn = 213.9 nm, Ca = 422.7 nm, and K = 766.6 nm. Three repetitions were carried out.

FUNCTIONAL PROPERTIES

FATTY ACID PROFILES

Samples of 50 mg were dissolved using 10 ml hexane in 20 ml reaction vials with screw caps. Then, 100 µl of 2 # N sodium hydroxide in methanol solution (11.2 g in 100 ml) was added and, after sealing the caps, the vials were vortexed for about 30 s and then centrifuged at 3000 rpm during 5 min. The supernatant was transferred into a new vial to obtain a 2-ml autosampler (Agilent 7863; Agilent Technologies, Wilmington, USA) (AOAC, 2000). Analyses were performed with a gas chromatograph (Agilent 6890; Agilent Technologies, Wilmington, USA) coupled to a Flame Ionizing Detector (FID). The column used was HP-88 (J&W 112-88A7; Agilent Technologies, Wilmington, USA) 100 m × 0.25 mm ID, 0.2 µm. The FID was set as follows: 250 °C, admitting 1 µl of sample, with a separation time of 1/50, carrier gas A: hydrogen, carrier gas B: helium, and a pressure charge of 1 ml/min. Furnace A temperature was set with the following program: 120 °C during 1 min; ramping 10 °C/min up to 175 °C; then ramping 5 °C/min up to 210 °C during 5 min; ramping again 5 °C/min up to 230 °C, and then remaining for 5 min. Furnace B temperature was set as follows: 175 °C for 10 min; ramping 3 °C/min up to 220 °C, and remaining for 5 min. The detector was set as follows: 280 °C; Hydrogen: 40 ml/min, and Air: 450 ml/min, with Helium as a carrier gas: 30 ml/min. Analysis was performed in three independent determinations. Standards for fatty acid methyl esters (FAME) were dissolved in hexane in order to reach 0.01 to 0.1 % (w/v) from the original concentration. Before running the analyses, the column was tested with a mixture of 37 compounds (SUPELCO #18919; SUPELCO Co., St. Louis, USA) with around 100 mg of methyl esters of C4 to C24 fatty acids with a relative concentration of 2 to 4 %. Prior to their analysis, the samples were dissolved in 10 ml hexane, obtaining a final concentration of around 0.2 to 0.4 mg/ml per FAME.

TOTAL PHENOLIC CONTENT

For this determination, the Folin-Ciocalteau method was employed (Singleton et al., 1999). Briefly, 100 mg of ground seed and 10 ml of 30 % methanol were vortexed with a Falcon tube. After that, the tubes were centrifuged at 8000 rpm for 10 min and the supernatant was filtered using a Whatman filter until all of the oil extracts were recovered. In a fresh tube, 125 µl of extract, 500 µl of deionized water, and 125 µl of Folin-Ciocalteau reagent were mixed and left in the dark for 6 min. After that, 1.25 ml of # 7 % Na_2CO_3 and 1 ml of deionized water were added and mixed, and the tubes were placed back into the dark for 1.5 h at room temperature. Spectrophotometric readings at 750 nm were performed (6405 UV/Vis; Jenway, Stone, Staffordshire, UK). Because total phenolic content was estimated in terms of mg of gallic acid equivalents (GAE)/100 g sample (mg GAE/100 g), a standard curve was constructed, with dilutions of gallic acid (GA) in 30 % methanol in the range of 0.00625 to 0.20 mg/ml. From all of the GA solutions, 125 µl were treated as mentioned for the extracts. Absorbance was plotted against concentration to obtain the

equation for phenolic content determination. Analysis was performed in three independent tests.

ANTIOXIDANT CAPACITY (OXYGEN RADICAL ABSORBANCE CAPACITY, ORAC)

Antioxidant capacity was determined through the ORAC method (OU et al., 2001). First, a phosphate buffer (0.16 M NaH_2PO_4, pH 7.4) was prepared and stored in an amber flask at 4 °C. A fluorescein stock solution 0.5315 mM (5 mg in 25 ml) and a subsequent solution 8.185 × 10-5 mM (7.7 µl of the fluorescein solution stock in 50 ml) was also prepared. Next, 1.5 ml poured into a spectrophotometer cuvette together with 0.75 ml of the sample of the oil seed diluted in the phosphate buffer were placed in a hot bath at 37 °C for 5 minutes. After that, 0.75 ml of AAPH [2, 2' (2-amidinopropano)-HCl] solution (0.415 g AAPH in 10 ml) was added and mixed with the pipette. Readings were taken immediately and every min thereafter, in a fluorometer (Invitrogen, Carlsbad, USA) ($\lambda_{excitation}$ = 493 nm, $\lambda_{emission}$ = 515 nm), placing the sample back into the hot bath between readings until the value obtained was about 10 % of the initial one. The blank samples were required for every reading. The obtained values were calculated as the area under the standard curve (AUC) of Trolox and reported as µmol of Trolox equivalents (TE) per gram of sample (µmol TE/g). Analysis was performed in three independent determinations.

STATISTICAL ANALYSES

All evaluations were carried out under a completely randomized design. Results were analyzed by analysis of variance (ANOVA) and a means comparison with a Tukey test at $p \leq 0.05$ using Statgraphics Centurion XVL (ver. 16.1.03; Statpoint Technologies, Inc., Warrenton, USA).

RESULTS AND DISCUSSION

CHEMICAL ANALYSIS

Ether extract, total dietary fiber, ash, protein, and carbohydrate contents are expressed as percentages on the dry basis of *Opuntia* seeds from 11 genotypes (Table 1). Ether extract ranged from 10.4 % ('Xoconostle Cuaresmeño') to 16.8 % ('Cardona'); the highest values obtained are higher than those reported for O. *ficus-indica* within the range of 8.15 % and 9.88 % (Ramadan and Mörsel, 2003; Guzmán-Maldonado et al., 2010) but appear to be lower than the 17.1 % reported by Sawaya et al. (1983). Total dietary fiber ranged from 67.1 % ('Cristalina') to 59.2 % ('Tapón Aguanoso'), superior to that reported for O. *ficus-indica:* 40.6 % and 54.2 %, respectively (Felker et al., 1997; Sawaya et al., 1983). Ashes ranged from 1.81 % ('San Juanera') to 0.79 % ('Rosa de Castilla'), lower than the 3 %, 5.9 %, and 5.3 % of O. *ficus-indica* (Felker et al., 1997; Nassar, 2008; Sawaya et al., 1983). Protein content ranged from 4.7 % ('Cristalina') to 8.7 % ('Amarilla Montesa'); these values are lower than those of 10.7 % and 11.7 % reported for O. *ficus-indica* by (Felker et al., 1997). On the other hand, Salim et al. (2009) found a slightly lower protein content (4.48 %) in seeds of fruit of *Opuntia ficus-indica*. Carbohydrate content was within the range of 4.8 % ('Amarilla Montesa') to 10.5 % ('Xoconostle Cuaresmeño'). In *O. ficus-indica*, Felker et al. (1997) found a value of 5.35 % for carbohydrates, which lies within the range of this work.

Ca, K, and Fe contents were outstanding in 'Xoconostle Cuaresmeño' (Table 2). Calcium content in this cultivar was three quarters (74.4 %) higher than that of 'Tapón Aguanoso'. Potassium was nearly one and one half times (exceeding 48.2 %) compared with that of 'Rojo Pelón', and iron nearly doubled (exceeding 98 %) that of 'Pico Chulo'. Zinc content ranged from 1.84 mg/100 g ('Cristalina') to 10.35 mg/100 g ('Selección Hidalgo'). Further information comprises that 'Xoconostle Cuaresmeño' is a wild genotype. Other work differ substantially with our results. For example, Salim et al. (2009) found higher values of calcium and potassium (21.1 mg/100 g and 78.6 mg/100 g, respectively) in *O. ficus-indica* seeds, while El Kossori et al. (1998) found a similar content of iron and zinc (12.1 mg/100 g and 4.16 mg/100 g) in *O. ficus-indica* seeds. This latter datum is lower than that found in 'Selección Hidalgo' (Table 2), with 10.35 mg/100 g in this study.

Table 1: Proximate analysis (%) of eleven outstanding *Opuntia* genotypes[a]

Genotype	Ether extract	Total dietary fiber	Ashes	Crude protein	Carbohydrates
Pico Chulo	14.23 ± 0.98^{b}	61.28 ± 3.63^{bcdefg}	1.33 ± 0.03^{a}	8.34 ± 0.75^{ab}	8.10 ± 1.27^{bcdef}
Selección Hidalgo	13.11 ± 1.10^{bc}	64.44 ± 1.32^{abc}	1.36 ± 0.13^{a}	5.13 ± 0.09^{i}	8.79 ± 1.22^{b}
Tapón Aguanoso	15.87 ± 0.48^{a}	59.15 ± 1.58^{efhij}	1.12 ± 0.09b	7.88 ± 0.53^{abc}	8.65 ± 0.48^{bc}
Cristalina	13.26 ± 0.97^{bc}	67.08 ± 2.32^{a}	0.79 ± 0.07b	4.66 ± 0.40^{ij}	6.96 ± 1.12^{defgh}
Rojo Vigor	15.14 ± 0.46^{b}	64.36 ± 1.11^{abc}	1.64 ± 0.11^{a}	6.50 ± 0.0^{efg}	5.83 ± 0.8^{jij}
Rojo Pelón	12.62 ± 0.32^{bc}	65.25 ± 4.36^{ab}	1.41 ± 0.09^{a}	7.28 ± 0.13^{cde}	6.49 ± 0.46^{ghi}
Cardona	16.77 ± 0.52^{a}	60.05 ± 1.19^{cdefghi}	1.44 ± 0.08^{a}	7.59 ± 0.57^{bcd}	7.46 ± 0.61^{bcdef}
San Juanera	15.30 ± 0.63^{ab}	60.85 ± 2.11^{bcdefgh}	1.81 ± 0.08^{a}	6.72 ± 0.31^{def}	8.36 ± 0.47^{bcde}
Rosa de Castilla	11.61 ± 0.87^{bcd}	64.22 ± 1.68^{abcd}	1.69 ± 0.02^{a}	6.34 ± 0.57^{fgh}	8.51 ± 1.01^{bcd}
Amarilla Montesa	15.30 ± 0.33^{ab}	63.32 ± 1.52^{abcdef}	1.66 ± 0.11^{a}	8.69 ± 0.0^{a}	4.78 ± 0.87^{j}
X. Cuaresmeño	10.40 ± 0.27^{bcd}	63.96 ± 2.07^{abcde}	1.14 ± 0.01^{b}	7.86 0.11^{abc}	10.50 ± 0.34^{a}

[a]Results are expressed as Mean ± SD; different letters within the same column are significantly different (Tukey, $p < 0.05$); determinations were made in triplicate.

Table 2: Mineral content (mg/100 g sample) of seed from eleven elite *Opuntia* genotypes[a]

Genotype	Calcium	Potassium	Iron	Zinc
Pico Chulo	0.14 ± 0.0^{ij}	0.24 ± 0.01^{bc}	2.85 ± 0.04^{h}	3.16 ± 0.11^{d}
Selección Hidalgo	0.20 ± .01^{fgh}	0.16 ± 0.01^{hi}	12.65 ± 0.64^{b}	10.35 ± 0.49^{a}
Tapón Aguanoso	0.11 ± 0.0^{k}	0.24 ± 0.01^{bc}	6.26 ± 0.17^{e}	3.50 ± 0.07^{d}
Cristalina	0.17 ± 0.01^{hi}	0.23 ± 0.0^{cd}	6.97 ± 0.19^{e}	1.84 ± 0.03^{f}
Rojo Vigor	0.27 ± 0.01^{c}	0.20 ± 0.01^{ef}	10.65 ± 0.07^{c}	6.38 ± 0.18^{b}
Rojo Pelón	0.23 ± 0.0^{e}	0.15 ± 0.0^{i}	6.23 ± 0.53^{e}	3.48 ± 0.18^{d}
Cardona	0.23 ± 0.04^{ef}	0.17 ± 0.01^{gh}	4.91 ± 0.42^{f}	2.63 ± 0.18^{e}
San Juanera	0.31 ± 0.0^{b}	0.21 ± 0.01^{e}	6.74 ± 0.66^{e}	4.53 ± 0.30^{c}
Rosa de Castilla	0.26 ± 0.01^{cd}	0.18 ± 0.01^{fg}	4.02 ± 0.23^{g}	1.80 ± 0.06^{f}
Amarilla Montesa	0.21 ± 0.0^{efg}	0.25 ± 0.0^{b}	9.73 ± 0.25^{d}	4.70 ± 0.03^{c}
X. Cuaresmeño	0.43 ± 0.0^{a}	0.29 ± 0.0^{a}	17.48 ± 0.39^{a}	2.16± 0.05^{ef}

[a]Results are expressed as Mean ± SD from three replicas; Means with different letters within the same column are significantly different (Tukey, $p < 0.05$).

FUNCTIONAL PROPERTIES

FATTY ACIDS PROFILES

In relation to the profile of fatty acids (Fig. 1), five types of fatty acids were found, palmitic (C16:0) and stearic (C18:0, saturated), oleic (C18:1, monounsaturated), linoleic and linolenic (C18:2 and C18:3, respectively, polyunsaturated). Table 3a and 3b summarize the values of palmitic, stearic, oleic, linoleic, and linolenic acids that were detected in the different fractions of *Opuntia* seeds. Average concentrations were the following: linolenic acid 67.59 %; palmitic acid 12.17 %; oleic acid 11.19 %; linoleic acid 4.69 % and stearic acid 2.95 %. Three genotypes ('Rosa de Castilla', 'Selección Hidalgo', and 'Tapón Aguanoso') showed highest contents of palmitic acid, 14.28 %, and of stearic acid, 3.79 %, respectively. The 'Rojo Vigor' genotype demonstrated the highest value (16.75 %) for unsaturated fatty acids. Two genotypes exhibited highest polyunsaturated fatty acids: 'Xoconostle Cuaresmeño' (linoleic, 73.09 %) and 'Rojo Vigor' (linolenic, 5.59 %). These results support the possibility of using Opuntia seeds as a source of linolenic acid, in that it was reported as up to 74 % on a dry basis (Fawzy et al., 2003; Krifa et al., 1993; Sawaya et al., 1983). Oleic and palmitic acids were found at 12.8 % and 7.2 %, respectively, and small amounts of lauric and myristic acid were found in *Opuntia stricta* (Ennouri et al., 2005). Similar data were reported by (El Mannoubi et al. (2009) in *O. ficus-indica* as follows: linolenic, 60.69 %; oleic, 21.42 %, and palmitic acid, 12.76 %.

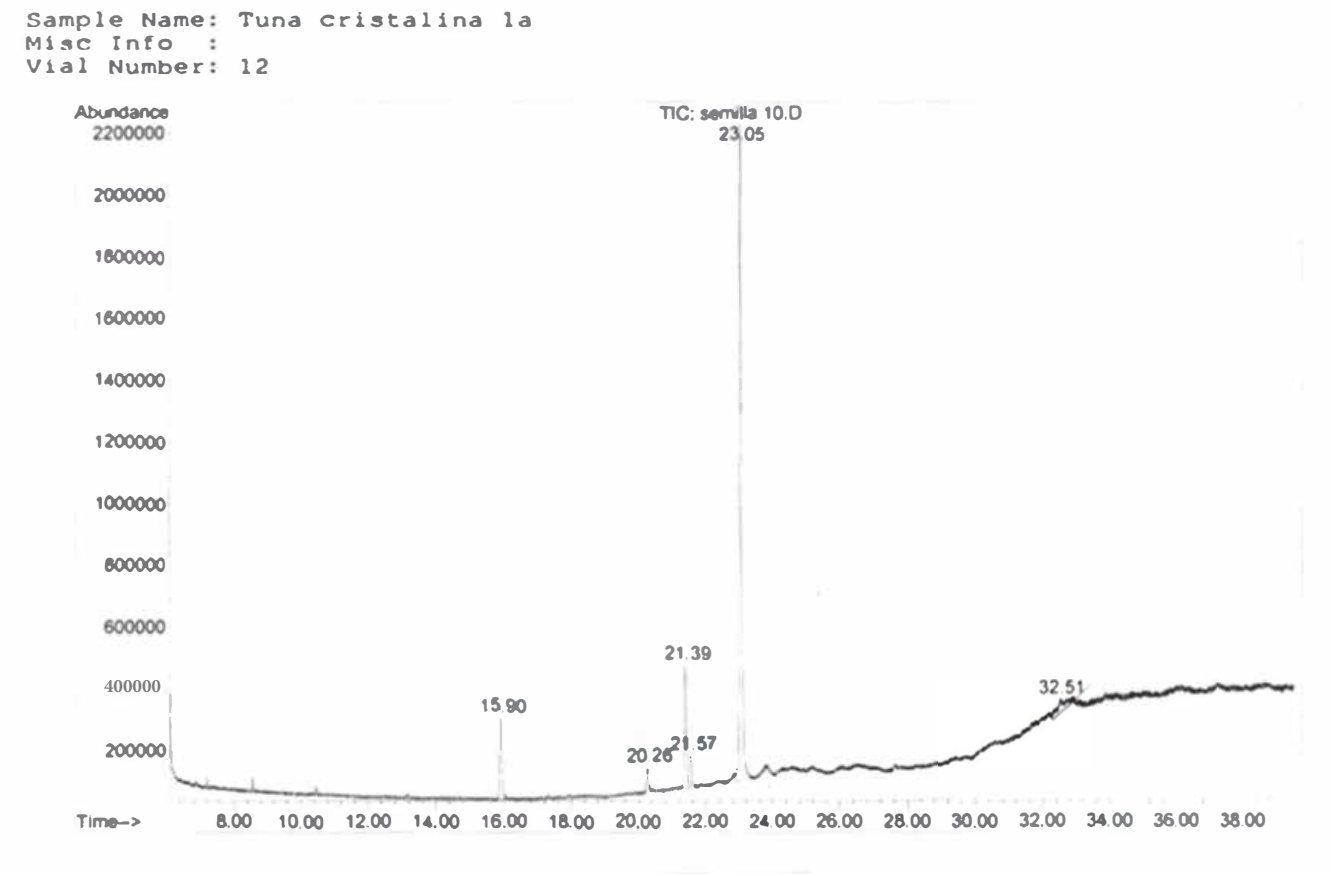

Fig. 1: Fatty acid profile of *Opuntia albicarpa* Scheinvar seeds

Table 3a: Fatty acids found in seed oil from outstanding *Opuntia* genotypes

	Contents (%)				
Genotype	**Palmitic**	**Stearic**	**Oleic**	**Linoleic**	**Linolenic**
A	11.89 ± 0.11^{d}	2.75 ± 0.45^{b}	11.32 ± 0.21^{de}	69.52 ± 0.25^{a}	4.52 ± 0.12^{b}
B	13.23 ± 0.19^{b}	3.79 ± 0.17^{a}	11.38 ± 0.43^{d}	66.75 ± 0.19^{b}	4.85 ± 0.12^{b}
C	11.81 ± 0.01^{d}	3.79 ± 0.03^{a}	10.38 ± 0.04^{ef}	70.32 ± 0.00^{a}	3.70 ± 0.00^{c}
D	11.11 ± 0.05^{e}	2.88 ± 0.66^{b}	11.48 ± 1.17^{d}	67.57 ± 0.91^{b}	4.52 ± 0.76^{b}
E	13.23 ± 0.17^{b}	2.82 ± 0.14^{b}	14.46 ± 0.30^{b}	63.90 ± 0.04^{b}	5.59 ± 0.06^{a}
F	12.59 ± 0.15^{c}	2.92 ± 0.52^{b}	9.75 ± 0.25^{f}	69.38 ± 0.75^{a}	5.37 ± 0.17^{a}
G	11.90 ± 0.20^{d}	2.72 ± 0.01^{b}	16.75 ± 0.14^{a}	64 ± 0.27^{b}	4.64 ± 0.07^{b}
H	11.51 ± 0.50^{d}	2.28 ± 0.11^{b}	11.12 ± 0.14^{e}	69.74 ± 0.58^{a}	5.35 ± 0.04^{a}
I	14.28 ± 0.06^{a}	3.21 ± 0.29^{a}	12.22 ± 0.08^{d}	65.10 ± 0.02^{b}	5.19 ± 0.13^{a}
J	11.37 ± 0.01^{de}	2.61 ± 0.12^{b}	13.20 ± 0.14^{c}	63.24 ± 7.35^{b}	4.57 ± 0.00^{b}
K	10.97 ± 0.03^{e}	2.63 ± 0.07^{b}	9.98 ± 0.54^{f}	73.09 ± 0.56^{a}	3.34 ± 0.12^{c}
Average	12.17	2.95	11.19	67.59	4.69

*A. Pico chulo, B. Selección Hidalgo, C. Tapón Aguanoso, D. Cristalina, E. Rojo Vigor, F. Rojo Pelón, G. Cardona, H. San Juanera, I. Rosa de Castilla, J. Amarilla Montesa and K. X. Cuaresmeño; results are expressed as Mean ± SD, different letters within the same column are significantly different (Tukey, $p < 0.05$); determinations were made in triplicate.

Table 3b: Fatty acids found in seed oil from outstanding *Opuntia* genotypes

	Ratio		
Genotype	**Sat/Insat**	**Oleic/Linoleic**	**Linoleic/Linolenic**
A	0.17 ± 0.00	0.16 ± 0.00	15.38 ± 0.46
B	0.21 ± 0.01	0.17 ± 0.01	13.76 ± 0.38
C	0.18 ± 0.00	0.15 ± 0.00	19.02 ± 0.02
D	0.17 ± 0.00	0.17 ± 0.02	15.14 ± 2.34
E	0.19 ± 0.00	0.23 ± 0.00	11.44 ± 0.12
F	0.18 ± 0.01	0.14 ± 0.01	12.93 ± 0.27
G	0.17 ± 0.00	0.26 ± 0.00	13.80 ± 0.15
H	0.16 ± 0.01	0.16 ± 0.00	13.03 ± 0.20
I	0.21 ± 0.00	0.19 ± 0.00	12.56 ± 0.30
J	0.17 ± 0.02	0.21 ± 0.03	13.83 ± 1.62
K	0.16 ± 0.00	0.14 ± 0.01	21.92 ± 0.95
Average	0.18	0.18	14.83

* A. Pico chulo, B. Selección Hidalgo, C. Tapón Aguanoso, D. Cristalina, E. Rojo Vigor, F. Rojo Pelón, G. Cardona, H. San Juanera, I. Rosa de Castilla, J. Amarilla Montesa and K. X. Cuaresmeño; results are expressed as Mean ± SD, different letters within the same column are significantly different (Tukey, $p < 0.05$); determinations were made in triplicate.

Also Tlili et al. (2011) showed that O. *ficus-indica* seeds contain 83 % of unsaturated fatty acids; 26 % of the latter are monounsaturated and 57 % polyunsaturated. According to these results, the pattern of *Opuntia* seeds is very similar to those of sunflower and grape seeds (Tan and Che Man, 2000).

Seed oil from *Opuntia* appears to have a better quality as compared with that obtained from other species. Working with the seed oil of black raspberry, Parry and Yu (2004) found 35 % of linolenic acid with antioxidant capacity. Blueberry seed oil is rich in essential fatty acids, such as linoleic (35 to 44 %) and α-linolenic (23 to 35 %), as found by several authors (Heeg and Bernard, 2002; Parker et al., 2003). Also Parry and Yu (2004) found 62.8 % of linoleic acid in seeds from a marion berry, which is similar to that found in *Opuntia* seeds. The American Heart Association (AHA) strongly recommends including oleic acid in the diet, as 15 to 16 % of total energy, having an oleic/linoleic ratio ranging from 1:1 to 3:1. Ratios found in seed oil from *Opuntia* genotypes are close to those recommended; therefore, these could be beneficial to human health (Martin et al., 2011).

TOTAL PHENOLIC CONTENT AND ANTIOXIDANT CAPACITY

The Oxygen Radical Absorbance Capacity (ORAC) test is an *in vitro* assay to measure the total antioxidant power of substances, measuring both lipophilic and hydrophilic extracts (Prior et al., 2003). The ORAC assay measures the antioxidant inhibition of peroxyl radical-induced oxidation, therefore reflecting the classical radical chain-breaking antioxidant activity by hydrogen atom transfer (Huang et al., 2005). TEAC and the phenolic soluble content were determined in *Opuntia* fruit and are specified in Table 4. Highly contrasting results in antioxidant capacity were found within the range between 5.53 ('Xoconostle Cuaresmeño') to 51.68 mMol/l EqTrolox ('San Juanera'). The majority of yellow-colored flesh fruit had seeds with higher antioxidant capacity; this is important since there are no reports, to our knowledge, on the antioxidant capacity of the oil extract of *Opuntia* seeds. Total phenolics from 'Xoconostle Cuaresmeño' were 141.81 mg GAE/100 g, which is 57.17 % higher than those of 'Pico Chulo' (60.73 mg GAE/100 g). Two wild genotypes revealed highest total phenolics; no condensed tannins were found in any of the analyzed genotypes. Similar TEAC values were found (Cardador-Martínez et al., 2011) in 'Pelón Liso', 'Cristalina', and 'Montesa' genotypes, but these had a high total phenolic concentration (337 to 460 mg GAE/100 ml). Chang et al. (2008) reported a phenolic content of 212.8 mg/100 g sample for cactus pear seeds.

Table 4: Antioxidant capacity and total soluble phenolics content from outstanding *Opuntia* genotypes[a]

Genotype	Antioxidant capacity (µMol/l EqTrolox)	Total phenolics (mg GAE/100 mg)
Pico Chulo	43.62 ± 0.22^{b}	60.73 ± 0.61^{g}
Selección Hidalgo	18.75 ± 1.57^{f}	82.61 ± 0.61^{c}
Tapón Aguanoso	13.18 ± 0.86^{g}	84.33 ± 0.61^{c}
Cristalina	13.06 ± 0.37^{g}	65.66 ± 2.12^{e}
Rojo Vigor	39.09 ± 0.49^{c}	107.49 ± 0.00^{b}
Rojo Pelón	18.23 ± 0.08^{f}	67.59 ± 1.21^{e}
Cardona	24.46 ± 1.84^{e}	109.64 ± 1.82^{b}
San Juanera	51.68 ± 0.17^{a}	74.67 ± 0.30^{d}
Rosa de Castilla	30.54 ± 2.90^{d}	74.46 ± 0.61^{d}
Amarilla Montesa	15.20 ± 0.18^{g}	63.73 ± 0.61^{f}
Xoconostle Cuaresmeño	5.53± 0.01^{h}	141.81 ± 1.21^{a}

[a] Results are expressed as Mean ± SD from three replicas; Means followed by different letters within the same column are significantly different (Tukey, $p < 0.05$).

In fact, Betancourt et al. (2017) found a very strong correlation between phenolic content and antioxidant activity measured by TEAC. Similar relationships were observed by Moussa-Ayoub et al. (2016) in *Opuntia dillenii* juices when these authors compared total phenolic content and antioxidant activity, and by Sawicki et al. (2016), who demonstrated that the antioxidant capacity of red beetroot was positively and significantly correlated with the betalain content. Chang et al. (2008) reported Trolox Equivalent Antioxidant Capacity (TEAC) values of 2.15 Mm and ORAC values of 0.18 mM for cactus pear seeds.

Welti-Chanes et al. (2019) found a total of 14 betalains in Mexican and Spanish prickly pear, where betanin and indicaxanthin were the most abundant, recording a maximal concentration of betacyanins (ranging from 1372 to 2176 μg/g dry whole fruit), and betaxanthin (ranging from 435 to 488 μg/g dry whole fruit), and the 17 phenolics detected corresponded mostly to flavonoid (isorhamnetin, quercetin, and kaempferol) glycosides and a phenolic acid, piscidic acid; highest phenolic content was 49.012 μg/g dry peel.

CONCLUSIONS

In conclusion, the ground seed of *Opuntia* can be considered a good quality source of protein (8.69 %), fiber (67.08 %), and oil (16.77 %) for use as a food or raw material for industrial purposes. Polyunsaturated fatty acids were prevalent in seed oil, especially linoleic (73.09 %), which was highest in 'Xoconostle Cuaresmeño'.These polyunsaturated fatty acids and the oleic/linoleic ratio of *Opuntia* seed oil reveal that seed oil can be considered a nutraceutic product, contributing to the avoidance of chronic degenerative diseases. Also, the methanolic extracts from *Opuntia* seeds exhibited marked activity in ORAC and had high total phenolic content. Antioxidant capacity was highest in 'San Juanera' seeds, demonstrating 51.68 μMol TE/l. Therefore, there is great promise for the utilization of *Opuntia* seeds for creating novel beneficial health products for nutraceutical markets in the future.

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