Nutritional and antioxidant properties of pulp and seeds of two xoconostle cultivars (*Opuntia joconostle* F.A.C. Weber ex Diguet **and** *Opuntia matudae* Scheinvar**) of high consumption in Mexico**

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ABSTRACT

The genus *Opuntia* embraces different species of cactus, and many of them produce acid fruits known as xoconostle, which are considered valuable vegetable foods in Latin America. Xoconostle fruit contains an edible thick-acid-freshly mesocarp, and seeds that are considered as by-products. Given the high potential of its use and consumption, and the lack of information about its detailed chemical composition and bioactive compounds, the aim of this study was to evaluate the nutritional and antioxidant properties of pulp and seeds of two highly consumed commercial cultivars of xoconostle fruits (*Opuntia joconostle* F.A.C. Weber ex Diguet, cv. Cuaresmeño, and *Opuntia matudae* Scheinvar, cv. Rosa).

This investigation shows that the pulp of the studied xoconostle cultivars had an appreciable amount of soluble fiber and antioxidant compounds such as ascorbic acid, while the seeds are a source of fiber, phenolics, flavonoids, PUFAs and tocopherols (specially γ-tocopherol), which provide a good antioxidant capacity. In the light of these results, xoconostle fruits should be considered of great interest for either promoting the conventional consumption, and also as sources of bioactive compounds for the addition to other food products, so that all the nutrients present are fully used, instead of being discarded.

KEYWORDS: *Opuntia*; xoconostle; PUFAs; vitamins; antioxidant capacity, seeds by-product.

1. Introduction

The genus *Opuntia* embraces about 1500 species of cactus and many of them produce sweet (cactus pear) or acid fruits (xoconostle), which growth in arid and semi-arid climates, being considered valuable vegetable foods in Latin America. Xoconostle fruit is a piriform berry and exhibits an apical depression or receptacle. It is composed by the epicarp (the skin), the mesocarp (pulp), and the endocarp (where the seeds are tightly packed together in a mucilaginous structure), as it can be seen in **Fig. 1** (Reyes-Agüero, Aguirre, & Valiente-Banuet, 2006). This fruit may remain in the plant for several months without deteriorating, and it can even be kept for several weeks in a dry and cool environment without losing flavour, color or moisture (Zabaleta-Beckler, Olivares-Orozco, Montiel-Solero, Chimal-Hernández, & Scheinvar, 2001). It has a great relevance because in arid and semiarid zones the accessibility to other vegetables is low. The mesocarp (pulp) is the edible part of this fruit, and is used as a condiment in the mexican cuisine, as well as in the elaboration of candies, jellies and beverages. It contains readily absorbable sugars, dietary fiber, ascorbic acid, polyphenols, carotenoids and betacyanin pigments, that have been related to its healthy benefits such as hypoglycaemic and hypolipidemic action, and antioxidant properties (Pimienta-Barrios, Méndez-Morán, Ramírez-Hernández, García, & Domínguez-Arias, 2008; Paiz et al., 2010; Bender, 2003; Schaffer, Schmitt-Schillig, Müller, & Eckert, 2005; Phillips et al., 2010; Osorio-Esquivel, Ortiz-Moreno, Álvarez, Dorantes-Álvarez, & Giusti, 2011). In the other hand, the seeds of xoconostle are considered as potential food by-products, since they present high content of unsaturated fatty acids, specially polyunsaturated fatty acids (PUFAs), which are associated with a reduced risk of developing cardiovascular, inflammatory and autoimmune diseases (Simopoulus, 2002). These health benefits are associated with the fact that fatty acids from n-3 and n-6

series, such as linoleic (LA, C18:2n-6) and α-linolenic (ALA, C18:3n-3) acids are the biosynthetic precursors of eicosanoids, which take part in a wide range of metabolic functions.

Furthermore, vitamin E and tocopherols (α, β, γ and δ-tocopherol) are antioxidant nutrients that play important roles in health by the inactivation of free radicals produced through normal cellular activity and from various stressors. Tocopherols act as antioxidants by their capacity to scavenge lipid peroxyl radicals of unsaturated lipid molecules, preventing propagation of lipid peroxidation (Traber, 2007). Due to its role as free radicals scavenger, vitamin E is also believed to protect against degenerative processes, such as cancer and cardiovascular diseases (Burton & Traber, 1990). It has been reported that γ-tocopherol and its physiological metabolite 2,7,8-trimethyl-2-(β-carboxyethyl)-6-hydroxychroman (γ-CEHC), inhibit COX-2-catalyzed formation of PGE2, inducing anti-inflammatory properties (Jiang, Elson-Schwab, Courtemanche, & Ames, 2000; Barreira, Alves, Casal, & Ferreira, 2009). Regarding nutritional composition and antioxidant properties, xoconostle fruits could be an attractive target for food industry while seeds, instead of being discarded, could be recovered for their high nutritional value, as sources of PUFAs, tocopherols and dietary fiber (Prieto-García, et al., 2006). Given the high potential of use and consumption of xoconostle fruits and the lack of information about its nutritional composition, the aim of this study was to evaluate the nutritional and antioxidant properties of pulp and seeds of two commercial varieties of xoconostle. Although there are some previously studies on other Opuntia fruits (Kuti, 2004; Chang, Hsieh, & Yen, 2008; Álvarez & Peña-Valdivia, 2009; Ayadi, Abdelmaksoud, Ennouri, & Attia, 2009; Moussa-Ayoub et al., 2011), and on the xoconostle fruits (Osorio-Esquivel, Ortiz-Moreno, Álvarez, Dorantes-Álvarez, Giusti, 2011; Prieto-García et al., 2006; Guzmán-Maldonado et al., 2010), this is the

first report that highlights the nutritional composition and antioxidant potential of the seeds, being important to evaluate the possibility of using them in food industry as functional ingredients. Furthermore, the pulp, mainly in those varieties of higher consumption in Mexico, could also be considered as functional foods.

2. Material and Methods.

2.1. Standards and reagents

The eluents *n*-hexane 95% and ethyl acetate 99.98% were of HPLC grade from Lab-Scan (Lisbon, Portugal). Methanol was of analytical grade purity and supplied by Pronalab (Lisbon, Portugal). The fatty acids methyl ester (FAME) reference standard mixture 37 (standard 47885-U) as well as other individual fatty acid isomers, tocopherol standards (α, β, γ and δ-isoforms), glucose, fructose, sucrose, organic acid standards (L (+)-ascorbic, oxalic, malic, citric and succinic acids), and gallic acid (for phenolics determination) were purchased from Sigma (St. Louis, MO, USA). Glutamic acid (used in organic acids analysis) and L-Cystein (used in vitamin C analysis) were purchased from Merck (Darmstadt, Germany). Racemic tocol (for tocopherols analysis) in *n*-hexane, 50 mg/mL, was purchased from Matreya (PA, USA). The 2,2-diphenyl-1-picrylhydrazyl (DPPH) used in antioxidant activity evaluation was obtained from Alfa Aesar (Ward Hill, MA, USA). Water was treated in a Milli-Q water purification system (TGI Pure Water Systems, USA).

2.2. Material

Fruits of *O. joconostle* F.A.C. Weber ex Diguet cv. Cuaresmeño (white skinned), and *O. matudae* Scheinvar cv. Rosa (pink skinned), were provided by a Mexican association (CoMeNTuna). The fruits were manually harvested in spring 2009 from a growing area in Mexico (Hidalgo state), when the characteristics of maturation (size and skin colours) were according to conventional standards for this product. The thin skin (epicarp) was removed, and then the mesocarp (the edible pulp) was separated of the endocarp with seeds (**Fig. 1**), and cut into small pieces. Both parts (mesocarp and endocarp) were freeze-dried. The seeds were cleaned by removing any mucilaginous material or pulp. The two lyophilized fractions (pulp and seeds) were ground separately, passed through a 1.0-mesh sieve, and stored in the dark at -20 ºC until analysis. Total vitamin C analysis was carried out in fresh material.

2.3. Proximate Analysis.

AOAC (Association of Analytical Communities) official methods (AOAC, 2005) were used for analysis of moisture (Method 925.09), total protein (Method 950.48), fat (Method 983.23) and ash (Method 930.05) (AOAC, 2005).

2.4. Dietary fiber: Soluble and insoluble fiber

Soluble (SDF) and insoluble dietary fiber (IDF) was determined according to AOAC enzymatic-gravimetric method (AOAC, 2005). Total dietary fiber (TDF) was the sum of SDF and IDF.

2.5. Soluble sugars.

Ethanol soluble carbohydrates were extracted with 80% ethanol at 60 ºC and soluble sugars profile was determined by HPLC (Sánchez-Mata, Cámara-Hurtado, & Díez-Marqués, 2002), using a Waters system equipped with a refractive index (RI) detector and the column used was a Luna 5mm NH_2 100 R, 250 mm × 4.60 mm (Phenomenex, Torrance, CA, USA). The flow rate of the acetonitrile/water solvent (80:20) was 0.9 mL/min. Quantification was based on the RI signal response, and the resultant peak areas in the chromatograms were plotted against concentrations obtained from standards.

2.6. Fatty acids

Fatty acids (obtained after Soxhlet extraction) were methylated with 5 mL of methanol:sulphuric acid 95%:toluene 2:1:1 (v/v/v) for, at least, 12 h in a bath at 50 ºC and 160 rpm; to obtain phase separation 3 mL of deionised water were added; the fatty acids methyl esters (FAME) were recovered by strongly shaking with 3 mL of diethyl ether, and the upper phase was passed through a micro-column of anhydrous sodium sulphate to eliminate the water. The sample was recovered in a vial with Teflon and filtered through a 0.2 µm Whatman nylon filter. Fatty acids were determined by gas-liquid chromatography with flame ionization detection (GC-FID)/capillary column as described previously by the authors (Morales et al., 2011a). The analysis was carried out with a DANI model GC 1000 instrument (Milan, Italy) equipped with a split/splitless injector and a flame ionization detector (FID at 260 ºC). The column used was a Macherey-Nagel (Duren, Germany) (50% cyanopropyl-methyl, 50% phenylmethylpolysiloxane; 30 m×0.32 mm ID × 0.25 µm d_f). The oven temperature

program was as follows: the initial temperature of the column was 50 ºC, held for 2 min, then a 30ºC/min ramp to 125 ºC, 5ºC/min ramp to 160 ºC, 20ºC/min ramp to 180 ºC, 3ºC/min ramp to 200 ºC, 20ºC/min ramp to 220 ºC and held for 15 min. The carrier gas (hydrogen) flow-rate was 4.0 mL/min (0.61 bar), measured at 50 ºC. Split injection (1:40) was carried out at 250 ºC. Fatty acid identification was made by comparing the relative retention times of FAME peaks from samples with standards. The results were recorded and processed using CSW 1.7 software (DataApex 1.7).

2.7. Vitamin C

The quantification of ascorbic acid (AA) was carried out by a previously validated HPLC method (Ruiz-Rodríguez et al., 2011). Briefly, samples were extracted with 4.5% metaphosphoric acid, and dehydroascorbic acid was reduced to AA in the extract, using 4% L-cysteine at pH 7. The HPLC equipment used was a liquid chromatographer (Micron Analítica, Madrid, Spain) equipped with an isocratic pump (model PU-II) and an AS-1555 automatic injector (Jasco AS-1555 Intelligent simple, Japan). The column used was a Sphereclone ODS 250 × 4.60, 5 µm (Phenomenex, Torrance, CA, USA) a UV-visible detector (Thermo Separation Specta Series UV100). The HPLC conditions were: 1.8 mM H_2SO_4 in distilled water (pH = 2.6) as solvent, with a flow rate of 0.9 mL/min and UV detection at 245 nm. Data were analyzed using a Biocrom 2000 3.0 software. Quantification was based on the UV signal response, and the resultant peak areas in the chromatograms were plotted against concentrations obtained from standards.

2.8. Tocopherols composition

Butylated hydroxytoluene (BHT) solution in hexane (10 mg/mL; 100 µL) and tocol (internal standard, IS) solution in hexane (50 µg/mL; 400 µL) were added to the sample prior to the extraction procedure. Samples (~500 mg) were homogenized with methanol (4 mL) by vortex mixing (1 min). Subsequently, hexane (4 mL) was added and again vortex mixed for 1 min. Saturated NaCl aqueous solution (2 mL) was added, the mixture was homogenized (1 min), centrifuged (5 min, 4,000*g*) and the clear upper layer was carefully transferred to a vial. The sample was re-extracted twice with *n*-hexane. The combined extracts were taken to dryness under a nitrogen stream, redissolved in 2 mL of *n*-hexane, dehydrated with anhydrous sodium sulphate and filtered through 0.2 µm nylon filters and transferred into a dark injection vial. Tocopherols content was determined following a procedure previously described by Morales et al. (2011b). The HPLC equipment consisted of an integrated system with a Smartline pump 1000 (Knauer, Germany), a degasser system Smartline manager 5000, an AS-2057 auto-sampler and a 2500 UV detector at 295 nm (Knauer, Germany) connected in series with a FP-2020 fluorescence detector (Jasco, Japan) programmed for excitation at 290 nm and emission at 330 nm. The column used was a normal-phase 250 mm × 4.6 mm i.d., 5 µm, Polyamide II, with a 10 mm × 4 mm i.d. guard column of the same material (YMC Waters, Dinslaken, Germany), operating at 30ºC. The mobile phase used was a mixture of *n*-hexane and ethyl acetate (70:30, v/v) at a flow rate of 1 mL/min. Tocopherols identification was made by comparing the relative retention times of sample peaks with standards. Quantification was based on the fluorescence signal response, using the internal standard method.

2.9. Determination of total phenolics and flavonoids

Extracts preparation. A fine dried powder (1 g) was extracted by stirring with 40 mL of

methanol at 25 °C for 1 h and filtered through Whatman No. 4 filter paper. The residue was then extracted with one additional 40 mL portion of methanol. The combined methanolic extracts were evaporated at 35 °C under reduced pressure (rotary evaporator Büchi R-210 R-210; Flawil, Switzerland), re-dissolved in methanol at a concentration of 5 mg/mL, and stored at 4 °C (2 days) for further use.

Total phenolics were estimated based on procedures described by Wolfe Wu, & Liu (2003) with some modifications. An aliquot of the extract solution (0.5 mL) was mixed with Folin–Ciocalteu reagent (2.5 mL, previously diluted with water 1:10 v/v) and sodium carbonate (75 g/l, 2 mL). The tubes were vortexed for 15 s and allowed to stand for 30 min at 40 °C for colour development. Absorbance was then measured at 765 nm (AnalytikJena 200 spectrophotometer, Jena, Germany). Gallic acid was used to calculate the standard curve (9.4×10^{-3} - 1.5×10^{-1} mg/mL).

Flavonoids content was determined using the method of Jia, Tang, & Wu (1993), with some modifications. An aliquot (0.5 mL) of the extract solution was mixed with distilled water (2 mL) and subsequently with $NaNO_2$ solution (5%, 0.15 mL). After 6 min, $AlCl_3$ solution (10%, 0.15 mL) was added and allowed to stand further 6 min, thereafter, NaOH solution (4%, 2 mL) was added to the mixture. Immediately, distilled water was added to bring the final volume to 5 mL. Then the mixture was properly mixed and allowed to stand for 15 min. The intensity of pink colour was measured at 510nm. (+)-Catechin was used to calculate the standard curve (4.5×10^{-3} - 2.9×10^{-1} mg/mL).

2.10. Evaluation of antioxidant activity

DPPH radical-scavenging activity. This methodology was performed using an ELX800 Microplate Reader (Bio-Tek Instruments, Inc.), according to Morales et al. (2011b). The

reaction mixture in each one of the 96-wells consisted of one of the different concentrations of the extracts (30 μl) and aqueous methanolic solution (80:20 v/v, 270 μl) containing DPPH radicals (6×10^{-5} mol/L). The mixture was left to stand for 60 min in the dark. The reduction of the DPPH radical was determined by measuring the absorbance at 515 nm. The radical-scavenging activity (RSA) was calculated as a percentage of DPPH discolouration using the equation: % RSA = $[(A_{DPPH} - A_S)/A_{DPPH}] \times 100$, where A_S is the absorbance of the solution when the sample extract has been added at a particular level, and A_{DPPH} is the absorbance of the DPPH solution. The extract concentration providing 50% of radicals scavenging activity (EC_{50}) was calculated from the graph of RSA percentage against extract concentration.

Reducing power. Different concentrations of the extracts (0.5 mL) were mixed with sodium phosphate buffer (200 mmol/l, pH 6.6, 0.5 mL) and potassium ferricyanide (1% w/v, 0.5 mL) in Eppendorf tubes. The mixture was incubated at 50 °C for 20 min, and trichloroacetic acid (10% w/v, 0.5 mL) was added. Afterwards, the mixture (0.8 mL) was poured in the 48-wells microplates, as also deionised water (0.8 mL) and ferric chloride (0.1% w/v, 0.16 mL), and the absorbance was measured at 690 nm in the Microplate Reader described above (Morales et al., 2011b). The extract concentration providing 0.5 of absorbance (EC_{50}- 50% of the maximal absorbance, 1) was calculated from the graph of absorbance at 690 nm against extract concentration.

Inhibition of β-carotene bleaching. A solution of β-carotene was prepared by dissolving β-carotene (2 mg) in chloroform (10 mL). Two millilitres of this solution were pipetted into a round-bottom flask. After the chloroform was removed at 40 °C under vacuum, linoleic acid (40 mg), Tween 80 emulsifier (400 mg), and distilled water (100 mL) were

added to the flask with vigorous shaking. Aliquots (4.8 mL) of this emulsion were transferred into different test tubes containing different concentrations of the extracts (0.2 mL). The tubes were shaken and incubated at 50 °C in a water bath (Morales et al., 2011b). As soon as the emulsion was added to each tube, the zero time absorbance was measured at 470 nm. β-Carotene bleaching inhibition was calculated using the following equation: (β-carotene absorbance after 2 h of essay/initial β-carotene absorbance) × 100. The extract concentration providing 50% antioxidant activity (EC_{50}) was calculated by interpolation from the graph of β-carotene bleaching inhibition percentage against extract concentration.

2.11. Statistical analysis

Analysis of variance (ANOVA), followed by Duncan`s test, was conducted using Statgraphics Plus 5.1. software to analyze data, at a confidence level of 95 %. Values were expressed as means and standard deviations of triplicate analyses (n=3).

3. Results and discussion

3.1. Nutritional composition

Proximal composition of *O. joconostle* and *O. matudae* is listed in **Table 1**. Results of pulp and seeds are separately presented, as seeds represent a high proportion of the complete fruit (21 to 23%). The pulp of xoconostle was characterized by an appreciable content of digestible carbohydrates, while the seeds had a significant predominance of non-digestible compounds (insoluble fiber dietary), protein, fat and ash contents. Proximal composition in pulp was similar for both cultivars, with the exception of

soluble sugars and ash. The seeds had significant differences among both species, with lower moisture content in *O. matudae* and a higher content of nutrients (protein, fat, soluble sugars, fiber and ash) compared with *O. joconostle.*

One of the main characteristics of this fruit is its sour taste (Guzmán-Maldonado et al., 2010) and its low content of soluble sugars. The content of soluble sugars in pulp (1.56 ± 0.17 and 2.02 ± 0.09 g/100 fw, in *O. matudae* and *O. joconostle* respectively) was low compared with other *Opuntia* fruits (cactus pear) (Ayadi et al., 2009), or with other conventional fruits. The soluble sugars were the most important contributors to the total available carbohydrates fraction. The profile of the soluble sugars in both fractions of the fruit (pulp and seeds) showed a clear predominance of fructose, while glucose and sucrose appeared as minor compounds (**Table 1**).

In comparison with other fruits, the xoconostle pulp provides a good percentage of total fiber (2 % approximately), mainly as insoluble fiber. Alvarez & Peña-Valdivia (2009) reported that the distribution of fiber fractions in xoconostle was different according to the stages of maturation; in particular, mucilage and pectin increased (3 or 4 times) with the maturation of the fruit. The Recommended Dietary Allowance (RDA) established by dietary fiber (FAO, 2003), indicate the consumption between 25 and 30 g of fiber per day. Furthermore, it is recommended that a third of total fiber (approximately 10 g) should be soluble fiber, and the distribution of fiber in the xoconostle pulp is in agreement with the nutritional recommendations. According to these recommendations, 100 g of edible pulp of xoconostle provides up to 5 and 8 % of the daily fiber requirement for adults (1.74 ± 0.07 and 2.31 ± 0.12 g/100 g fw in *O. matudae* and *O. joconostle*, respectively), with a very adequate soluble/insoluble ratio. On the other hand, seeds revealed the highest content of dietary fiber (19.22 and 30.17 g/100g in *O. joconostle* and *O. matudae*, respectively) mainly as insoluble fiber in similar form of

other conventional fruits as apple, pear, pineapple and strawberry (Pak, 2003; Ramulu & Udayasekhara, 2003). In comparison with other species of the genus *Opuntia* the fiber content of xoconostle fruit is higher (Ramírez-Moreno et al., 2011; Díaz, Rodríguez & Díaz, 2007), making it ideal to be added to other foods to improve fiber intake of the population.

The amount of fat in xoconostle seeds (2.45 ± 0.05 and 3.52 ± 0.12 g/100 g fw in *O. joconostle* and *O. matudae*, respectively) was higher compared with the pulp (0.03 and 0.04 g/100 g fw in O. *joconostle* and *O. matudae*, respectively). The amount of fat present in seeds is nutritionally interesting and makes it a good potential source of dietary oil. Sawaya & Khan (1982) reported the high quality of oil in other *Opuntia* species in terms of fatty acids composition (82% unsaturated fatty acids), representing a major contribution to the dietary intake of essential fatty acids for population who traditionally include these fruits in the diet. The individual fatty acids content in pulp and seeds of the xoconostle cultivars in this study are presented in Table 2. At least nineteen fatty acids were identified and quantified in the seeds of both cultivars, while in the pulp only sixteen fatty acids were characterized. The beneficial effects attributed to the n-3 PUFAs is due to their anti-agregant effects in cardiovascular diseases. Therefore the ratio of dietary ALA to LA is very important from a nutritional point of view (Guil, & Rodriguez, 1999). Adequate intakes for male adults must be around 14-17 g per day of linoleic acid (LA) and 1.6 g per day of α-linolenic acid (ALA), whereas for females the adequate intakes are lower, around 11-12 g per day of LA and 1.1 g per day of ALA (Trumbo, Schlicker, Yates, & Poos, 2002). LA (C18:2n6) was found at 72.49 and 79.15 % in the seeds of Cuaresmeño and Rosa cultivars, respectively (Table 2), similar values to the ones found in *Opuntia* fruits from other species (*O. ficus-indica*) (Sawaya & Khan, 1982; Ramadan, & Mörsel, 2003).

Two saturated fatty acids (SFA), palmitic acid (PA, C16:0) and octanoic acid (C8:0) were found in significant amounts. In fact, PA was the main SFA found in all the samples, except in the pulp of *O. joconostle* fruit pulp, whose main SFA was octanoic acid (C8:0, 21.35%), with percentages of 9.42 and 12.74% in seed and pulp of *O. matudae* respectively, while *O. joconostle* presented 12.35 and 15.03% in seed and pulp, respectively (**Table 2**). Comparing with other authors, the percentages of PA were lower than the ones described by Ramadan & Mörsel (2003) for cactus pear (*O. ficus–indica*). The mentioned variability could be explained by different climatic and soil conditions as well as by time of harvest and postharvest conditions (Prohens et al. 2005). In the case of monounsaturated fatty acids (MUFA), oleic acid (OA, 18:1n9) was the most representative one, with percentages of 2.45 and 3.68% in pulp of *O. matudae* and *O. joconostle,* respectively, and 7.82 and 9.72% in seeds of *O. joconostle* and *O. matudae*, respectively. Being *O. joconostle* fruits the one that had the highest content. The highest LA content was found in the seeds (72.49 and 79.15% in *O. joconostle* and *O. matudae*, respectively), whereas the ALA content was higher in the pulp of the fruit (7.67 and 8.64% in *O. joconostle* and *O. matudae*, respectively).

The percentages of each group of fatty acids (SFA, MUFA and PUFA) and PUFA/SFA and n-3/n-6 ratios, calculated in pulp and seed of the studied cultivars are also shown in **Table 2**. As can be observed, the seeds provide the lowest SFA (12.01 ± 1.43 to 16.86 ± 0.17%, in *O. matudae* and *O. joconostle*, respectively), the highest MUFA (7.64 ± 0.86 to 10.28 ± 0.03%, in *O. matudae* and *O. joconostle,* respectively) and PUFA (72.85 ± 0.14 to 80.26 ± 1.83%, in *O. joconostle* and *O. matudae,* respectively) contents; in this way, seeds of xoconostle fruits were good sources of healthy unsaturated fatty acids as also stated by Sawaya & Khan (1982) for cactus pear seeds oil. All samples presented a good ratio PUFA/SFA, higher than 0.45: pulp (1.28 ± 0.74 and 2.88 ± 0.03 for *O.*

joconostle and *O. matudae,* respectively) and seeds (4.32 ± 0.05 and 6.67 ± 0.73 for *O. joconostle* and *O. matudae*, respectively); these results are interesting since diets rich in n-6 PUFA and low in SFA have been shown to be cardio-protective (McGee, Ree, & Yano, 1984). Moreover, the samples presented low n-3/n-6 ratios (less than 0.20); it is becoming increasingly clear that both n-3 and n-6 PUFA have independent health effects in the body, and as intakes of n-6 PUFA are within the guidelines for a healthy diet, concerns about the n-3 to n-6 ratio are driven by low intakes of n-3 rather than high intakes of n-6 (Ward & Singh, 2005). Furthermore, the optimal balance between dietary ALA and LA, which are the two main fatty acids influencing this ratio, may contribute to reduce the prevalence of different diseases such as asthma atherosclerosis and potential lung cancer (Oddym et al., 2004).

3.2. Antioxidant compounds and antioxidant properties

The contents of vitamin C, vitamin E and antioxidant activity of analysed samples are presented in **Table 3**. The methodology applied for vitamin C analysis allowed the quantification of the two active forms: ascorbic acid (AA) and dehidroascorbic acid (DHAA). The total content of vitamin C as AA was characterized in pulp of each fruit, with values of 20 ± 0.32 and 31.67 ± 0.67 mg/100 g fw in *O. joconostle* and *O. matudae*, respectively. These values was in agreement with Guzmán-Maldonado et al. (2010) who reported an ascorbic acid content of 31.8 mg/100 g fw for pulp of *O. matudae* xoconostle, but are lower than the values reported by Corral-Aguayo, Yahia, Carrillo-López, & González-Aguilar (2008) and Kuti (2004) for other cactus pear fruits (around 45 mg/100 g fw). The vitamin C in the pulp of xoconostle was mostly in the reduced form (AA), as no detectable amounts of DHAA were found, which could mean

a higher contribution to antioxidant activity. Vitamin C was not detected in xoconostle seeds, in the same way found in previous studies for cactus pear (Corral-Aguayo, Yahia, Carrillo-López, & González-Aguilar, 2008).

Total tocopherols content was higher in seeds of both cultivars than in the pulp (3.23 ± 0.18 and 6.71 ± 0.38 mg/100 g fw in *O. joconostle* and *O. matudae*, respectively). The four isoforms (α, β, γ and δ- tocopherols) were identified. The seeds presented a high amount of γ-tocopherol (3.07 and 6.42 mg/100g fw in *O. joconostle* and *O. matudae*, respectively), whereas α-tocopherol was the main isoform in pulp, with relatively low values (0.10 and 0.16 mg/100g fw in *O. matudae* and *O. joconostle*, respectively) (**Table 3**). This data is quite important because until now, there was only few available data on tocopherols content in cactus pear species (*O. ficus–indica* L.) (Ramadan & Mörsel, 2006; Yahia & Mondragon, 2006) and not in xoconostle fruits, whose by the high amount in the seeds could be consider for the food industry as a functional ingredient.

Xoconostle seeds presented the highest phenolics and flavonoids contents, particularly *O. matudae* (phenolics 59.48 mg GAE/g of extract and flavonoids 58.40 mg CE/g of extract) (**Table 3**). The total polyphenol content in these cultivars were three fold higher than the ones reported by other authors in other cultivars of *Opuntia matudae* xoconostle (Guzmán-Maldonado et al., 2010) and *Opuntia joconostle* fruits (Osorio-Esquivel, Ortiz-Moreno, Álvarez, Dorantes-Álvarez, & Giusti, 2011), and even higher than the contents found in cactus pear fruits (Chang et al., 2008). Phenolic compounds help to protect plants against ultraviolet light and act as defences against pathogenic microorganisms in plants. This type of protection could be necessary for xoconostle fruits, that may remain in the plant for several months without deterioration, and could explain its higher phenolics and flavonoids contents than other fruits of *Opuntia*

(Chang, Hsieh, & Yen, 2008) or even, the higher flavonoids content found in peel or epicarp (0.68 mg CE/g) in comparison with the pulp or mesocarp (around 0.35 mg CE/g) described in other studies (Osorio-Esquivel, Ortiz-Moreno, Álvarez, Dorantes-Álvarez, & Giusti, 2011). Flavonoids act as antioxidants, which markedly delay or prevent oxidation of the substrate, and they are strongly correlated to antioxidant activity. The consumption of xoconostle fruits may contribute to increase the amount of antioxidants in the diet.

The antioxidant properties of the studied fruits were evaluated by DPPH radical scavenging capacity, reducing power (Fe^{3+} into Fe^{2+}) and inhibition of lipid peroxidation using β-carotene–linoleate model system by neutralising the linoleate-free radical and other free radicals formed in the system, which attack the highly unsaturated β-carotene models (Morales, et al., 2011b; Barros, Carvalho, Sá Morais, & Ferreira, 2010). DPPH free radicals can be used to evaluate the antioxidant activity in a relatively short time. The highest antioxidant properties of seeds are in agreement with their highest phenolics and flavonoids contents (**Table 3**). The seeds of *O. joconostle* fruits presented the highest DPPH scavenging activity (EC_{50} 1.53 ± 0.05 mg/mL) and reducing power (EC_{50} 0.27 ± 0.04 mg/mL) activities, whereas the pulp of both fruits presented the lowest phenolics content and the worst antioxidant activity for DPPH and reducing power assays. On the other hand, these samples presented the highest antioxidant capacity for lipid peroxidation inhibition; with values for β-carotene bleaching inhibition assay of 0.02 ± 0.00 and 0.32 ± 0.02 mg/mL for pulp of *O. matudae* and *O. joconostle* fruits, respectively.

4. Conclusions

This investigation shows that the pulp of the studied xoconostle cultivars had an appreciable amount of soluble fiber and antioxidant compounds such as ascorbic acid, while the seeds are a source of fiber, phenolics, flavonoids, PUFAs and tocopherols (specially γ-tocopherol), which provide a good antioxidant capacity. The most remarkable features of the cultivars of xoconostle studied were that *O. matudae* fruits showed higher ascorbic acid in the pulp, and higher fiber and flavonoids contents in the seeds than *O. joconostle*, which had a pulp richer in soluble sugars and higher antioxidant activity in the seeds. For those reasons, these fruits should be considered of great interest for either promoting the conventional consumption, and as sources of bioactive compounds for the addition to other food products, so that all the nutrients present are fully used instead of being discarded.

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Table 1. Nutritional composition of cultivated xoconostle fruits.[A]

Nutritional parameters	*Opuntia joconostle*, cv. Cuaresmeño		*Opuntia matudae*, cv. Rosa	
	Pulp	Seeds	Pulp	Seeds
Moisture	93.24 ± 0.02 [c]	73.95 ± 1.09 [b]	94.11 ± 0.00 [c]	60.44 ± 0.66 [a]
Protein	0.66 ± 0.01 [a]	2.12 ± 0.00 [b]	0.56 ± 0.00 [a]	3.45 ± 0.02 [c]
Fat	0.03 ± 0.0 [a]	2.45 ± 0.05 [b]	0.04 ± 0.00 [a]	3.52 ± 0.12 [c]
Total available carbohydrates [B]	3.69 [c]	1.71 [b]	3.93 [d]	1.56 [a]
Soluble sugars	2.02 ± 0.09 [c]	0.95 ± 0.09 [a]	1.56 ± 0.17 [b]	1.47 ± 0.19 [b]
Fructose	1.38 ± 0.03 [c]	0.71 ± 0.07 [a]	0.87 ± 0.03 [ab]	0.99 ± 0.12 [b]
Glucose	0.37 ± 0.05 [b]	0.15 ± 0.01 [a]	0.35 ± 0.13 [b]	0.34 ± 0.05 [b]
Sucrose	0.27 ± 0.01 [c]	0.09 ± 0.00 [a]	0.34 ± 0.01 [d]	0.14 ± 0.02 [b]
Total dietary fiber	2.31 ± 0.12 [b]	19.22 ± 0.15 [c]	1.74 ± 0.07 [a]	30.17 ± 0.64 [d]
Insoluble fiber	1.45 ± 0.07 [b]	18.85 ± 0.12 [c]	1.16 ± 0.01 [a]	29.04 ± 0.57 [d]
Soluble fiber	0.86 ± 0.05 [c]	0.36 ± 0.03 [a]	0.58 ± 0.07 [b]	1.13 ± 0.07 [d]
Ash	0.07 ± 0.00 [a]	0.54 ± 0.01 [c]	0.11 ± 0.00 [b]	0.85 ± 0.04 [d]

[A]Results were expressed as g/100 g of fresh weight (fw). Mean ± SD, n=3. In each row, different letters mean statistically significant difference ($p \leq 0.05$).

[B] Total available carbohydrates were calculated as the difference of moisture, protein, fat, ash and fiber values (Barros et al., 2010).

Table 2. Fatty acids composition of cultivated xoconostle fruits.[A]

Fatty acids	*Opuntia joconostle*, cv. Cuaresmeño		*Opuntia matudae*, cv. Rosa	
	Pulp	Seeds	Pulp	Seeds
C6:0	nd	0.57 ± 0.03	0.24 ± 0.00	0.09 ± 0.02
C8:0	31.35 ± 3.55	0.01 ± 0.00	8.04 ± 0.67	traces
C10:0	0.16 ± 0.10	0.01 ± 0.00	0.05 ± 0.00	traces
C12:0	0.82 ± 0.41	0.09 ± 0.00	0.19 ± 0.01	traces
C13:0	nd	0.01 ± 0.00	nd	traces
C14:0	0.84 ± 0.19	0.04 ± 0.00	0.28 ± 0.00	0.04 ± 0.00
C14:1	nd	nd	nd	traces
C15:0	0.32 ± 0.02	0.01 ± 0.00	0.48 ± 0.04	0.01 ± 0.00
C15:1	nd	0.01 ± 0.00	nd	0.01 ± 0.00
C16:0	15.03 ± 1.59	12.35 ± 0.40	12.74 ± 0.18	9.42 ± 0.78
C16:1	0.48 ± 0.06	0.41 ± 0.01	0.50 ± 0.00	0.31 ± 0.03
C17:0	2.08 ± 0.12	0.04 ± 0.00	nd	0.02 ± 0.00
C18:0	2.81 ± 1.04	3.28 ± 0.18	2.05 ± 0.02	2.04 ± 0.34
C18:1n9c	3.68 ± 1.29	9.72 ± 0.03	2.45 ± 0.06	7.82 ± 0.84
C18:2n6c	32.70 ± 2.30	72.49 ± 0.13	64.29 ± 0.46	79.15 ± 1.86
C18:3n3c	8.64 ± 1.97	0.36 ± 0.01	7.67 ± 0.21	0.41 ± 0.04
C20:0	0.25 ± 0.15	0.26 ± 0.01	0.15 ± 0.06	0.19 ± 0.01
C20:1	nd	0.07 ± 0.02	nd	0.05 ± 0.00
C22:0	0.30 ± 0.12	0.19 ± 0.02	0.23 ± 0.06	0.04 ± 0.01
C23:0	0.10 ± 0.08	nd	nd	0.10 ± 0.02
C22:1n9c	nd	0.05 ± 0.01	nd	0.04 ± 0.01
C23:0	nd	nd	nd	0.03 ± 0.00
C24:0	0.38 ± 0.26	0.09 ± 0.03	0.53 ± 0.23	0.06 ± 0.00
C24:1	nd	0.02 ± 0.00	0.09 ± 0.03	0.01 ± 0.00
SFA (% of total FA)	54.43 ± 0.92^{c}	16.86 ± 0.17^{a}	25.00 ± 0.22^{b}	12.10 ± 1.43^{a}
MUFA (% of total FA)	4.16 ± 1.23^{a}	10.28 ± 0.03^{c}	3.05 ± 0.03^{a}	7.64 ± 0.86^{b}
PUFA (% of total FA)	41.41 ± 0.32^{a}	72.85 ± 0.14^{b}	71.95 ± 0.25^{b}	80.26 ± 1.83^{c}
PUFA/SFA (% of total FA)	1.28 ± 0.74^{a}	4.32 ± 0.05^{b}	2.88 ± 0.03^{a}	6.67 ± 0.73^{c}
n-3/n-6	0.20 ± 0.01^{b}	0.01 ± 0.00^{a}	0.12 ± 0.00^{b}	0.01 ± 0.00^{a}

[A]Results were expressed as relative percentage of each fatty acid. Mean ± SD, n=3. In each row, different letters mean statistically significant difference ($p \leq 0.05$).

Traces (≤ 0.005 %); nd (not detected).

Table 3. Antioxidant compounds and antioxidant properties (EC_{50} values) of cultivated xoconostle.

Antioxidant compounds	*Opuntia joconostle*, cv. Cuaresmeño		*Opuntia matudae,* cv. Rosa	
	Pulp	Seeds	Pulp	Seeds
Ascorbic acid[A]	20.63 ± 0.32 [a]	nd	31.67 ± 0.67 [b]	nd
Total tocopherols[A]	0.22 ± 0.01 [b]	3.23 ± 0.18 [c]	0.14 ± 0.00 [a]	6.71 ± 0.38 [d]
α-tocopherol[A]	0.16 ± 0.01 [b]	0.09 ± 0.02 [a]	0.10 ± 0.00 [a]	0.19 ± 0.01 [c]
β-tocopherol[A]	0.01 ± 0.00 [a]	0.01 ± 0.00 [a]	Traces	0.02 ± 0.00 [b]
γ-tocopherol[A]	0.05 ± 0.00 [a]	3.07 ± 0.15 [b]	0.03 ± 0.00 [a]	6.43 ± 0.37 [c]
δ-tocopherol[A]	Traces	0.06 ± 0.01 [b]	0.01 ± 0.00 [a]	0.08 ± 0.00 [c]
Total Phenolics [B]	38.57 ± 6.87 [b]	50.43 ± 4.86 [c]	33.71 ± 2.09 [b]	59.48 ± 0.69 [d]
Total Flavonoids[C]	3.93 ± 0.19 [b]	24.18 ± 1.69 [c]	0.86 ± 0.09 [a]	58.40 ± 0.78 [d]
Antioxidant activity EC_{50} values				
DPPH scavenging activity[D]	5.14 ± 0.20 [c]	1.53 ± 0.05 [a]	> 16 [d]	1.88 ± 0.11 [b]
Reducing power[D]	3.16 ± 0.12[c]	0.27 ± 0.04 [a]	5.35 ± 0.63 [d]	1.58 ± 0.01 [b]
β-carotene bleaching inhibition[D]	0.32 ± 0.02 [b]	2.11 ± 0.30 [c]	0.02 ± 0.00 [a]	4.75 ± 0.03 [d]

[A]Results were expressed as mg/100 g of fresh weight (fw).

[B] Results were expressed as mg of gallic acid equivalents (GAE)/g of extract.

[C] Results were expressed as mg of catechin equivalents (CE)/g of extract.

[D]Results were expressed as mg/mL of extract.

Mean ± SD, n=3. In each row, different letters mean statistically significant difference ($p \leq 0.05$).

nd (not detected).

Traces (≤ 0.005 mg/100 g fw).

Figure 1. Picture of *Opuntia joconostle* (cv. Cuaresmeño) fruits. The whole fruit (a) and its parts (b) are shown, epicarp, mesocarp (edible part) and endocarp (mucilaginous part with seeds).