Xoconostle fruit (*Opuntia matudae* Scheinvar cv. Rosa) by-products as potential functional ingredients

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ABSTRACT

There is a lack of information on the potential use of xoconostle cultivars as sources of antioxidants for food, pharmaceutical and colorant industries. The aim of this study was to provide a phytochemical characterization and antioxidant activity evaluation of *Opuntia matudae* Scheinvar cv. Rosa by-products (epicarp and endocarp mucilage's), in order to evaluate their interest as sources of functional ingredients for human or animal foods. These by-products showed a high content in glucose, citric and linoleic acids, tocopherols, and isorhamnetin-*O*-(di-deoxyhexosyl-hexoside) (mainly in epicarp), and presented relevant antioxidant properties. The obtained results support the use of *O. matudae* Scheinvar cv. Rosa agro-industrial by-products as functional food ingredients, namely for antioxidant-enriched formulations, instead of being discarded.

KEYWORDS: *Opuntia matudae*; By-products; Functional ingredients; Polyphenols, PUFAs; Vitamins; Antioxidant capacity

Running title: ***Opuntia matudae* by-products as potential functional ingredients**

1. Introduction

Cactus pears (*Opuntia* spp.) are ubiquitous in Mexican semiarid regions (Gallegos-Vázquez, Scheinvar, Núñez-Colín, & Mondragón-Jacobo, 2012) due to their minimal water requirement, hardiness and adaptability to extreme temperature (Patel, 2013). Ten species producing xoconostle fruits have been described in Mexico (Scheinvar et al., 2009), nine of these belong to the genus Opuntia (*O. heliabravoana* Scheinvar, *O. elizondoana* E. Sánchez and Villaseñor, *O. joconostle* F.A.C. Weber, *O. matudae* Scheinvar, *O. spinulifera* Salm-Dyck, *O. leucotricha* DC, *O. zamudioi* Scheinvar, *O. durangensis* Britton and Rose, *O. oligacantha* C.F. Förster). *Opuntia joconostle* F.A.C. Weber ex Diguet cv. Cuaresmeño is the most exploited and marketed species followed by *Opuntia matudae* Scheinvar cv. Rosa. The main regions of xoconostle cv. Cuaresmeño production are Puebla, Estado de México, Hidalgo and Guanajuato, with approximately 10000 ton per annum (Guzman-Maldonado et al. 2010), while there is no statistical information about the production of most of xoconoslte cultivars, such as *cv*. Rosa.

The fruits of the studied cultivar are very prized for their coloury, fleshy and acidic pulp (mesocarp), characterized by a deep dark purple endocarp, a mucilaginous part with small seeds (**Figure 1**) (Osorio-Esquivel et al., 2011; Scheinvar, Filardo, Olalde, & Zavaleta, 2009), even with a more attractive pink colour in comparison with other cultivars. The mesocarp (pulp) is the edible fraction, traditionally used as a condiment in sauces or other dishes of the Mexican cuisine, as well as in the elaboration of candies, jellies and beverages. For its consumption, the mesocarp needs to be cleaned and separated from the endocarp (with a mucilaginous structure that surrounds the seeds of the fruit) and seed by-products, being discarded together with the peel (epicarp) (**Figure 1**).

Xoconostle (acidic cactus pear) and its by-products have recently received considerable attention from health professionals as well as from consumers regarding the discovery of their health-promoting potential, such as antihypoglycemic, antihyperlipidemic, hypocholesterolemic, anti-inflammatory, antidiuretic, antiulcerogenic and immunostimulating activity, as well as in some cancers prevention (Díaz-Vela, Totosaus, Cuz-Guerrero, & Pérez-Chabela, 2013; Chavez-Santoscoy, Gutierrez-Uribe, & Serna-Saldívar, 2009; Morales et al., 2012 and 2014; Osorio-Esquivel et al., 2011, 2012 and 2013; Sanchez-Gonzalez, Jaime-Fonseca, San Martin-Martínez, & Zepeda, 2013). The mesocarp of this xoconostle fruit has been studied by the content in antioxidant compounds, such as polyphenols (Osorio-Esquivel et al., 2013) ascorbic acid and tocopherols (Schwenke, 2002; Morales et al., 2012). Furthermore, Osorio-Esquivel et al. (2011) reported that the different parts of the fruit provide antioxidant activity in different percentages (62.96 % in pericarp, 42.27 % in mesocarp, and 51.70 % in endocarp).

Even though xoconostle fruit has nutritional characteristics with high nutraceutical value, this fruit is not considered as a conventional food in Mexico. However, the production of xoconostle and other products of *Opuntia* (cladodes, sweet prickly pear fruit) guarantee the subsistence of an important population sector in arid and semi-arid regions in Mexico where the accessibility to other vegetables is low. According with Hervert-Hernández, García, Rosado & Goñi (2011) the consumption of xoconostle in the diet of a Mexican rural population is low (0.62 g fresh matter of edible portion/day/person) in comparison with consumption of conventional vegetables such as tomatoes and potatoes (24.67 and 16.64 g fresh matter of edible portion/day/person respectively).

In these regions, the use of xoconostle is limited to the consumption of the fresh fruit, being only processed in small scale in artisanal form (jams, candies, juices and with power chilli) mainly due to the lack of knowledge about its nutritional potential (Sanchez-Gonzalez, Jaime-Fonseca, San Martin-Martinez, 2013), and due to the displacement of regional foods by processed products with low nutritional value.

In sweet cactus pear, 45% of the whole fruit are consider as by-products, mainly peel and the endocarp with seeds (Bensadón, Hervert-Hernadez, Sayago-Ayerdi & Goñi I (2010), being this proportion similar in the studied xoconostle fruits *cv*. Rosa (44%), since its peel (25%) and endocarp part (19%) are also considered as non-edible material. It is among the high amount of residues that are daily discarded, with the consequent loss of valuable nutrients and bioactive compounds present in this agro-industrial waste (Espírito Santo et al., 2012; Federici, Fava, Kalogerakisc, & Mantzavinos, 2009). The market in this field is competitive and the development of new by-product based ingredients is a challenge for food industry. Therefore, there is an increasing interest in considering not only the nutritional quality of these ingredients, but also its distribution, cost and other additional benefits, since the use of these materials as food ingredients would make them added-value products (Bensadón et al., 2010). In this sense, Chougui et al. (2015) applied *Opuntia ficus-indica* peel by-products to margarine due to its antioxidant properties.

Different authors (Morales et al., 2012 and 2014; Osorio-Esquivel et al., 2011; Prieto-García, et al., 2006) described that xoconostle (including cv. Cuaresmeño) and its by-products (seeds, pericarp and endocarp mucilage's) derived could be used as functional ingredients for food industry, namely antioxidant-enriched formulations including tocopherols, ascorbic acid and phenolic compounds, instead of being discarded. In this way, Pimienta-Barrios et al. (2008) described that the consumption of *O. joconostle*

pericarp promotes a reduction in cholesterol plasma levels, a gradual decrease in glucose and an increase in insulin plasmatic levels. Being the incorporation of this by-product an interesting way to functionalize foods with health promoting benefits. Furthermore, recent studies about these by-products have found that their functional and physico-chemical (e.g., viscosity) properties contribute to generate a new market niche and can be processed as jellies, liquors and caramelized peels using the peel and mesocarp (Gallegos-Vázquez et al., 2012).

In this scenario, research must be done to evaluate the potential use of xoconostle cultivars as sources of antioxidants that could be employed in foods for therapeutic treatments, colorants and others, increasing the missing value to these fruits. Therefore, the aim of this study was to provide a phytochemical characterization and bioactivity evaluation of *Opuntia matudae* Scheinvar cv. Rosa by-products (epicarp and endocarp mucilage's), in order to recover or promote their use as functional ingredients for human or animal foods.

2. Materials and Methods

2.1. Standards and reagents

Methanol was of analytical grade purity and supplied by Pronalab (Lisbon, Portugal). HPLC-grade acetonitrile, *n*-Hexane and ethyl acetate were obtained from Merck KgaA (Darmstadt, Germany) and Lab-Scan (Lisbon, Portugal), respectively. Formic and acetic acids were purchased from Prolabo (VWR International, France). The fatty acids methyl ester (FAME) reference standard mixture 37 (standard 47885-U) as well as other individual fatty acid isomers, tocopherol standards (!, , # and -isoforms), glucose, fructose, sucrose, and organic acid standards (L (+)-ascorbic, oxalic, malic, citric and

quinic acids) were purchased from Sigma (St. Louis, MO, USA). Phenolic compound standards (caffeic acid; ferulic acid; isorhamnetin-3-*O*-rutinoside; kaempferol 3-*O*-rutinoside and quercetin-3-*O*-rutinoside) were from Extrasynthese (Genay, France). Racemic tocol in *n*-hexane, 50 mg/mL, was purchased from Matreya (Plesant Gap, 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, Greenville, SC, USA).

2.2. Plant material

Fruits of *O. matudae* Scheinvar cv. Rosa were provided by a Mexican farmer association (CoMeNTuna) from commercial orchards at a place named Actopan, Hidalgo, located at latitude of 20° 16' 12" N, longitude of 98 ° 56' 42" W, and altitude of 2600 m above sea level. The plants are grown without any agronomical inputs, and as these fruits are non-climacteric, they could remain on the cladodes for over a year (Gallegos Vázquez et al., 2012; Avalos-Andrade, 2006). However, the fruits were collected in May 2013 by a native experienced picked, who used colour and size of the fruit as commercial maturation index (pink peel and purple pulp, 40 mm long). The fruits were selected with slight defects that shall not exceed 2%, bruising, sunspots, crusting, and blemished according prickly pear specifications (CODEX-STAN-186, 1993; NOM-FF-030-SCFI-1995). The fruits were weighed, washed with water in order to remove residues of dirt and insects and refrigerated at 4°C until sample preparation.

The thin peel (epicarp, that corresponds to the 25% of total fruit) was removed, and then the mesocarp (the edible pulp 56% of total fruit) was separated from the endocarp (19% of total fruit, formed by the mucilaginous part and seeds), and cut into small pieces. Different parts of the fruit are shown in **Figure 1**. Both parts (epicarp and endocarp)

were weighed again and freeze-dried. The endocarp was gently ground in a mortar and mucilaginous material (endocarp part which was analyzed) was separated from the seeds using a home strainer. The seeds remained intact because of the woody material that composes them. These two lyophilized fractions (peel and mucilaginous material of seeds) were ground separately, passed through a 0.5-mesh sieve, and stored in the dark at -20 ºC until analysis.

2.3. Hydrophilic phytochemicals

Free sugars. Free sugars were determined by high performance liquid chromatography coupled to a refraction index detector (HPLC-RI, Knauer, Smartline system 1000, Berlin, Germany), after an extraction procedure previously described by the authors (Barros et al., 2013), using melezitose as internal standard (IS). The equipment consisted of an integrated system with a pump (Knauer, Smartline system 1000, Berlin, Germany), degasser system (Smartline manager 5000), auto-sampler (AS-2057 Jasco, Easton, MD, USA) and an RI detector (Knauer Smartline 2300, Berlin, Germany). Data were analysed using Clarity 2.4 Software (DataApex, Prague, The Czech Republic). The chromatographic separation was achieved with a Eurospher 100-5 NH2 column (4.6 ! 250 mm, 5 mm, Knauer, Berlin, Germany) operating at 30 ºC (7971 R Grace oven). The mobile phase was acetonitrile/deionized water, 70:30 (v/v) at a flow rate of 1 mL/min. The compounds were identified by chromatographic comparisons with authentic standards. Quantification was performed using the internal standard method and sugar contents were further expressed in g per 100 g of dry weight (dw).

Organic acids. Organic acids namely oxalic, quinic, malic, ascorbic and citiric acid, were determined following a procedure previously described (Pereira, Barros, Carvalho,

& Ferreira, 2013; Sarmento et al., 2015). The analysis was performed using a Shimadzu 20A series UFLC (Shimadzu Corporation, Kyoto, Japan). Separation was achieved on a SphereClone (Phenomenex, Torrance, CA, USA) reverse phase C_{18} column (5 μm, 250 mm % 4.6 mm i.d.) thermostatted at 35 ºC. The elution was performed with sulphuric acid (3.6 mM) using a flow rate of 0.8 mL/min. Detection was carried out in a photodiode array (PDA), using 215 and 245 nm (for ascorbic acid) as preferred wavelengths. The organic acids found were quantified by comparison of the area of their peaks with calibration curves obtained from commercial standards of each compound: oxalic acid (!=9!10^{6n}# + 377946; I^2=0.994); quinic acid (!=612327# + 16563; I^2=1); malic acid (!=863548# + 55591; I^2=0.999); ascorbic acid (!=1!10^8x + 751815; I^2=0.999) and citric acid (!=1!10^6# + 16276; I^2=1). For quantitative analysis, calibration curves were prepared from different standard compounds. The results were expressed in g per 100 g of dry weight.

Phenolic compounds. Phenolic extraction was performed using the plant material (1 g) stirring with 30 mL of methanol:water (80:20, *v/v*) at 25 ºC and 150 rpm for 1 h, and filtered through Whatman No. 4 paper. The residue was then extracted with one additional 30 mL portion of the hydroalcoholic mixture. The combined extracts were evaporated at 35 ºC under reduced pressure (rotary evaporator Büchi R-210, Flawil, Switzerland) and then further lyophilized (FreeZone 4.5, Labconco, Kansas, USA).

Phenolic compounds were determined by HPLC (Hewlett-Packard 1100, Agilent Technologies, Santa Clara, CA, USA) as previously described by the authors (Barros et al., 2013). Double online detection was carried out in a PDA using 280 nm and 370 nm as preferred wavelengths and in a mass spectrometer (API 3200 Qtrap, Applied Biosystems, Darmstadt, Germany) connected to the HPLC system via the PDA cell

outlet. The phenolic compounds were identified by comparing their retention time, UV-vis and mass spectra with those obtained from standard compounds, when available. Otherwise, peaks were tentatively identified from their UV and mass spectra and comparing the obtained information with data reported in the literature. For quantitative analysis, a calibration curve for each available phenolic standard was constructed based on the UV signal: caffeic acid (y=611.9x-4.5733; R^2=0.999); ferulic acid (y=505.97x-64.578; R^2=0.999); isorhamnetin-3-*O*-rutinoside (y= 327.42x + 313.78; R^2=0.999); kaempferol 3-*O*-rutinoside (y=239.16x-10.587; R^2=1) and quercetin-3-*O*-rutinoside (y=281.98x-0.3459; R^2=1). For the identified phenolic compounds for which a commercial standard was not available, the quantification was performed through the calibration curve of other compound from the same phenolic group. The results were expressed in mg per g of methanolic extract.

Betacyanins. Samples (1 g) were extracted with 30 mL of methanol containing 0.5% trifluoroacetic acid (TFA), and filtered through a Whatman nº 4 paper. The residue was then re-extracted twice with additional 30 mL portions of 0.5% TFA in methanol. The combined extracts were evaporated at 35 ºC to remove the methanol, and re-dissolved in water. For purification, extracts were deposited onto a C-18 SepPak® Vac 3 cc cartridge (Phenomenex), previously activated with methanol followed by water; sugars and more polar substances were removed by passing through 10 mL of water and betalain/betacyanin pigments were further eluted with 5 mL of methanol:water (80:20, v/v) containing 0.1% TFA. The extract was concentrated under vacuum, lyophilized, re-dissolved in 1 mL of 20% aqueous methanol and filtered through a 0.22-"m disposable LC filter disk for HPLC analysis. Betacyanins were determined by HPLC as previously described by the authors (Roriz et al., 2015). Double detection was carried out in the

PDA, using 520 nm as the preferred wavelength, and in a mass spectrometer (MS) connected to the HPLC system via the PDA cell outlet. The betacyanins were tentatively identified by comparing their UV-vis and mass spectra with available data information reported in the literature. The quantification was performed using a calibration curve of gomphrenin (!=254.35#+244.69; $!^2$=0.999). The results were expressed in mg per g of extract.

2.4. Lipophilic phytochemicals

Total fat and fatty acids. Fat was obtained after Soxhlet extraction and fatty acids were determined by gas–liquid chromatography with flame ionization detection (GC-FID) as described previously by the authors (Morales et al., 2012; Sarmento et al., 2015). Analyses were carried out with a DANI model GC 1000 instrument (Milan, Italy) equipped with a split/splitless injector, a flame ionization detector (FID at 260 ºC) and a Macherey-Nagel (Duren, Germany) column (50% cyanopropyl-methyl-50% phenylmethylpolysiloxane, 30 m x 0.32 mm ID x 0.25 µm df). The oven temperature program was as follows: the initial temperature of the column was 50 ºC, held for 2 min, then a 30 ºC/min ramp to 125 ºC, 5 ºC/min ramp to 160 ºC, 20 ºC/ min ramp to 180 ºC, 3 ºC/min ramp to 200 ºC, 20 ºC/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 Clarity Software (DataApex, Prague, The Czech Republic) and expressed in relative percentage of each fatty acid.

Tocopherols. Tocopherols were determined following a procedure previously described

by the authors (Morales et al., 2013). Prior to the extraction procedure, BHT (butylhydroxytoluene) solution in hexane (10 mg/mL; 100 μL) and internal standard (IS) solution in hexane (tocol; 2.0 μg/mL; 250 μL) were added to the sample prior to the extraction procedure. Analyses were performed by HPLC (equipment described above) using a fluorescence detector (FP-2020; Jasco; Easton, MD, USA) programmed for excitation at 290 nm and emission at 330 nm. The chromatographic separation was achieved with a Polyamide II (250 x 4.6 mm) normal-phase column from YMC Waters operating at 30 ºC. 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, and the injection volume was 20 "L. The compounds were identified by chromatographic comparisons with authentic standards. Quantification was based on the fluorescence signal response of each standard, using the IS (tocol) method and by using calibration curves obtained from commercial standards of each compound. The results were expressed in mg per 100 g of dry weight.

2.5. Antioxidant activity

Methanolic 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 10 mg/mL, and stored at 4 °C (2 days) for further use.

DPPH radical-scavenging activity. This methodology was performed using an ELX800 Microplate Reader (Bio-Tek Instruments, Inc., Winooski, VT, USA), according to Sarmento et al. (2015). The reaction mixture in each one of the 96-wells consisted of one of the different concentrations (range from 0.156 to 10 mg/mL methanolic extract)

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}] % 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 well of a 48-well microplate, 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 (Sarmento et al., 2015). The extract concentration providing 0.5 of absorbance (EC_{50}- 50% of the maximal absorbance) was calculated from the graph of absorbance at 690 nm against extract concentration.

Inhibition of !-carotene bleaching. A solution of -carotene was prepared by dissolving -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 (Sarmento et

al., 2015). As soon as the emulsion was added to each tube, the zero time absorbance was measured at 470 nm. -carotene bleaching inhibition was calculated using the following equation: (-carotene absorbance after 2 h of essay/initial -carotene absorbance) % 100. The extract concentration providing 50% antioxidant activity (EC_{50}) was calculated by interpolation from the graph of -carotene bleaching inhibition percentage against extract concentration.

2.6. Statistical analysis

Three samples were used for each part and all the assays were carried out in triplicate. The results are expressed as mean values and standard deviation (SD) and each parameter was compared by means of a Student's *t*-test to determine the significant difference among samples, with # = 0.05. This treatment was carried out using SPSS v. 22.0 program.

3. Results and discussion

3.1. Hydrophilic phytochemicals of xoconostle by-products

Free sugars of both by-products are shown in **Table 1**. Glucose was the major compound in the endocarp, followed by fructose and sucrose. The same was previously reported for the endocarp fraction of other xoconostle variety (*O. joconostle* cv. Cuaresmeño; Morales et al., 2014), while fructose was the major soluble sugar in *O. matudae* Scheinvar *cv. Rosado* pulp and seeds (Morales et al., 2012). In the case of epicarp (peel), free sugars were found in lower amounts, with the predominance of sucrose.

Oxalic, quinic, malic and citric acids were quantified in the endocarp and epicarp, being citric acid the major organic acid in both fractions (**Table 1**). The same compound was

also previously reported as the major organic acid in endocarp of *O. joconostle* cv. Cuaresmeño, but in lower amount (20.98 mg/100 g, Morales et al., 2014). The other organic acids were found in higher concentration in the endocarp. Vitamin C, characterized as ascorbic acid, was also found in the analysed by-products; this bioactive compound was found in much lower levels comparing with the edible part of these fruits (20 – 31.8 mg of ascorbic acid/100 g fresh weight (fw) of pulp; Guzmán-Maldonado et al., 2010; Morales et al., 2012). Otherwise, other *Opuntia* by-products such as seeds did not present this bioactive component (Morales et al., 2012).

The phenolic profile of *O. matudae* Scheinvar *cv. Rosado* epicarp, recorded at 370 nm, is shown in **Figure 2**; peak characteristics, tentative identities and quantification are presented in **Table 2**. Phenolic compounds were mostly detected in the epicarp and hardly in the endocarp. In global, fourteen compounds were detected, including two phenolic acid derivatives, eight flavonoids, and four betacyanins. With the exception of compound 1, all the others have already been described in the epicarp and endocarp of *O. joconostle* F.A.C. Weber ex Diguet, cv. Cuaresmeño previously studied (Morales et al., 2014). All flavonoids corresponded to flavonol derivatives, most of them deriving from isorhamnetin but with different patterns of sugar substitution. Among them, compounds **7** (isorhamnetin-3-*O*-rutinoside) and **10** (isorhamnetin-3-*O*-glucoside) were positively identified according to its retention time, mass and UV-vis characteristics by comparison with commercial standards.

Compounds **1** and **2** were identified as ferulic and caffeic acid hexosides according to their pseudomolecular ions $[M-H]^-$ at *m/z* 341 and 355 and MS^2 fragment released at *m/z* 179 and 193 ($[M-H-162]^-$, loss of an hexosyl moiety), respectively. Compound **3** ($[M-H]^-$ at *m/z* 755) should correspond to a quercetin derivative bearing two deoxyhexosyl and one hexosyl residues. The fact that only one MS^2 fragment was

released, corresponding to the aglycone (i.e., *m/z* at 301, quercetin), would suggest that the three sugars constitutes a trisaccharide, so this compound was assigned as a quercetin di-deoxyhexosyl-hexoside. Similar reasoning applies for compounds **4** ([M-H]$^-$ at *m/z* 739), **5** and **6** (both with [M-H]$^-$ at *m/z* 769) that should correspond to kaempferol and isorhamnetin derivatives bearing two deoxyhexosyl and one hexosyl residues. The fact that two compounds (5 and 6) showed the same UV and mass characteristics could be explained by the existence of different linkage among sugar residues in the possible trisaccharide substituent, although the location of sugar moieties on different positions of the aglycone cannot be discarded, either. Similarly, compounds **8** and **9** with the same pseudomolecular ion as peak 7 (isorhamnetin-3-*O*-rutinoside) might be explained different linkages between the two sugar moieties or their substitution on different positions of the aglycone.

Compounds **11** and **12** were identified as betanidin and isobetanidin-5-*!*-$-glucoside (i.e., betanin and isobetanin); these compounds were also identified by Osorio-Esquivel et al. (2011). Other two betacyanins were observed (compounds **13** and **14**) in these by-products, being identified as 2-descarboxy-betanin and 2-descarboxy-isobetanin, respectively. These type of betacyanins normally present $\%_{max}$ around 533 nm with a correct protonated molecular ion [M+H]$^+$ at m/z 345 (base peak) (Kobayashi, Schmidt, Wray & Schliemann, 2001; Strack, Vogt & Schliemann, 2003).

The epicarp presented higher concentration of phenolic compounds and betacyanins (5.87 and 0.47 mg/g extract, respectively) than endocarp, being isorhamnetin-*O*-di-deoxyhexosyl-hexoside (compound **6**) the most abundant compound found (3.17 mg/g extract). Morales et al. (2014) also studied the epicarp and endocarp of another *Opuntia* species; the main phenolic compounds were similar, but the species study herein presented a different profile (lower variety of phenolic compounds and different

quantities). Flavonols were also found as the mayor polyphenols in other *Opuntia* sp. by-products (*O. ficus-indica* peel), namely isorahamnetin derivatives (Yeddes et al., 2013; Chougi et al., 2015). However, in this by-product, betacyanins were indentified but not quantified (nq).

3.2. Lipophilic phytochemicals of xoconostle by-products

Table 3 shows fat and fatty acids profile of the analysed by-products. The total fat amount in xoconostle was similar in both by-products, and lower compared with those reported for by-products from other xoconostle varieties (2.28 and 3.36 g/100 g, in Cuaresmeño endocarp and epicarp, respectively; Morales et al., 2014).

SFA were the predominant lipid fraction in epicarp, followed by PUFA fraction, which was the prevalent fraction in endocarp by-product; MUFA were found in similar percentages in both by-products (**Table 3**). All samples presented a good ratio PUFA/SFA (higher than 0.45), as also happened for similar Cuaresmeño by-products (Morales et al., 2014). The results are interesting since diets rich in PUFA have been shown to be cardio-protective (McGee, Ree & Yano, 1984).

Twenty-one and twenty-two individual fatty acids were identified in the endocarp and epicarp, respectively. Regarding SFA, palmitic acid (PA, C16:0) and myristic acid (C14:0) were found in relevant proportion both in epicarp and endocarp. Similar percentages were reported for Cuaresmeño by-products (15.55 and 26.30% of PA in peel and endocarp, respectively; Morales et al., 2014), as well as in *O. matudae* fruit seeds and pulp (9.42 and 12.74 %, respectively; Morales et al., 2012). Nevertheless, these percentages were lower than those reported by Ramadan & Mörsel (2003) for another cactus pear (*O. ficus-indica*) (around 30% of PA). Oleic acid (OA, 18:1n9) was the most representative MUFA, with similar values in both by-products (around 8-9%),

whereas different OA percentages were found in similar by-products from the Cuaresmeño variety (5.30 and 10.95 for epicarp and endocarp, respectively; Morales et al., 2014). However, lower percentages of OA were determined in the edible part (mesocarp) of the same fruits (OA: 3.68%, Morales et al., 2012). Linoleic acid (LA) was the most representative PUFA; it was present in greater proportion in endocarp than in epicarp, as also observed in Cuaresmeño by-products (LA: 27.15 and 14.70%, in endocarp and epicarp, respectively; Morales et al., 2014).

Regarding total tocopherol values (showed in **Table 3)**, the epicarp contains much higher levels (21.44 mg/100 g) than endocarp (3.42 mg/100 g), as well as than the edible part of the fruit (mesocarp, 0.14 mg/100 g fw, Morales et al., 2012). The four isoforms (#, , # and - tocopherols) were identified in *O. matudae* endocarp, while - tocopherol was not found in epicarp. !-Tocopherol was the major isoform in both by-products (**Table 3**). This is in agreement with tocopherols composition in similar *O. joconostle* by-products (Morales et al., 2014), as well as in the edible pulp of the fruit (Morales et al., 2012), where !-tocopherol was also the main isoform, while #-tocopherol was the major tocopherol isoform in *O. matudae* seeds (Morales et al., 2012). Thus, the use of these by-products as food ingredients, especially epicarp, could be of interest for food industry.

3.3. Antioxidant properties of xoconostle by-products

The antioxidant properties of the studied *O. matudae* by-products were evaluated by DPPH radical scavenging capacity, reducing power (Fe^{3+} into Fe^{2+}), and inhibition of lipid peroxidation using $-carotene–linoleate model system (**Table 4**). The epicarp of *O. matudae* fruits presented higher antioxidant capacity (lower EC_{50} values) in all the assays, which could be related with the highest concentration of phenolic compounds

present in this by-product. The highest activity was observed in the $-carotene bleaching inhibition assay, for both by-products. This relatively high $-carotene bleaching inhibition could be associated to the presence of tocopherols, which are known to be related with lipid peroxidation inhibition (Nogala-Kalucka, Kupczyk, Polewski, Siger & Dwiecki, 2007). Comparing with other parts of the fruit previously studied by the authors (Morales et al., 2012), the epicarp also presented higher DPPH scavenging activity, reducing power and $-carotene bleaching inhibition than seeds (1.88, 1.58 and 4.75 mg/mL, respectively). Our results are also in accordance with those reported by Osorio-Esquivel et al. (2011) for xoconostle fruits (*Opuntia joconostle*), in which peel presented higher DPPH scavenging activity (higher DPPH percentage inhibition) than endocarp (without seeds) (62.96 and 51.70%, respectively, at 0.06 mg/mL of methanolic extract). Moreover, the studied epicarp fraction showed higher DPPH scavenging activity and reducing power than the sweet cactus pear (*O. ficus-indica*) peel by-product, with EC_{50} 77.81 mg/mL and EC_{50} 1.03 mg/mL, respectively (Chougi et al., 2015).

4. Conclusions

Overall, the present study contributes to promote *xoconostle* agro-industrial by-products as functional food ingredients, namely for antioxidant-enriched formulations, due to their richness in bioactive compounds (#-tocopherol, ascorbic acid and phenolic compounds), as well as relevant antioxidant properties. The next step, for future studies, could be focus on rheological and physico-chemical properties of the studied by-products, as well as on the technological approach for food incorporation, in order to establish the suitability of this by-product for food industry uses.

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Table 2. Retention time (Rt), waveleghts of maximum absorption in visible region (λmax), mass spectral data, tentative identification and quantification of phenolic compounds and betacyanins in Xoconostle fruits (*Opuntia matudae* Scheinvar cv. Rosa) by-products.

Peak	Rt (min)	λ_{max} (nm)	Molecular ion $[M-H]^-$ (*m/z*)	MS^2 (*m/z*)	Tentative identification	Quantification (mg/g extract)		Student's *t*-test *p*-value
						Endocarp	**Epicarp**	
1	5.6	326	341	179(100)	Caffeic acid hexoside	nd	0.21 ± 0.02	-
2	9.7	330	355	193(100)	Ferulic acid glucoside	nd	0.26 ± 0.03	-
3	15.2	358	755	301(100)	Quercetin-*O*-(di-deoxyhexosyl-hexoside)	nd	0.10 ± 0.01	-
4	17.2	350	739	285(100)	Kaempferol-*O*-(di-deoxyhexosyl-hexoside)	nd	0.11 ± 0.01	-
5	17.9	356	769	315(100)	Isorhamnetin-*O*-(di-deoxyhexosyl-hexoside)	0.01 ± 0.00	0.34 ± 0.02	<0.001
6	18.2	356	769	315(100)	Isorhamnetin-*O*-(di-deoxyhexosyl-hexoside)	0.04 ± 0.01	3.17 ± 0.03	<0.001
7	20.4	358	623	315(100)	Isorhamnetin-3-*O*-rutinoside	nd	0.08 ± 0.01	-
8	22.8	354	623	315(100)	Isorhamnetin-*O*-(deoxyhexosyl-hexoside)	nd	0.74 ± 0.03	-
9	23.4	356	623	315(100)	Isorhamnetin-*O*-(deoxyhexosyl-hexoside)	nd	0.78 ± 0.02	-
10	24.3	354	477	315(100)	Isorhamnetin-3-*O*-glucoside	nd	0.08 ± 0.01	-
					Total phenolic acid	nd	0.47 ± 0.05	-
					Total flavonols	0.05 ± 0.01	5.40 ± 0.02	<0.001
					Total phenolic compounds	0.05 ± 0.01	5.87 ± 0.07	<0.001
					Betacyanins			
Peak	Rt (min)	λ_{max}	Molecular ion	MS^2	Tentative identification	Quantification (mg/g extract)		

13	8.2	530	507	345(100)	2-Descarboxy-betanin	0.02 ± 0.00	0.07 ± 0.01	<0.001
14	11.3	532	507	345(100)	2-Descarboxy-isobetanin	0.07 ± 0.01	0.03 ± 0.01	<0.001
					Total betacyanins	0.31 ± 0.02	0.47 ± 0.04	0.003

nd (not detected).

Table 3. Tocopherols and fatty acids in Xoconostle (*Opuntia matudae* Scheinvar by-products (Mean ± SD, n=3).

	Endocarp	**Epicarp**	Student's *t*-test *p*-value
Fatty acids (percentage)			
C6:0	0.10 ± 0.00	0.10 ± 0.00	-
C8:0	0.15 ± 0.04	0.30 ± 0.00	0.007
C10:0	0.15 ± 0.04	0.40 ± 0.00	0.001
C12:0	0.75 ± 0.04	4.35 ± 0.12	<0.001
C13:0	0.10 ± 0.00	0.15 ± 0.04	0.158
C14:0	2.40 ± 0.24	4.50 ± 0.08	<0.001
C15:0	1.80 ± 0.16	1.30 ± 0.08	0.018
C16:0	23.35 ± 0.36	21.85 ± 0.20	0.007
C16:1	3.80 ± 0.81	3.20 ± 0.24	0.376
C17:0	1.15 ± 0.04	1.00 ± 0.00	0.007
C17:1	0.55 ± 0.04	0.35 ± 0.04	0.008
C18:0	2.90 ± 0.08	3.90 ± 0.08	<0.001
C18:1n9	8.15 ± 0.04	8.85 ± 0.20	0.009
C18:2n6	38.35 ± 1.34	24.05 ± 0.04	<0.001
C18:3n3	12.60 ± 0.04	14.30 ± 0.04	<0.001
C20:0	0.30 ± 0.00	1.05 ± 0.04	<0.001
C20:1	0.45 ± 0.04	0.75 ± 0.12	<0.001
C20:3n3+C21:0	0.20 ± 0.00	0.20 ± 0.00	-
C20:5n3	0.15 ± 0.04	1.95 ± 0.28	<0.001
C22:0	0.55 ± 0.04	1.90 ± 0.00	0.001
C22:1n9	0.50 ± 0.00	nd	-
C22:2	0.20 ± 0.00	1.30 ± 0.08	<0.001
C23:0	0.25 ± 0.04	2.05 ± 0.04	<0.001
C24:0	1.10 ± 0.08	2.20 ± 0.16	0.001
Total fat (g/100 g dw)	1.75 ± 0.05	1.78 ± 0.01	0.759
SFA	35.05 ± 0.53	45.05 ± 0.28	<0.001
MUFA	13.45 ± 0.77	13.15 ± 0.04	0.614
PUFA	51.50 ± 1.42	41.80 ± 0.28	0.001
Tocopherols (mg/100 g dw)			
α- tocopherol	2.74 ± 0.04	20.14 ± 0.10	<0.001
β-tocopherol	0.09 ± 0.01	nd	-
γ-tocopherol	0.46 ± 0.02	0.78 ± 0.01	<0.001
δ-tocopherol	0.13 ± 0.00	0.52 ± 0.01	<0.001
Total tocopherols	3.42 ± 0.07	21.44 ± 0.12	<0.001

nd (not detected). SFA- saturated fatty acids; MUFA- monounsaturated fatty acids; PUFA- po fatty acids. dw- dry weight.

Table 4. Antioxidant activity of Xoconoste (*Opuntia matudae* Scheinvar *cv.* Rosa) by-produts (Mean ± SD, n=3).

EC_{50} values (mg/mL of extract)	**Endocarp**	**Epicarp**	Student's *t*-test *p*-value
DPPH scavenging activity	7.95 ± 0.16	1.56 ± 0.02	<0.001
Reducing power	4.14 ± 0.08	0.80 ± 0.02	<0.001
β-carotene bleaching inhibition	0.46 ± 0.03	0.11 ± 0.01	<0.001

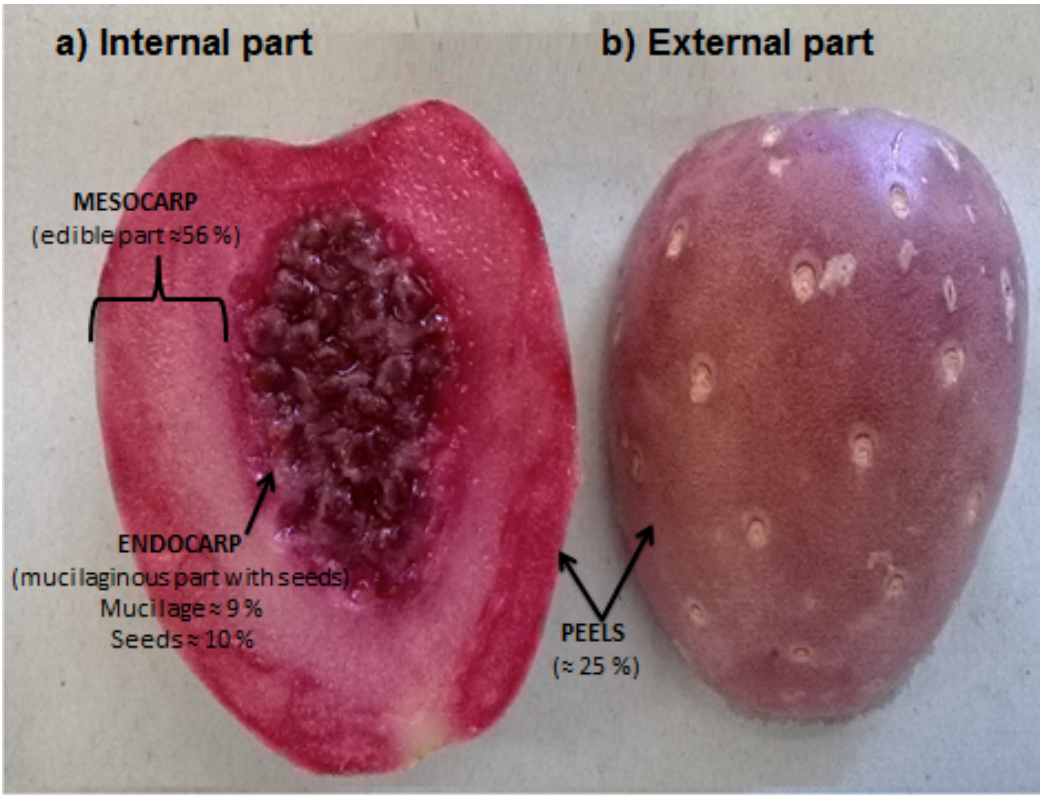
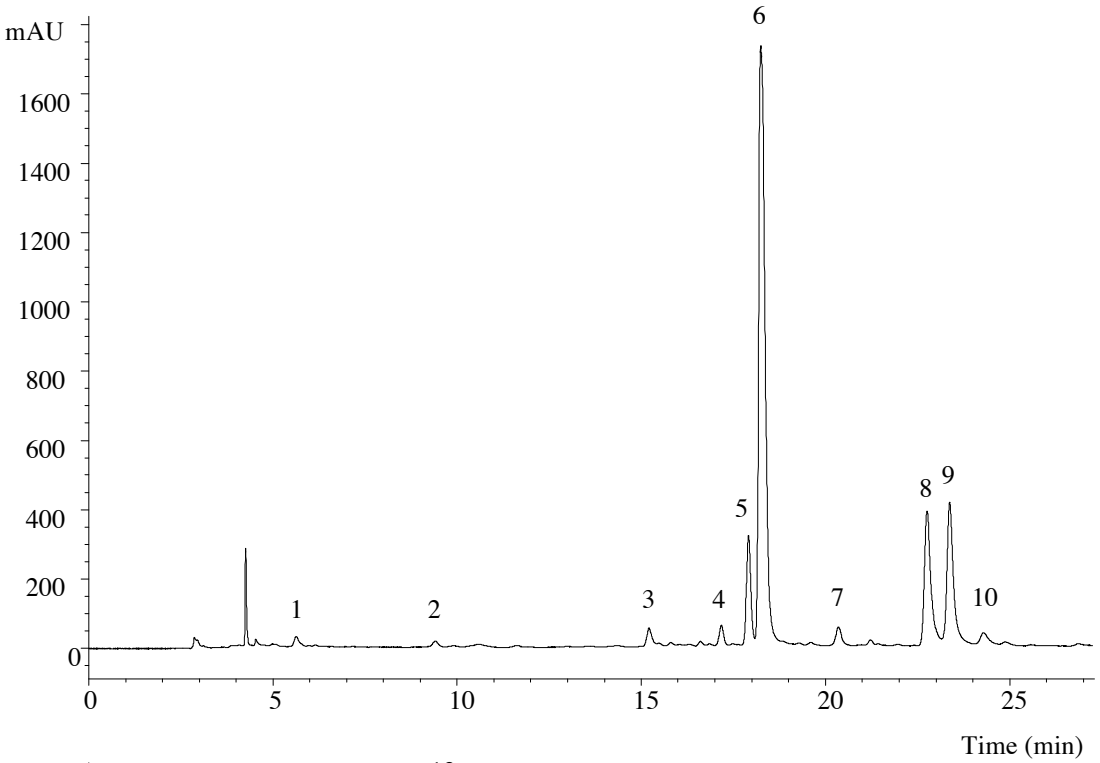


Figure 1. Xoconostle fruit (*Opuntia matudae* Scheinvar *cv.* Rosa), epicarp (peels), mesocarp (edible part) and endocarp (mucilaginous part with seeds)


mAU
1600
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1000
800
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Time (min)
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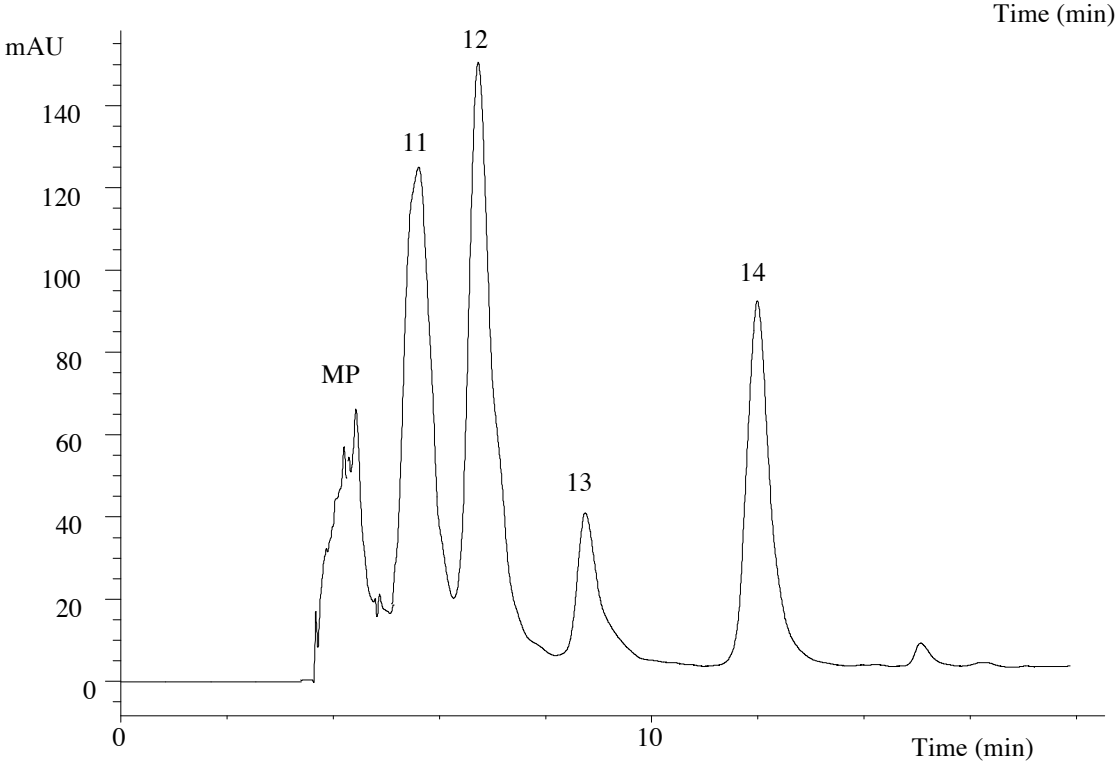
A


mAU
140
120
100
80
60
40
20
0
0
10
Time (min)
MP
11
12
13
14

B

Figure 2. Profile of xoconostle by-products (*Opuntia matudae* Scheinvar *cv.* Rosa) in: (A) flavonoids of epicarp recorded at 370 nm and (B) betacyanins of endocarp recorded at 520 nm.