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Chemical characterization and biological activities of two varieties of xoconostle fruits *Opuntia joconostle* F.A.C. Weber ex Diguet and *Opuntia matudae* Scheinvar

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The present work focusses on the chemical characterization and bioactive properties of *Opuntia joconostle* F.A.C. Weber ex Diguet and *Opuntia matudae* Scheinvar fruits. This research showed that xoconostle cv. Cuaresmeño (*O. joconostle*) and xoconostle cv. Rosa (*O. matudae*) are a good source of PUFAs and tocopherols. Moreover, both fruits revealed the presence of ten phenolic compounds (*e.g.*, ferulic acid hexoside, quercetin-*O*-di-deoxyhexosyl-hexoside, and kaempferol-*O*-(di-deoxyhexosyl)-hexoside), as well as other organic acids (oxalic, malic, ascorbic and citric acids), and two betacyanins (betanin and isobetain). The hydroethanolic extracts of both fruits exhibited antioxidant activity, and inhibited the growth of several bacteria strains and of the yeast *Candida albicans*. As expected, xoconostle cv. Cuaresmeño was the fruit with highest antioxidant potential, since it was also the one that showed the highest content of bioactive compounds, with the exception of betacyanins. Overall, both fruits are revealed to be a good source of nutritive and bioactive compounds.

Introduction

Opuntia, usually called prickly pear, is a cactus genus and, as such, originates from the American continent. Although it grows in many arid parts of the world, it is especially abundant in Mexico, this country being considered the center of its biodiversity.[1] This genus includes the well-known *Opuntia ficus-indica* (L.) Mill., the most common and commercially important species in the Cactaceae family. Nevertheless, many potential biological activities have been attributed not only to this but also to other species from the *Opuntia* genus. These plants, especially their fruits (prickly pear), have aroused interest and popularity due to their nutritional value and biological activity associated with health benefits.[2] *Opuntia* fruits may be consumed in different ways: they can be eaten raw, or processed into an array of dehydrated, frozen, canned, and fermented delicacies (*e.g.*, candy, jam, marmalade, syrup, sauce, pies, smoothies, or health drinks).[3]

The present work is focused on the species *Opuntia joconostle* F.A.C. Weber ex Diguet and *Opuntia matudae* Scheinvar, namely on their acidic fruits, known as xoconostle. In fact, the number of studies involving xoconostle fruits is quite sparse when compared with other prickly pear fruits, such as the Indian fig (*O. ficus-indica*). Regarding the *Opuntia* species bearing acidic fruits, *O. joconostle* (xoconostle cv. Cuaresmeño) is the most exploited and marketed species, followed by *O. matudae* (xoconostle cv. Rosa) in Latin America.[4] Xoconostle is characterized by a light red-pink colour, succulent and pink white mesocarp, and a deep-red coloured endocarp that contains small brown seeds.[5] This sour fruit is highly prized for its colourful, fleshy and acidic mesocarp, unlike the cactus pear, which is appreciated for its light-sweet endocarp. Xoconostle fruits are consumed after removing the epicarp (peel), as well as the endocarp, since it is composed of seeds. This mesocarp is usually consumed in sauces, or other Mexican dishes.[6,7] Besides their nutritional interest, deriving from the fact that xoconostle fruits have a considerable amount of soluble fibre, minerals (*i.e.*, calcium, magnesium, iron, manganese, phosphorus, sulphur, zinc, and copper), polyunsaturated fatty acids or tocopherols (especially γ-tocopherol),[6,8] some studies have also attributed antioxidant properties to these species,[8,9] with *O. matudae* being considered a functional food.[8]

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In recent years, xoconostle mesocarp has been processed on a small scale in jams, candies, juices and with powdered chilies, while the peel (epicarp) and mucilage and seeds (endocarp) are considered as by-products of *O. joconostle* fruit processing.[10] However, these parts could be considered as an attractive target for food industry uses, considering the value of their nutritional and antioxidant properties,[5] without efficiency losses during the processing of this fruit.

Therefore, the present work was focused on the chemical characterization of two xoconostle cultivars (Cuaresmeño and Rosa), including fatty acids, tocopherols, organic acids and phenolic compounds, as well as regarding the presence of betacyanins (pigment compounds), that could be useful for the food industry as antioxidants and natural colourants. Moreover, the antioxidant and antimicrobial activities were also evaluated.

Materials and methods

Samples

The varieties of xoconostle fruits selected for this study were: *Opuntia joconostle* F.A.C. Weber ex Diguet cv. Cuaresmeño (XC) and *Opuntia matudae* Scheinvar cv. Rosa (XR). The samples were provided by a Mexican farmers' association (CoMeNTuna) of Actopan, Hidalgo, Mexico, located at a latitude of 20° 16′12″ N, longitude 98° 56′42″ W and altitude of 2600 m above sea level, in September 2015. These fruits were selected, weighed and measured. The whole fruits were washed, vertically sliced, frozen (−32 °C, 48 h), and subsequently lyophilized (4 days at −55 ± 1 °C with a vacuum pressure of 0.140 mbar). The samples were sieved to a particle size of 500 μm, homogenized and stored sheltered from light and moisture until the analyses were carried out.

Chemical characterization

Fatty acids. Fatty acids were determined by gas–liquid chromatography with flame ionization detection (GC-FID) as previously described by the authors.[11] 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 °C). A Macherey-Nagel (Duren, Germany) column (50% cyanopropyl–methyl/50% phenylmethylpolysiloxane, 30 m × 0.32 mm ID × 0.25 μm df) was used to separate the fatty acid methyl ester (FAME) compounds. The identification of fatty acids was performed by comparing the retention times of FAME standards with the samples. The results were expressed as relative percentages.

Tocopherols. The methodology used to determine tocopherols was described previously by the authors.[12] The analysis was performed using a HPLC system (Knauer, Smartline system 1000, Berlin, Germany) coupled to a fluorescence detector (FP-2020; Jasco, Easton, MD, USA). The tocopherol identification was performed by chromatographic comparisons with authentic standards and the quantification was based on the fluorescence signal response of each standard, using the internal standard (IS, tocol) method. The results were expressed in mg per 100 g of dry weight.

Organic acids. The organic acid profile was evaluated following a procedure already described,[13] using a Shimadzu 20A series UFLC (Shimadzu Corporation, Kyoto, Japan). The organic acid identification was performed by chromatographic comparisons with authentic standards and the quantification was made using calibration curves obtained from commercial standards using the peak areas (recorded at 215 and 245 nm). The results were expressed as mg per 100 g of dry weight.

Determination of phenolic and betacyanin compounds

Extraction procedure. The dried samples were extracted using 30 mL of aqueous ethanol (80 : 20, v/v) by magnetic stirring (25 °C, 150 rpm) for 1 h, and subsequently filtered (Whatman no. 4 paper) obtaining a hydroethanolic extract. The extraction procedure was repeated with an additional portion of solvent. The obtained extracts were combined, the ethanol was evaporated (rotary evaporator Büchi R-210, Flawil, Switzerland) and the residual aqueous phase was frozen and lyophilized.[14]

Determination of the phenolic compound profile. The analysis of phenolic compounds was performed following a procedure described by other authors[15] using a HPLC-DAD-ESI/MS (Dionex Ultimate 3000 UPLC, Thermo Scientific, San Jose, CA, USA). The phenolic compound identification was performed by comparing the chromatographic characteristics of available phenolic standards and using the information available in the literature. The quantification of the phenolic compounds was performed using calibration curves of the most similar available standards. The results were expressed as mg per g of extract.

Determination of the betacyanin profile. For betacyanin determination a HPLC-DAD-ESI/MS analysis was performed as previously described by the authors.[16] The detection was carried out with a diode array detector (DAD) using 530 nm as a reference wavelength, and with a Linear Ion Trap LTQ XL mass spectrometer (Thermo Finnigan, San Jose, CA, USA), equipped with an ESI source working in positive mode. The identification was performed by comparing the chromatographic characteristics of available betacyanin standards and using the information available in the literature. Quantification was performed by using a 5-level calibration curve from gomphrenin III (isolated from *Gomphrena globosa* L.).[16] The results for betacyanins were expressed as mg per g of extract.

Evaluation of the bioactive properties

Evaluation of the antioxidant activity. The antioxidant activity of the previously described extracts was assessed through four different assays: 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity, reducing power, β-carotene bleaching inhibition and thiobarbituric acid-reactive substances (TBARS).[17] Trolox was used as positive control and the results were expressed as EC_{50} values (mg mL^{-1}; sample concentration providing 50% of antioxidant activity or 0.5 of absorbance for the reducing power assay).

Evaluation of the antimicrobial activity. The evaluation of the antimicrobial activity was assessed through the microdilu-

tion method,[18] applied to ten strains of pathogenic bacteria and fungi. The microbial strains were clinical isolates donated from the Hospital Center of Trás-os-Montes e Alto Douro (Vila Real, Portugal). Five of these strains were Gram-negative bacteria – *Pseudomonas aeruginosa* (isolated from expectoration), *Escherichia coli*, *Klebsiella pneumonia*, *Proteus mirabilis* and *Morganella morganii* (all isolated from urine), and four Gram-positive bacteria – *Enterococcus faecalis* (isolated from urine), *Listeria monocytogenes* (isolated from cerebrospinal fluid), methicillin-sensitive *Staphylococcus aureus* (MSSA, isolated from wound exudate), and methicillin-resistant *S. aureus* (MRSA, isolated from expectoration). The fungus/yeast *Candida albicans* (isolated from urine) was also tested. The minimal inhibitory concentration (MIC) of the samples was calculated using the quick colorimetric assay with *p*-iodonitrotetrazolium chloride (INT). MIC was defined as the lowest concentration that inhibits the visible bacterial growth determined by changing colour from yellow to pink if the microorganisms were viable. The minimum bactericidal concentration (MBC) and minimal fungicidal concentration (MFC) were also determined. These were defined as the lowest concentrations required to kill the microorganisms.[19]

Statistical analysis

Three samples were used for each preparation and all the assays were carried out in triplicate. The results were expressed as mean values and standard deviation (SD). The results of each parameter were compared by means of a Student's *t*-test to determine the significant difference among samples, with $\alpha = 0.05$ (SPSS v. 23.0 program).

Results and discussion

Chemical characterization. From the performed analysis, it was possible to identify eighteen fatty acids in the xoconostle fruits (Table 1). When comparing both studied species, we found that the major fatty acids present in XC were also predominant in XR (although with some fluctuations in the relative percentage found). The major fatty acids were linoleic acid (C18:2n6), representing ~73% of total fatty acids; palmitic acid (C16:0), representing around 10–12% of total fatty acids; and oleic acid (C18:1n9), with a percentage of about 8–13%. Stearic (C18:0) and linolenic (C18:3n3) acids were present in both species, representing around 1–3% of total fatty acids. The other identified fatty acids were present at less than 1%. Given that the main fatty acid present was linoleic acid, this led to the prevalence of PUFAs (polyunsaturated fatty acids) in both species, followed by SFAs (saturated fatty acids) and MUFAs (monounsaturated fatty acids). Morales *et al.*[8] reported the fatty acid profile of xoconostle cv. Cuaresmeño and xoconostle cv. Rosa, comparing the fatty acids present in the mesocarp and endocarp. The obtained profiles were similar to our results regarding xoconostle cv. Rosa samples. However, for xoconostle cv. Cuaresmeño a higher percentage of caprylic acid (31%) was found. Palmitic acid (9–15%), linoleic acid (33–79%), and oleic acid (3–10%) were the next most prevalent fatty acids. Comparing the overall results obtained by Morales *et al.*[8] with the present work, the same trend was observed for xoconostle cv. Rosa, but a different trend was registered for xoconostle cv. Cuaresmeño, since the main fatty acids found by Morales *et al.* were SFAs, followed by PUFAs and MUFAs. Elsewhere, Morales *et al.*[4,5] reported the fatty acid profile of the same species but comparing the epicarp and endocarp and concluded that the same trend was observed for xoconostle cv. Rosa (PUFAs > SFAs > MUFAs), but for xoconostle cv. Cuaresmeño the predominant fatty acids were SFAs, followed by PUFAs and MUFAs. Since the samples were supplied by the same Mexican farmers' association (CoMeNTuna), there may have been changes in the cultivation conditions, or differences in the state of maturity of the fruits, the time, collection or different climatic conditions. These plants are grown without any agronomical inputs and, as these fruits are non-climacteric, they could remain on cladodes for over a year. For this

Table 1 Fatty acid composition (%) and tocopherol content (mg per 100 g dw) of the studied species (mean ± SD)

	Xoconostle cv. Cuaresmeño	Xoconostle cv. Rosa	Student's *t*-test *p*-Value
Fatty acids			
C6:0	0.07 ± 0.001	0.0270 ± 0.0001	<0.001
C8.0	0.020 ± 0.001	0.0060 ± 0.0001	<0.001
C10:0	0.39 ± 0.02	0.142 ± 0.005	<0.001
C12:0	0.223 ± 0.004	0.067 ± 0.001	<0.001
C14:0	0.277 ± 0.003	0.127 ± 0.001	<0.001
C15:0	0.071 ± 0.001	0.030 ± 0.001	<0.001
C16:0	11.95 ± 0.03	9.64 ± 0.01	<0.001
C16:1	0.284 ± 0.002	0.192 ± 0.001	<0.001
C17:0	0.1050 ± 0.0001	0.076 ± 0.004	<0.001
C18:0	2.763 ± 0.004	2.47 ± 0.01	<0.001
C18:1n9	8.18 ± 0.02	12.63 ± 0.01	<0.001
C18:2n6	73.52 ± 0.01	73.05 ± 0.01	<0.001
C18:3n3	1.06 ± 0.05	1.18 ± 0.01	0.003
C20:0	0.25 ± 0.01	0.178 ± 0.004	<0.001
C20:1	0.031 ± 0.001	0.092 ± 0.005	<0.001
C22:0	0.70 ± 0.04	0.38 ± 0.01	<0.001
C23:0	nd	0.0370 ± 0.0001	—
C24:0	0.11 ± 0.01	0.078 ± 0.004	<0.001
Total SFA (% of total FA)	16.93 ± 0.08	13.25 ± 0.03	<0.001
Total MUFA (% of total FA)	8.49 ± 0.02	12.51 ± 0.01	<0.001
Total PUFA (% of total FA)	74.58 ± 0.06	74.24 ± 0.01	<0.001
Tocopherols			
α-Tocopherol	7.2 ± 0.2	0.40 ± 0.01	<0.001
β-Tocopherol	0.117 ± 0.003	0.132 ± 0.005	0.003
γ-Tocopherol	4.1 ± 0.2	4.28 ± 0.005	0.044
δ-Tocopherol	0.078 ± 0.001	nd	—
Total tocopherol	11.5 ± 0.4	7.31 ± 0.03	<0.001

Caproic acid (C6:0); caprylic acid (C8:0); capric acid (C10:0); lauric acid (C12:0); myristic acid (C14:0); pentadecanoic acid (C15:0); palmitic acid (C16:0); palmitoleic acid (C16:1); heptadecanoic acid (C17:0); stearic acid (C18:0); oleic acid (C18:1n9); linoleic acid (C18:2n6); α-linolenic acid (C18:3n3); arachidic acid (C20:0); eicosenoic acid (C20:1); behenic acid (C22:0); tricosanoic acid (C23:0); lignoceric acid (C24:0); SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids. nd – not detected.

reason, and even when collected by specialized personnel, harvesting the fruits according to their state of maturity and visual features, this can lead to differences in the final chemical composition of the fruits. Therefore, as already established for other fresh fruits (*e.g.*, apples, pears, tomatoes, *etc.*), it is important to implement maturity indices also for these less exploited fruits.

Regarding the tocopherol composition (Table 1), all four vitamers were found in XC (11.5 mg per 100 g dry weight (dw)), while XR only revealed α-, β- and γ-tocopherol (7.31 mg per 100 g dw). In the first species α-tocopherol was the prevailing vitamer (7.2 mg per 100 g dw), while in XR higher levels of γ-tocopherol were found (4.28 mg per 100 g dw). Morales *et al.*[8] also detected the four vitamers of tocopherols in both species' mesocarp and endocarp, with a total content of tocopherols of approximately 16 mg per 100 g dw for xoconostle cv. Cuaresmeño and 19 mg per 100 g dw for xoconostle cv. Rosa. However, some authors[5] have reported much higher amounts, especially of α-tocopherol, in the epicarp of xoconostle cv. Cuaresmeño (26.86 mg per 100 g dw). Some authors have also reported such a profile for xoconostle cv. Rosa, with higher amounts of tocopherols in the epicarp of the fruit, mainly due to the presence of high quantities of α-tocopherol (20.14 mg per 100 g dw).[4] The differences between the obtained results can be explained by factors that influence tocopherol production, namely the seasonal variations limiting the time of plant growth and seed maturation, and the environmental conditions.[20]

Regarding the organic acids, both species revealed the presence of oxalic acid (0.2–0.4 mg per 100 g dw), malic acid (1.1–2.4 mg per 100 g dw), ascorbic acid (0.2–0.4 mg per 100 g dw), and citric acid (15–21 mg per 100 g dw). Other studies have also found detectable levels of ascorbic acid present in xoconostle cv. Cuaresmeño and cv. Rosa. Morales *et al.*[8] detected approximately 0.3 mg per 100 g dw of ascorbic acid in xoconostle cv. Cuaresmeño and 0.5 mg per 100 g dw of ascorbic acid in xoconostle cv. Rosa. When those authors studied and compared the epicarp and endocarp, they also found oxalic, quinic, malic, and citric acids, these together being the total organic acids found in the endocarp of xoconostle cv. Cuaresmeño (22.41 mg per 100 g dw) and in xoconostle cv. Rosa (29.84 mg per 100 g dw).[4,5] While XC presented a similar quantity of organic acids herein, XR revealed a lower amount of the sum of organic acids. This could be explained by the reasons mentioned above, regarding possible changes in fruit production/harvesting, which may influence the production of compounds by either the primary metabolism or secondary metabolism.

Phenolic and betacyanin compounds. Tables 3 and 4 present the peak characteristics, tentative identification and quantification of the phenolic compounds and betacyanins, respectively, present in the hydroethanolic extracts of XC and XR. Twelve different compounds were identified (Table 3), including ten phenolic compounds (of which two were phenolic acids (eucomic acid and ferulic acid hexoside) and eight were flavonoids (isorhamnetin, quercetin and kaempferol glycoside derivatives)), and two betacyanins (betanidin derivatives). All of the identified compounds have been previously described by other authors in *Opuntia* spp. samples.[4,5,14,21] Regarding phenolic compounds, all the identified peaks were

Table 2 Organic acid composition (mg per 100 dw) of the studied species (mean ± SD)

	Xoconostle cv. Cuaresmeño	Xoconostle cv. Rosa	Student's *t*-test *p*-Value
Oxalic acid	0.161 ± 0.002	0.40 ± 0.01	<0.001
Malic acid	1.12 ± 0.02	2.4 ± 0.1	<0.001
Ascorbic acid	0.39 ± 0.01	0.233 ± 0.003	<0.001
Citric acid	21.08 ± 0.01	15.0 ± 0.5	<0.001
Total organic acids	22.76 ± 0.05	18.1 ± 0.6	<0.001

Table 3 Retention time (Rt), wavelengths of maximum absorption in the visible region (λ_{max}), mass spectral data and tentative identification of the phenolic compounds and betacyanins present in the studied species

Peak	Rt (min)	λ_{max} (nm)	Pseudomolecular ion $[M - H]^-$ (*m*/*z*)	MS^2 (*m*/*z*)	Tentative identification
Phenolic compounds					
1	6.5	233/276	239	179 (100), 149 (69)	Eucomic acid
2	8.7	329	355	193 (100), 175 (22), 149 (60)	Ferulic acid hexoside
3	14.5	346	755	301 (100)	Quercetin-*O*-(di-deoxyhexosyl-hexoside)
4	16.2	342	739	285 (100)	Kaempferol-*O*-(di-deoxyhexosyl-hexoside)
5	16.9	354	769	315 (100)	Isorhamnetin-*O*-(di-deoxyhexosyl-hexoside)
6	17.3	354	769	315 (100)	Isorhamnetin-*O*-(di-deoxyhexosyl-hexoside)
7	19.0	354	623	315 (100)	Isorhamnetin-*O*-(deoxyhexosyl-hexoside)
8	19.5	352	609	315 (100)	Isorhamnetin-*O*-(pentosyl-hexoside)
9	21.4	352	623	315 (100)	Isorhamnetin-*O*-(deoxyhexosyl-hexoside)
10	22.0	351	623	315 (100)	Isorhamnetin-3-*O*-rutinoside
Peak	Rt (min)	λ_{max} (nm)	Pseudomolecular ion $[M + H]^+$ (*m*/*z*)	MS^2 (*m*/*z*)	Tentative identification
Betacyanins					
11	22.8	534	551	389 (100)	Betanidin-5-*O*-glucoside (betanin)
12	24.0	533	551	389 (100)	Isobetanidin-5-*O*-glucoside (isobetain)

Table 4 Phenolic compound and betacyanin composition (mg g^{-1} extract) of the studied species (mean ± SD)

Peak	Xoconostle cv. Cuaresmeño	Xoconostle cv. Rosa	Student's *t*-test *p*-value
Phenolic compounds			
1^{A}	0.22 ± 0.02	0.279 ± 0.008	0.002
2^{B}	0.143 ± 0.009	0.131 ± 0.002	0.036
3^{C}	0.928 ± 0.002	0.9029 ± 0.0003	<0.001
4^{C}	0.939 ± 0.001	0.9021 ± 0.0002	<0.001
5^{C}	1.029 ± 0.003	0.969 ± 0.002	<0.001
6^{C}	1.60 ± 0.04	1.160 ± 0.004	<0.001
7^{C}	0.938 ± 0.005	0.9083 ± 0.0003	<0.001
8^{C}	nd	0.900 ± 0.001	—
9^{C}	1.0371 ± 0.0003	0.9110 ± 0.0001	<0.001
10^{C}	1.031 ± 0.008	0.935 ± 0.001	<0.001
TPC	7.9 ± 0.1	7.719 ± 0.005	0.003
Betacyanins			
11^{D}	0.382 ± 0.007	4.0 ± 0.2	<0.001
12^{D}	0.34 ± 0.002	2.6 ± 0.2	<0.001
TB	0.726 ± 0.005	6.58 ± 0.04	<0.001

nd – not detected. TPC – total phenolic compounds; TB – total betacyanins. Standard calibration curves: A: *p*-hydroxybenzoic acid (y = 208604x + 173 056, R^2 = 0.9995); B: ferulic acid (y = 633126x − 185 462; R^2 = 0.9999); C: quercetin-3-*O*-glucoside (y = 34843x − 160 173; R^2 = 0.9989); D: gomphrenin III (y = 14670x − 19 725; R^2 = 0.9989).

present in both species, except for compound 8 (isorhamnetin-*O*-(pentosyl-hexoside)), which was not found in XC. Nevertheless, both XC and XR revealed very similar total phenolic compounds (7.9 and 7.7 mg g^{-1} extract), and all the identified compounds were found in similar amounts (Table 4), with slightly higher amounts of compound 6 (isorhamnetin-*O*-(di-deoxyhexosyl-hexoside)). There have been some studies regarding the phenolic composition of xoconostle. Morales *et al.*[4] also detected several compounds in xoconostle cv. Rosa endocarp and epicarp, including ferulic acid hexoside, quercetin-*O*-(di-deoxyhexosyl-hexoside), kaempferol-*O*-(di-deoxyhexosyl-hexoside), isorhamnetin-*O*-(di-deoxyhexosyl-hexoside) (compounds 5 and 6), and isorhamnetin-3-*O*-rutinoside. The total content of phenolic compounds presented by this species in Morales *et al.* (not including the mesocarp) was ≈6 mg g^{-1} extract, which is similar to the value reported in this study. Regarding xoconostle cv. Cuaresmeño, Morales *et al.*[5] reported the presence of the same compounds previously referred to. However, other compounds were also identified, with total phenolic compound levels of ≈19 mg g^{-1} extract. Osorio-Esquivel *et al.*[10] identified protocatechuic acid, 4-hydroxybenzoic acid, caffeic acid, vanillic acid, syringic acid, rutin, and quercetin in *O. joconostle* fruits (whole fruit). In another study,[6] the authors reported the presence of gallic acid, vanillic acid, 4-hydroxybenzoic acid, catechin, epicatechin, and vanillin in xoconostle cv. Cuaresmeño. Since phenolic compounds are secondary molecules of the plant's metabolism, whose production is often associated with stress phenomena, it is normal to have a variability in their profile, even when studying the same species. However, it can be concluded that both species may be a source of different phenolic compounds, more or less abundant, depending on the production conditions and harvesting time. It should be kept in mind that the extraction conditions also have a great influence on the compounds/quantities present in the extracts. Hence, these differences may also be due to the type of extracts under study.

The betacyanins identified in the studied species were betanidin-5-*O*-glucoside and isobetanidin-5-*O*-glucoside (Table 3). Inspecting Table 4, we can see that the XR species showed a much higher quantity of these compounds than the XC species (6.58 mg g^{-1} extract and 0.73 mg g^{-1} extract, respectively). Since these compounds are pigments responsible for the colouring of the fruits, this could explain the observed differences between the studied fruits, namely that XR has a more intense colour than XC. However, it must not be forgotten that these compounds are also known for their antioxidant potential, and their production may be associated with stress conditions to which the species may have been exposed. Osorio-Esquivel *et al.*[10] also identified betanidin-5-*O*-β-glucoside and isobetanidin-5-*O*-β-glucoside in *O. joconostle* fruits. However, the authors also reported the presence of phyllocactin. Morales *et al.*[5] detected betanidin-*O*-hexoside but in non-quantifiable quantities in xoconostle cv. Cuaresmeño, and betanin, isobetanin, 2-descarboxy-betanin, and 2-descarboxy-isobetanin in the endocarp and epicarp of xoconostle cv. Rosa.[4] Betacyanins are indole-derived pigments responsible for the reddish to violet colours of fruits. Therefore, xoconostle appears to be a good source of colouring agents, which may be useful for the food or textile industries, namely for the formulation of novel natural-based colouring agents.

Bioactive properties of the hydroethanolic extracts. The antioxidant properties of the studied fruit extracts are presented in Table 5. Both species revealed antioxidant potential, XC having the most promising results (lower EC_{50} values for all the performed assays). Morales *et al.*[8] also performed three antioxidant assays (DPPH radical scavenging activity, reducing power and β-carotene bleaching inhibition) for xoconostle cv. Cuaresmeño and xoconostle cv. Rosa mesocarp and endocarp (methanolic extracts). The authors concluded that the mesocarp presented lower antioxidant potential in comparison to the endocarp, except for the β-carotene bleaching inhibition assay. This was probably related to the compounds present in the different parts of the fruits, namely the phenolic compounds, whose levels were higher in seeds (50–60 mg of gallic acid equivalents (GAE) per g of extract). In the present study XC presented the highest content of total tocopherols (Table 1), ascorbic acid (Table 2), and total phenolic compounds (Table 4). Therefore, these compounds may be responsible for the antioxidant properties of these fruits. Comparing the endocarp with the epicarp, Morales *et al.*[4] showed that the mesocarp of xoconostle cv. Rosa had a higher antioxidant potential than the endocarp for all three performed assays. However, the endocarp of xoconostle cv. Cuaresmeño revealed higher reducing power, in comparison with the fruit epicarp.[5] Osorio-Esquivel *et al.*[10] also reported the antioxidant potential of *O. joconostle* fruits (methanolic extracts, phenolic compound fraction and betalain fraction), concluding that the pericarp

Table 5 Antioxidant properties (EC_{50}; mg mL^{-1}) of the hydroethanolic extract obtained from each studied species (mean ± SD)

Activity	Assay	Xoconostle cv. Cuaresmeño	Xoconostle cv. Rosa	Student's *t*-test *p*-value
Reducing power	Ferricyanide/Prussian blue	1.82 ± 0.07	8.9 ± 0.6	<0.001
Radical scavenging activity	DPPH scavenging activity	1.5 ± 0.1	3.8 ± 0.2	<0.001
Lipid peroxidation inhibition	β-carotene/linoleate	0.21 ± 0.02	0.83 ± 0.08	<0.001
	TBARS	0.12 ± 0.01	0.15 ± 0.04	0.012

The antioxidant activity was expressed as EC_{50} values, which means that higher values correspond to lower reducing power or antioxidant potential. Trolox EC_{50} values: 0.041 mg mL^{-1} (reducing power), 0.042 mg mL^{-1} (DPPH scavenging activity), 0.018 mg mL^{-1} (β-carotene bleaching inhibition) and 0.023 mg mL^{-1} (TBARS inhibition).

Table 6 Antimicrobial activity (MIC, MBC and MFC, mg mL^{-1}) of the hydroethanolic extract obtained from each studied species

	Xoconostle cv. Cuaresmeño		Xoconostle cv. Rosa		Ampicillin (20 mg mL^{-1})		Imipenem (1 mg mL^{-1})		Vancomycin (1 mg mL^{-1})		Fluconazole (1 mg mL^{-1})	
Antibacterial activity	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Gram-negative bacteria												
Escherichia coli	5	>20	5	>20	<0.15	<0.15	<0.0078	<0.0078	nt	nt	nt	nt
Klebsiella pneumoniae	10	>20	10	>20	10	20	<0.0078	<0.0078	nt	nt	nt	nt
Morganella morganii	5	>20	5	>20	20	>20	<0.0078	<0.0078	nt	nt	nt	nt
Proteus mirabilis	10	>20	10	>20	<0.15	<0.15	<0.0078	<0.0078	nt	nt	nt	nt
Pseudomonas aeruginosa	5	>20	10	>20	>20	>20	0.5	1	nt	nt	nt	nt
Gram-positive bacteria												
Enterococcus faecalis	10	>20	20	>20	<0.15	<0.15	nt	nt	<0.0078	<0.0078	nt	nt
Listeria monocytogenes	>20	>20	>20	>20	<0.15	<0.15	nt	nt	nt	nt	nt	nt
MRSA	5	>20	5	>20	<0.15	<0.15	nt	nt	<0.0078	<0.0078	nt	nt
MSSA	5	20	5	20	<0.15	<0.15	nt	nt	0.25	0.5	nt	nt
Antifungal activity	**MIC**	**MFC**	**MIC**	**MFC**								
Candida albicans	10	>20	10	>20	nt	nt	nt	nt	nt	nt	0.06	0.06

MRSA – Methicillin-resistant *Staphylococcus aureus*; MSSA – methicillin-susceptible *Staphylococcus aureus*; MIC – minimal inhibitory concentration; MBC – minimum bactericidal concentration; MFC – minimal fungicidal concentration: nt – not tested.

and the whole xoconostle fruit presented higher percentages of DPPH radical inhibitors than the mesocarp and endocarp. With the results obtained in the current work, and these few studies available in the literature, we can conclude that the whole xoconostle fruits have effective antioxidant properties, which could be further exploited and confirmed in future works (*e.g.*, *in vivo* assays).

Regarding the antimicrobial potential of the xoconostle fruits, both species revealed antimicrobial properties against Gram-negative and Gram-positive bacteria, except against *Listeria monocytogenes* (Table 6). The obtained MICs were between 5 and 20 mg mL^{-1}. Regarding the bactericidal effects of the fruits, both XC and XR revealed MBCs of 20 mg mL^{-1} for MSSA. However, they were not effective against the other bacteria strains (at the tested concentrations). The same was observed for *Candida albicans*, revealing an MIC of 10 mg mL^{-1} and MFC > 20 mg mL^{-1} for both species. Hayek and Ibrahim[22] also tested the antimicrobial activity of xoconostle pear juice (obtained from the species *O. matudae*), concluding that it was able to inhibit the growth of *E. coli* O157:H7 and could provide a natural means for pathogenic contamination prevention. As far as we know, no other studies have been performed testing this kind of biological activity of *O. joconostle* or *O. matudae*.

Conclusions

Although one of the most studied species from the genus *Opuntia* is *O. ficus-indica*, which produces sweet prickly pears, other varieties producing acidic fruits also have important chemical and functional properties. The study performed herein confirms such properties, since it demonstrates that both xoconostle cv. Cuaresmeño and xoconostle cv. Rosa are a good source of PUFAs and tocopherols, as well as phenolic compounds and other organic acids, together with betacyanins. Some of these compounds, as well as others that may be present in these species, confer antioxidant and antimicrobial properties, showing that xoconostle may potentially be an excellent option as a dietary supplement or as a new source of interesting compounds (nutritive, bioactive compounds, and colouring agents). Moreover, the extracts obtained from both fruits inhibited the growth of several bacterial and yeast strains, and since there is very little information on this topic, it would be interesting to deepen these studies.

Conflicts of interest

There are no conflicts to declare.

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