Extensive profiling of three varieties of *Opuntia* spp. fruit for innovative food ingredients

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ABSTRACT

Consumer interest in the use of natural ingredients is creating a growing trend in the food industry, leading to research into the development of natural products such as colorants, antimicrobials and antioxidant compounds. This work involves an extensive morphological (using physico-chemical assays), chemical (antioxidant activity assays) and microbiological (Gram-positive and negative strains) characterization of prickly peras (*Opuntia ficus-indica* (OFI) var. sanguigna, gialla and *Opuntia engelmannii*) fruits. Through chromatographic assays, these species have shown interesting contents of hydrophilic (sugars, organic acids and betalains) and lipophilic (tocopherols and fatty acids) compounds. While *Opuntia engelmannii* exhibited higher content of betacyanins and mucilage, OFI varieties sanguigna and gialla displayed greater organic acid content. The sanguigna variety also showed the highest α-tocopherol content. All this compounds could be the responsible of enhancing the bioactivity of this variety, which can be observed in its antimicrobial potential, tested in the studied strains too. Results revealed that *Opuntia* spp. could be used as a nutraceutical and/or food additive, maintaining and promoting health and life quality.

1. Introduction

Consumers, in today's food market, are becoming increasingly conscious of high-quality healthy foods, leading to a demand for the exclusion of synthetic food additives in groceries. These consumers expect to see more natural ingredients in their food products following the food authorities warnings on the reduction of daily intake levels of synthetic additives and the addition of these ingredients to the Redbook 2000 (FDA, 2007). This provides scientific information about the toxicological effects of the consumption of additives, but often leads to misunderstanding by consumers.

Scientists are constantly looking for better alternatives to synthetic food additives and functional properties of the ingredients employed by food industries. Some researchers like Almeida et al. (2011), directed their investigations towards the revalorization of exotic fruit juices and extracts. These substances contain biomolecules that could be applied as unpurified extracts or isolated molecules, or as a possible substitute for synthetic additives. Thus, by employing these new natural ingredients, consumers will be able to choose for healthier products which could improve their overall well-being, as well as their contribution to the prevention of some diseases (Devalaraja, Jain, & Yadav, 2011). Additionally, food industries would be able to publish clearer labelling that could have a beneficial impact on their sales (Osborn, 2015).

The prickly pear (*Opuntia* spp.) is an important crop to study due to its adaptability to difficult growing conditions (arid and semiarid zones). Although this species is native to Mexico, it has spread and been cultivated across the world (Novoa, Le Roux, Robertson, Wilson, & Richardson, 2015). The genus *Opuntia* is reported to have almost 300 different varieties (FAO, 2002), between domesticated and wild species. *Opuntia ficus-indica* (OFI) is one of the five most cultivated species for fruit production (Griffith, 2004), but there are also other wild species such as *Opuntia engelmannii* that could be potentially used in the extraction of natural ingredients, such as colorants.

Prickly pears shows a wide range of colour due to the presence of betalains, this molecules are water-soluble, nitrogen-containing pigments present in a limited number of families of the plant order Caryophyllales (Strack, Vogt, & Schliemann, 2003). There are two types

of betalains, red/violet betacyanins and yellow/orange betaxanthins (Esquivel, 2016), creating an interesting palette of natural colouring agents. There is growing interest in betalains, partially due to their good stability between the pH values of 3 and 7 (Herbach, Stintzing, & Carle, 2006), and their ability to protect against oxidative stress (Azeredo, 2009; Strack et al., 2003). Although, the antioxidant properties of betalains could be related to other bioactive molecules. Tocopherols, organic acids, reducing sugars and polyunsaturated fatty acids (PUFA) might have a synergistic effect with the aforementioned dyes (Pereira et al., 2014).

Therefore, the aim of this research was to carry out an extensive physical, chemical and microbiological characterization of OFI var. sanguigna (OS) and gialla (OG) and *Opuntia engelmannii* (OE) fruits, as possible fruit to be used in the food industry as natural ingredients.

2. Material and methods

2.1. Sample preparation

Cactus pear fruits (OFI var. sanguigna - OS and gialla - OG) were collected in July–August 2016 in Sicily, Italy and were purchased from a local market in Bragança, Portugal. Fruit from these species were separated according to their inherent colour orange-red (pulp and peel) and red-violet, obtaining two different samples. Wild prickly pear fruit (*Opuntia engelmannii*- OE) were collected in Bragança, Portugal (GPS coordinates: 41.797344, − 6.772735) in early September 2016. Dr. Carlos Aguiar of the School of Agriculture, Polytechnic Institute of Bragança (Trás-os-Montes, Portugal), confirmed the botanical identifications and voucher specimens were deposited.

Within 24 h, the fruit were washed with distilled water in order to remove glochids, and then air-dried on the countertop of the laboratory. Afterwards, all the fruits (three samples of each) were peeled and the resulting pulp was lyophilized (LabConco, Frezone − 105 °C, 4.5 L Cascade Benchtop Freeze Dry System, Kansas, MO, USA), crushed in a porcelain mortar, and stored in a cool, dry place until use.

2.2. Morphological parameters

Fruit size: the length and width of the entire fruit and pulp were measured with a calliper. The whole fruit, pulp and peel where weighed separately. For colour detection, fresh and lyophilized pulp and peel were measured with a colorimeter (model CR-400; Konica Minolta Sensing Inc., Japan), previously calibrated using the standard white plate. Using illuminant C and a diaphragm opening of 8 mm, the CIE L* a* b* colour space values were registered with a computerized system using the colour data software "Spectra Magic Nx" (version CM-S100W 2.03.0006).

2.3. Chemical characterization

2.3.1. Proximal nutritional composition

Chemical and nutritional parameters (protein, fat, moisture, ash, carbohydrates and energy) were determined only for the edible part of the fruit (pulp). Samples were analysed according to the AOAC procedures (AOAC, 2016) Crude protein content (N × 6.25) was determined by the macro-Kjeldahl method (AOAC, 991.02). Crude fat (AOAC, 989.05) was estimated by extracting a known weight of powdered sample with petroleum ether, using a Soxhlet apparatus. Moisture content was determined by the weight difference before and after the drying process (oven at 100 °C); the ash content (AOAC, 935.42) was determined by incineration at 550 ± 15 °C. Total carbohydrates (including fibre) were calculated by difference [total carbohydrates (g/100 g) = 100 − (g fat + g protein + g ash)]. Total energy was calculated according to the following equation: Energy (kcal/100 g) = 4 × (g proteins + g carbohydrates) + 9 × (g fat).

2.3.2. Hydrophilic compounds

2.3.2.1. Soluble sugars.
Sugars were determined in defatted samples by high performance liquid chromatography coupled to a refraction index detector (HPLC-RI, Knauer, Smartline system 1000, Berlin, Germany) following a procedure described by Pereira, Barros, Carvalho, and Ferreira (2011). Mobile phase consisted of acetonitrile:water mixture (70:30 v/v, acetonitrile HPLC-grade, Lab-Scan, Lisbon, Portugal) and separation was achieved using a Eurospher 100-5 NH$_2$ column (4.6 × 250 mm, 5 μm, Knauer). The results were recorded and processed using Clarity 2.4 software (DataApex, Prague, Czech Republic).

2.3.2.2. Organic acids.
Organic acids were determined by an optimized procedure previously described by Barros, Pereira, and Ferreira (2013) and the analysis was performed using a Shimadzu 20A series UFLC (Shimadzu Corporation, Kyoto, Japan) coupled to a diode array detector (DAD), using 215 and 245 nm (for ascorbic acid) as the preferred wavelengths. The results were recorded and processed using a Shimadzu's LCsolution software (Shimadzu Corporation, Kyoto, Japan).

The sugars and organic acids were identified by comparing their retention times with standard compounds, and quantificárion was conducted by comparison with dose–response curves constructed from authentic standards. For sugar determination, melezitose was used as the internal standard. Results were expressed as g and mg per 100 g of pulp fresh weight (FW), for sugars and organic acids, respectively.

2.3.2.3. Betalains.
The profile of these compounds was determined by LC-DAD-ESI/MSn (Dionex Ultimate 3000 UPLC, Thermo Scientific, San Jose, CA, USA). The lyophilized pulps were re-dissolved in water at a concentration of 150 mg/mL. Chromatographic separation was achieved with a Waters Spherisorb S3 ODS-2 C18 (3 μm, 4.6 mm × 150 mm, Waters, Milford, MA, USA) column working at 35 °C. The solvents used were: (A) 0.1% trifluoroacetic acid (TFA) in water, and (B) acetonitrile. The gradient elution followed these parameters: from 0% to 10% B for 15 min, from 10% to 15% B for 5 min, from 15 to 18% B for 5 min, from 18 to 50% B for 8 min, and from 50 to 0% B for 12 min. The resulting total run time was 45 min, using a flow rate of 0.5 mL/min. Detection was carried out in the DAD using 480 nm (for betaxanthins) and 530 nm (for betacyanins), as the preferred wavelengths, and in a mass spectrometer (MS). MS detection was performed using positive mode, with a Linear Ion Trap LTQ XL mass spectrometer (ThermoFinnigan, San Jose, CA, USA) equipped with an ESI source. Nitrogen served as the sheath gas (50 psi); the system was operated with a spray voltage of 4.8 kV, a source temperature of 320 °C, and a capillary voltage of 39 V. The tube lens offset was kept at a voltage of 140 V. The full scan covered the mass range from m/z 100 to 1500. The collision energy used was 24 (arbitrary units). Data acquisition was carried out with the Xcalibur® data system (ThermoFinnigan, San Jose, CA, USA).

Identification of the betalain compounds (betacyanins and betaxanthins) was performed by comparing the obtained information with available data reported in the literature, providing a tentative identification. For quantitative analysis, a calibration curve using an isolated compound gomphrenin III (isolated from *Gomphrena globosa* L.) was constructed based on the UV signal (y = 14,670x − 19,725, R^2 = 0.9997). The results for betacyanins were expressed as mg per 100 g of pulp fresh weight (FW), and the results for betaxanthins were expressed as a relative percentage (%) of the areas recorded at 480 nm.

2.3.3. Lipophilic compounds

2.3.3.1. Fatty acids.
Fatty acid determination was achieved *via* the transesterification procedure described previously by Guimarães et al. (2013). The analysis was performed in gas chromatography (GC DANI 1000; Contone, Switzerland) equipment with flame ionization detection. Results were expressed as relative percentages of each fatty acid.

2.3.3.2. Tocopherols. The four isoforms of tocopherols were analysed according to the previously described procedure (Heleno, Barros, Sousa, Martins, & Ferreira, 2010). Analysis was performed using a HPLC system (Knauer, Smartline system 1000, Berlin, Germany), coupled to a fluorescence detector (FP-2020; Jasco, Easton, MD, USA). Quantification was based on the fluorescence signal response of each standard, using the internal standard (tocol) method and using calibration curves obtained from commercial standards of each isoform. The results were expressed in µg per 100 g of pulp fresh weight (FW).

2.4. Antimicrobial effect of fruit pulp

Antibacterial activity was performed using the lyophilized pulps redissolved in water at a concentration of 10 mg/mL and following a procedure previously reported by Reis et al. (2014). Four Gram-positive bacteria: *Staphylococcus aureus* (ATCC 6538), *Bacillus cereus* (clinical isolate), *Micrococcus flavus* (ATCC10240), and *Listeria monocytogenes* (NCTC7973) and four Gram-negative bacteria: *Escherichia coli* (ATCC 35210), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella typhimurium* (ATCC 13311), and *Enterobacter cloacae* (ATCC 35030) were used. While for antifungal assays, the following microfungi were used: *Aspergillus fumigatus* (ATCC1022), *Aspergillus ochraceus* (ATCC12066), *Aspergillus versicolor* (ATCC11730), *Aspergillus niger* (ATCC6275), *Penicillium funiculosum* (ATCC 36839), *Penicillium ochrochloron* (ATCC9112), *Penicillium verrucosum* var. *cyclopium* (food isolate), and *Trichoderma viride* (IAM 5061).

Each fresh overnight culture of bacteria was adjusted spectrophotometrically (625 nm) to a concentration of 1×10^5 CFU/mL. Dilutions of inocula were cultured on solid medium to verify the absence of contamination and check the validity of each inoculum. Different dilutions of the aqueous extract were added to the wells containing 100 µL of Tryptic Soy Broth (TSB) and afterwards, 10 µL of inoculum was added to all wells. The microplates were incubated for 24 h at 37 °C. The MIC of the samples was detected following the addition of 40 µL of iodonitrotetrazolium chloride (INT) (0.2 mg/mL) and incubation at 37 °C for 30 min. The lowest concentration that produced a significant inhibition (around 50%) of the growth of the bacteria in comparison with the positive control was identified as the MIC. The minimum inhibitory concentrations (MICs, mg/mL) obtained from the susceptibility testing of various bacteria to tested extracts were determined also by a colorimetric microbial viability assay based on the reduction of the INT colour and compared with a positive control for each bacterial strain. The minimum bactericidal concentrations (MBC) were determined by serial sub-cultivation of 10 µL into microplates containing 100 µL of TSB. The lowest concentration that showed no growth after this sub-culturing was read as the MBC.

The fungal spores were washed from the surface of agar plates with sterile 0.85% saline containing 0.1% Tween 80 (v/v). The spore suspension was adjusted with sterile saline to a concentration of approximately 1.0×10^5 in a final volume of 100 µL per well. The inocula were stored at 4 °C for further use. Dilutions of each inoculum were cultured on solid MA to verify the absence of contamination and to check the validity of the inoculum. MIC determination was also performed by a serial dilution technique using 96-well microtitre plates. The investigated sample was dissolved in water and added to broth malt medium with a fungal inoculum. The microplates were incubated for 72 h at 28 °C. The lowest concentrations without visible growth (as assessed using a binocular microscope) were defined as the MICs. The minimum fungicidal concentrations (MFCs) were determined by serial sub-cultivation of 2 µL in microtitre plates containing 100 µL of malt broth per well and further incubation for 72 h at 28 °C. The lowest concentration with no visible growth was defined as the MFC, indicating 99.5% killing of the original inoculum.

Standard drugs, namely streptomycin and ampicillin, bifonazole and ketoconazole (in a range of 0.01 to 5 mg/mL) were used as positive controls, while 5% DMSO was used as the negative control. Samples

were tested in duplicate and experiments were repeated three times.

Bacterial and fungal organisms were obtained from the Mycological Laboratory, Department of Plant Physiology, Institute for Biological Research "Sinisa Stanković", University of Belgrade, Serbia and the results were expressed in mg/mL.

2.5. Statistical analysis

All the extractions and assays were performed in triplicate. Results were expressed as mean values and standard deviations (SD), analysed using a one-way analysis of variance (ANOVA) followed by Tukey's HSD Test with $p = 0.05$. The treatment was carried out using IBM SPSS Statistics for Windows, version 23.0 (IBM Corp., Armonk, New York, USA).

3. Results and discussion

3.1. Morphological parameters and nutritional composition

According to the data summarized in Table 1 (morphologic parameters), the Italian fruit varieties of OFI, gialla (OG) and sanguigna (OS), are notably heavier in pulp weight compared to the Portuguese species *Opuntia engelmannii* (OE), which is also the smallest variety. OG and OS pulps were about 5 and 6-fold heavier, respectively. The OS variety is around 13% heavier than fruits of the OG variety, although the pulp's length is reasonably similar, the difference can be perceived in the shape of the fruits, the fruit body of OG being more elliptical, while OS has a more rounded shape, which gives more volume to the fruits, consequently weight.

Additionally, in Table 1, colour characteristics are described. As mentioned above, betalains are the main molecules responsible of fruit coloration. Positive a* colour coordinates reflect tendencies to reddish colours, with the highest value for the OS variety, which displays an overall reddish pulp with some deep pink spots. On the other hand, positive values of b* coordinates exhibit yellowish colours, where the OG variety showed the highest values. OG pulp displayed a bright yellow colour in most of the fruit with several shiny orange stains. Finally, OE pulp showed lower values, particularly on the b*coordinates (blue-yellow) which mixed with the other coordinates, especially the low lightness, exhibiting a more matte purple colour. Correlation between the betalains content and the increase in lightness, L* was detected. In general, this parameter increased with higher betaxanthins content but decreased when betacyanins were predominant, the same tendency was observed in Stintzing et al. (2005) assays.

The macronutrient composition of *Opuntia* fruiting bodies is presented in Table 2. OFI var. gialla and sanguigna do not show significant differences in most of the macronutrients, excluding proteins and ash content. Higher percentages in proteins could be due to a greater concentration of pigments in the cell vacuoles, betalainic colorants being water-soluble nitrogen-containing pigments (Azeredo, 2009),

Table 1
Morphological parameters of *Opuntia* spp.

	OG	OS	OE
Pulp weight (g)	50 ± 6[a]	58 ± 5[a]	10.0 ± 0.9[b]
Pulp length (cm)	5.9 ± 0.4[a]	5.4 ± 0.1[a]	3.56 ± 0.18[b]
Pulp diameter (cm)	3.8 ± 0.4[a]	4.5 ± 0.4[a]	2.4 ± 0.1[b]
Colour coordinates			
a*	12 ± 1[c]	36.8 ± 0.4[a]	16 ± 0.8[b]
b*	49 ± 3[a]	19.8 ± 0.6[b]	4.9 ± 0.7[c]
L*	48.9 ± 0.9[a]	33 ± 1[b]	25 ± 1[c]
C*	50 ± 2[a]	41.8 ± 0.6[b]	16.8 ± 0.5[c]
h°	1.33 ± 0.03[a]	0.49 ± 0.01[b]	0.30 ± 0.05[c]

OG, *Opuntia ficus-indica* var. gialla; OS, *Opuntia ficus-indica* var. Sanguigna; OE, *Opuntia engelmannii*. In each row different letters mean significant differences ($p < 0.05$).

Table 2
Nutritional value and hydrophilic compounds of the studied *Opuntia* spp.

	OG	OS	OE
Moisture (%)	83.38 ± 0.07[a]	81 ± 2[a]	65 ± 2[b]
Fat (g/100 g FW)	0.037 ± 0.001[b]	0.063 ± 0.004[b]	0.38 ± 0.03[a]
Proteins (g/100 g FW)	0.52 ± 0.01[c]	0.84 ± 0.03[b]	1.62 ± 0.06[a]
Ash (g/100 g FW)	0.348 ± 0.004[c]	0.42 ± 0.02[b]	0.75 ± 0.02[a]
Carbohydrates (g/100 g FW)	15.68 ± 0.04[b]	18 ± 2[b]	33 ± 2[a]
Energy (kcal/100 g FW)	65.1 ± 0.1[b]	77 ± 7[b]	140 ± 6[a]
Sugars (g/100 g FW)			
Fructose	4.13 ± 0.02[b]	4.97 ± 0.07[a]	0.53 ± 0.01[c]
Glucose	5.53 ± 0.05[b]	6.30 ± 0.03[a]	0.83 ± 0.01[c]
Sucrose	0.133 ± 0.005[c]	0.157 ± 0.001[b]	0.31 ± 0.01[a]
Sum of free sugars	9.79 ± 0.07b	11.43 ± 0.09[a]	1.68 ± 0.02[c]
Organic acids (mg/100 g FW)			
Oxalic	26.7 ± 0.3[b]	29.5 ± 0.6[a]	tr
Quinic	28 ± 1[*]	35.6 ± 0.3[*]	nd
Malic	tr	tr	tr
Ascorbic	2.3 ± 0.1[b]	2.0 ± 0.1[b]	19.7 ± 0.1[a]
Citric	49.3 ± 0.7[a]	46.9 ± 0.1[b]	0.8 ± 0.01[c]
Succinic	184 ± 1[*]	131 ± 2[*]	nd
Fumaric	8.0 ± 0.1[*]	6.3 ± 0.2[*]	nd
Sum of organic acids	298.1 ± 0.8[a]	251 ± 2[b]	20.5 ± 0.1[c]

FW, pulp fresh weight; nd, not detected; tr, traces. OG, *Opuntia ficus-indica* var. Gialla; OS, *Opuntia ficus-indica* var. sanguigna; OE, *Opuntia engelmannii*. In each row different letters mean significant differences (*p* < 0.05).

[*] Statistical differences (< 0.001) were observed when *t*-Student test was applied.

direct correlation with betalainic concentration can be clearly observed, higher concentration of betalains in varieties, revealed a higher the concentration of proteins. Despite the differences shown between proteins and ash, these macronutrients along with moisture, fat and carbohydrates are in accordance with results reported by other authors like Angulo-Bejarano, Martínez-Cruz, and Paredes-López (2014).

Conversely, OE was statistically different from OG and OS, moisture in OE was around 17% inferior, while protein, ash and carbohydrates were 2-fold higher in OE and fat content was up to 10-fold higher. Once again, high protein content could be related to the greater pigment composition, as will be discussed later on in the betalainic profile of *Opuntia* spp. In addition, fat, ash and carbohydrate content could be superior, and moisture lower due to a higher ratio of seed/pulp compared to OG or OS. Chougui et al. (2013) have worked on the oil composition and characterization of *Opuntia* seeds and have shown that a higher ratio of seed/pulp increases the oil yield and the fat content. Likewise Jain, Grover, and Kaur (2016) reported that seeds are mainly composed of carbohydrates with a considerable amount of minerals and proteins, this information supports our hypothesis on the greater amount of macronutrients found in OE samples. To the best of our knowledge, no reports are available on the nutritional composition of *Opuntia engelmannii* species.

It is worthy to note, that besides the differences presented between the two different species, there are also additional factors that play an important role. The cultivar location may also influence the differences observed, due to environmental factors, such as soil, precipitation, sun exposure, among others. In the present study, different maturation times where observed regarding the Portuguese OE, which only reached their optimum maturation time two months after the Italians OG and OS.

3.2. Hydrophilic and lipophilic compounds

The free sugar content of *Opuntia* spp. is displayed in Table 2, OE has the lowest sugar content, followed by OG and OS in ascending order. It is worth highlighting that OE is around 10-fold lower sugar concentration compared to OFI fruit, this higher concentration is mainly due to the presence of glucose and fructose, the most

characteristic sugars in OFI (Kyriacou, Emmanouilidou, & Soteriou, 2016). Although all species were collected in the same period, OE species were probably not at their optimum ripeness. Despite the fact that OE has higher content in carbohydrates and lower sugar content compared to OG and OS, this could indicate that the majority of the carbohydrates could be fibres or longer polysaccharide chains like mucilage (Da-Costa-Rocha, Bonnlaender, Sievers, Pischel, & Heinrich, 2014). This hydrocolloid, forms molecular networks that are able to retain large amounts of water, which may slow down the absorption of glucose, cholesterol and biliary salts by increasing the viscosity of food in the gut (Del Socorro-Santos-Díaz, Barba De La Rosa, Héliès-Toussaint, Guéraud, & Nègre-Salvayre, 2017). The mucilage properties could be also potentially used by food, pharmaceutical and cosmetic industries as thickener agent.

The organic acid content of OE is 12 to 15-fold lower compared to OS and OG, respectively (Table 2). The main difference in the sum amount of the organic acids analysed in *Opuntia* spp., is due to the succinic acid content, this acid is by far the most abundant in OFI fruits, while it was not detected in OE. Farag, Maamoun, Ehrlich, Fahmy, and Wesjohann (2017), also identified succinic acid as the more abundant organic acid in Italian OFI varieties. Tretter, Patocs, and Chinopoulos (2016) described that succinic acid is an important metabolite involved in several signalling processes, not only in the mitochondria were it is generated, but in the cytoplasm as well as the extracellular space and it is also involved in the elimination of reactive oxygen species. This mechanism of action helped to understand part of the antioxidant effect of the OFI fruits performed in this study. Succinic acid is generally recognized as safe (FDA, 2017) and has different applications in the pharmaceutical and food industries, although it is primarily used as an acidity regulator (Ahn, Jang, & Lee, 2016). Organic acid profiles are not reported for *Opuntia* spp. in the literature, although, extensive assays have been performed on ascorbic acid for OFI. Variable values are found ranging from 3.5 to 45 mg /100 g edible pulp (Kuti, 2004; Stintzing et al., 2005). OG and OS showed lower levels of ascorbic acid, compared with reported data, while OE was almost 10-fold higher than the previously mentioned varieties.

The betalain profiles of OG, OS and OE are shown in Fig. S1 (Supplementary material). Data regarding retention time, λ_{max} in the visible region, molecular ion and main fragment ions observed in MS^2, obtained by HPLC-DAD-ESI/MS analysis regarding betalains identification and quantification are presented in Table 3. Compounds 1–3 were identified as betaxanthin derivatives, and compounds 4–7 as betacyanin derivatives. All of the identified compounds have been previously described in *Opuntia* spp. (Cejudo Bastante, Chaalal, Louaileche, Parrado, & Heredia, 2014; Mata et al., 2016), although, not all the compounds present in the same variety, were identified in the herein species. Betaxanthins were found in a higher percentage in OG, followed by OS and were not identified in OE. Nevertheless, betacyanidin content was lower in OG (11.32 mg/100 g of FW), followed by OS (215 mg/100 g FW) and the largest amounts were present in OE (sum content of 283 mg/100 g of FW), which presented 25-fold higher quantities then OG and 1.3-fold higher then OS. The hierarchical order observed agrees with the data found by other researchers, were the amount of betalains increased from yellow to red and to purple varieties (Farag et al., 2017). Several works have demonstrated the antiradical scavenging activity of betalains *in vitro* (Azeredo, 2009), which can contribute to the prevention of several degenerative diseases. Therefore, betalains could be a great substitutive of artificial dyes with additional bioactive activity.

Saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) are presented in Table 4. Samples were richer in PUFA with over 60% of the total fatty acids, where OE was notably higher than OS and OG. SFA were the second most abundant group followed by MUFA. In all the *Opuntia* species, linoleic palmitic and oleic acids were the main fatty acids present, respectively. Although plethora of information on the seed oils of *Opuntia*

Table 3

Retention time (Rt), wavelengths of maximum absorption in visible region (λ_{max}), mass spectral data, identification and quantification of betalains detected in *Opuntia* spp. pulp.

Peak	Identification	Rt (min)	λ_{max} (nm)	$[M + H]^+$ (m/z)	MS^2 (m/z)	Quantification		
						OG	OS	OE
Betaxanthins								
1	Muscaaurin	13.37	471	349	305 (100), 124 (10)	nq (5.7)	nd	nd
2	Indicaxanthin isomer I	24.47	477	309	263 (100), 219 (43), 188 (15)	nq (60.9)	nq (46.5)	nd
3	Indicaxanthin isomer II	25.63	477	309	263 (100), 219 (21), 188 (9)	nq (30.1)	nq (7.8)	nd
Betacyanins								
4	Betanidin-5-*O*-β-glucoside (betanin)[1]	23.26	534	551	389 (100), 345 (50), 150 (28)	11.32. ± 0.09[c] (3.4)	195.3 ± 0.9[b] (41.6)	225 ± 3[a] (80.76)
5	Isobetanin[1]	24.62	534	511	389 (100), 345 (73), 150 (46)	nd	nd	43 ± 1 (15.01)
6	Gomphrenin I[1]	25.73	535	551	507 (3), 389 (38), 345 (100), 301 (21)	nd	9.42 ± 0.07* (2.0)	7.4 ± 0.6* (2)
7	Betanidin[1]	28.16	523	389	343 (97), 150 (91)	nd	9.89 ± 0.03* (2.1)	7.5 ± 0.1* (2.13)

OG, *Opuntia ficus-indica* var. gialla; OS, *Opuntia ficus-indica* var. Sanguigna; OE, *Opuntia engelmannii*.

Amounts in samples outside brackets in mg/100 g pulp FW, amounts inside brackets in relative percentages. FW, Fresh weight; nq, not quantified; nd, not detected. Calibration curve: [1]gomphrenin III (y = 14,670x − 19,725). In each row different letters mean significant differences ($p < 0.05$).

  * Statistical differences (< 0.001) were observed when *t*-Student test was applied.

Table 4

Chemical composition in lipophilic compounds, tocopherols and fatty acids of *Opuntia* spp.

	OG	OS	OE
Fatty acids (%)			
C6:0	0.32 ± 0.08[a]	0.21 ± 0.02[b]	0.09 ± 0.01[c]
C8:0	0.22 ± 0.06[a]	0.07 ± 0.02[b]	0.044 ± 0.001[b]
C10:0	0.6 ± 0.2[a]	0.18 ± 0.02[b]	0.097 ± 0.001[b]
C12:0	0.7 ± 0.2[a]	0.23 ± 0.02[b]	0.087 ± 0.001[c]
C14:0	1.4 ± 0.3[a]	0.56 ± 0.03b	0.234 ± 0.001[c]
C15:0	0.47 ± 0.01[a]	0.16 ± 0.01[b]	0.055 ± 0.001[c]
C16:0	16 ± 1[a]	13.9 ± 0.1[b]	7.92 ± 0.03[c]
C16:1	0.31 ± 0.02[b]	0.504 ± 0.001[a]	0.298 ± 0.001[b]
C17:0	0.39 ± 0.04[a]	0.171 ± 0.001[b]	0.106 ± 0.001[c]
C18:0	6 ± 1[a]	3.95 ± 0.01[b]	3.58 ± 0.01[b]
C18:1n9	11.7 ± 0.3[a]	9.82 ± 0.03[c]	10.23 ± 0.01[b]
C18:2n6	59 ± 1[c]	68.3 ± 0.2[b]	75.73 ± 0.02[a]
C18:3n3	1.9 ± 0.9[a]	1.26 ± 0.05[ab]	0.71 ± 0.04[b]
C20:0	0.5 ± 0.1[a]	0.28 ± 0.01[b]	0.305 ± 0.001[b]
C20:1	0.116 ± 0.001	nd	0.144 ± 0.001
C22:0	0.636 ± 0.07[a]	0.38 ± 0.02[b]	0.242 ± 0.001[c]
C24:0	nd	nd	0.115 ± 0.001
SFA	27 ± 2[a]	20.1 ± 0.2[b]	12.75 ± 0.05[c]
MUFA	12.1 ± 0.3[a]	10.33 ± 0.03[c]	10.69 ± 0.01[b]
PUFA	61 ± 1[c]	69.6 ± 0.2[b]	76.45 ± 0.06[a]
Tocopherols (µg/100 g FW)			
α-Tocopherol	53 ± 1[b]	99 ± 3[a]	15.1 ± 0.3[c]
β-Tocopherol	2.97 ± 0.05*	5.2 ± 0.2*	nd
γ-Tocopherol	2.8 ± 0.4[c]	53 ± 1[a]	27 ± 0.9[b]
Sum of tocopherols	58.9 ± 0.4[b]	158 ± 1[a]	43.0 ± 0.6[c]

OG, *Opuntia ficus-indica* var. gialla; OS, *Opuntia ficus-indica* var. sanguigna; OE, *Opuntia engelmannii*.

FW, pulp fresh weight; nd, not detected; SFA, saturated fatty acids; MUFA, mono-unsaturated fatty acids; PUFA, polyunsaturated fatty acids. In each row different letters mean significant differences ($p < 0.05$).

  * Statistical differences (< 0.001) were observed when *t*-Student test was applied.

spp. are available, few publications have analysed the fatty acid content in the fruit, Farag et al. (2017) also report linoleic, palmitic and oleic acid as the major fatty acids present in OFI fruits. The essential fatty acids have given rise to great interest due to the health potential of PUFA. According to Timilsena, Wang, Adhikari, and Adhikari (2017), PUFA plays a vital role in maintaining health in humans by minimizing the risk of cardiovascular and neurodegenerative disease, arthritis, diabetes and certain types of cancer. Therefore, it is important to stress the possibility of finding richer PUFA matrices in other fruit, vegetables and seeds.

As shown in Table 4, OS was the variety that displayed the highest tocopherol content, α-tocopherol being the main isoform present, followed by γ- and β-tocopherol. δ-Tocopherol was analysed but not found in any variety. The lowest content in tocopherols was found in OE samples, where only α- and γ-isoforms were present, the latter isoform being the one that contributes most to the total content. Regarding OG, α-tocopherol was around 18-fold higher compared with β- and γ-to-copherol. As far as we know, there are no studies related to the tocopherol content in OFI, only Farag et al. (2017) describe the relative percentage of α-tocopherol in three varieties of OFI, but do not give concrete amounts with which a comparison could be performed. The concentration of tocopherols in the same genus, but different species was assayed by Morales, Ramírez-Moreno, Sanchez-Mata, Carvalho, and Ferreira (2012), with total tocopherol content ranging between 140 and 220 µg/100 g FW in *Opuntia joconostle* and *matudae*. Only the values obtained by OS are in concordance with those revealed by Morales et al. (2012), while samples OG and OE only displayed 59 and 43 µg/100 g, respectively. Tocopherols are the major lipid-soluble antioxidant in the cell antioxidant defence system, nonetheless, the human body is not able to synthesize these substances using its own metabolic pathways, therefore it has to be obtained from the diet (Sýs, Švecová, Švancara, & Metelka, 2017). Tocopherols function as a chain-breaking antioxidant, inhibiting the propagation of lipid peroxidation, preventing lipoproteins and cell membranes from oxidative damage by acting as singlet oxygen quencher and stabilizing chloroplast membranes (Takshak & Agrawal, 2015). Although OS has the highest content in total tocopherols, the amount present in 100 g of fruit only represents around 1% of the Reference Daily Intake (NIH, 2017).

3.3. Antimicrobial properties

Results showing pathogenic bacteria growth inhibition are presented in Table 5. The three varieties of *Opuntia* spp. showed different levels of antibacterial activity. The *Opuntia* variety OS and OE displayed activity against all the tested bacterial strains, being more active than the commercial controls ampicillin and streptomycin, for all the MIC values, with the exception of *Staphylococcus aureus*. When comparing the bactericidal (MBC) potential of OS with the commercial antibiotic streptomycin, only 5 out of the 8 tested strains had better performance (*Bacillus cereus*, *Micrococcus flavus*, *Escherichia coli*, *Enterobacter cloacae* and *Salmonella typhimurium*), but in the other three strains, the difference shown was very small. It is important to stress the strong effect of OS samples against *Micrococcus flavus*, this sample displayed 4-fold stronger inhibition compared to the best antibiotic tested as a positive control (streptomycin).

On the other hand, OE showed higher potential than the commercial

Table 5
Antibacterial and antifungal activity of *Opuntia* samples.

Bacteria (mg/mL)	Samples						Control			
	OS		OG		OE		C1		C2	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Bacillus cereus	0.075	0.15	0.10	0.15	0.10	0.15	0.10	0.20	0.25	0.40
Micrococcus flavus	0.050	0.07	–	–	0.20	0.30	0.20	0.30	0.25	0.40
Staphylococcus aureus	0.15	0.30	0.075	0.15	0.15	0.45	0.04	0.10	0.25	0.45
Listeria monocytogenes	0.15	0.45	0.10	0.15	0.15	0.45	0.20	0.30	0.40	0.50
Escherichia coli	0.10	0.15	–	–	0.20	0.30	0.20	0.30	0.40	0.50
Enterobacter cloacae	0.10	0.15	–	–	0.075	0.30	0.20	0.30	0.25	0.50
Pseudomonas aeruginosa	0.10	0.30	0.15	0.30	0.10	0.30	0.20	0.30	0.75	1.20
Salmonella typhimurium	0.10	0.45	0.20	0.45	0.30	0.45	0.25	0.50	0.40	0.75
Fungi (mg/mL)	OS		OG		OE		C3		C4	
	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
Aspergillus fumigatus	0.30	0.60	0.30	0.60	0.30	0.45	0.25	0.50	0.15	0.20
Aspergillus versicolor	0.30	0.45	0.30	0.45	0.15	0.30	0.20	0.50	0.10	0.20
Aspergillus ochraceus	0.20	0.45	0.30	0.45	0.20	0.30	1.50	2.00	0.15	0.20
Aspergillus niger	0.30	0.60	0.30	0.60	0.30	0.45	0.20	0.50	0.15	0.20
Trichoderma viride	0.10	0.20	0.075	0.15	0.0375	0.075	1.00	1.00	0.15	0.20
Penicillium funiculosum	0.30	0.45	0.30	0.45	0.30	0.45	0.20	0.50	0.20	0.25
Penicillium ochrochloron	0.50	0.75	0.075	0.15	0.30	0.45	2.50	3.50	0.20	0.25
Penicillium verrucosum var. cyclopium	0.30	0.60	0.30	0.60	0.30	0.60	0.20	0.30	0.10	0.20

OG, *Opuntia ficus-indica* var. gialla; OS, *Opuntia ficus-indica* var. sanguigna; OE, *Opuntia engelmannii*. C1, streptomycin; C2, ampicillin; C3, ketoconazole; C4, bifonazole; MIC, minimum inhibitory concentration; MBC, minimum bactericidal concentrations; MBC, minimum fungicidal concentrations; N.A., no activity.

antibiotic ampicillin against all 8 strains assayed. When this sample was compared to streptomycin, only 2 strains (*Staphylococcus aureus* and *Listeria monocytogenes*) stood out as the species with the highest resistance against the OE sample, otherwise for the remaining 6 strains OE showed a similar or better performance than the mentioned antibiotic.

The sample with the least potential was OG, which only had an effect on 5 out of 8 strains tested. Nevertheless, for 4 of the strains, the OG sample showed the same or better potential compared to the positive antibiotic controls.

The fungi positive controls used in this assay were ketoconazole and bifonazole (Table 5), the latter showing overall a stronger effect against the pathogenic fungal strains. Samples of OS, OG and OE exhibit fungistatic and fungicidal effects against all 8 strains tested. The minimum inhibitory concentrations (MIC) were similar or better than the concentration of ketoconazole control (except in *Trichoderma viride*, *Aspergillus ochraceus* and *Penicillium ochrochloron*), but none of the samples exhibited better performance than bifonazole. The minimum fungicidal concentrations (MFC) of the samples against bifonazole were always inferior, except for the *T. viride* strain against all the tested samples and in the *P. ochrochloron* strain against OG. The fungicidal power of OE against *T. viride* presented 3-fold lower concentrations (higher potential) than the fungicidal power displayed by bifonazole. Chahdoura et al. (2016), tested the antimicrobial activity from *Opuntia microdasys* flowers and reported lower effects on their samples compared to OS, OG and OE extracts. Polyphenols and other biofunctional molecules, such as betalains, have shown the capacity to induce cellular damage in pathogenical microorganisms (Azeredo, 2009; Sansano, Rivas, Pina-Pérez, Martinez, & Rodrigo, 2017).

Overall, fruit from OFI var. gialla and sanguigna and *Opuntia engelmannii* revealed the presence of importance hydrophilic and lipophilic compounds. This study allowed the characterization of two different Opuntia species and two varieties of OFI, and the correlation between the studied species. Moreover, with these results can permit the discovery of potential alternative natural additives in order to replace the synthetic ones, exerting their additive function plus inherent bioactive functionality, acting positively on the health and well-being of consumers. *Opuntia* samples have shown strong antimicrobial activity as well as antioxidant potential, providing a wide range of possibilities, from thickener (mucilage), acidity regulators (succinic acid),

lipid-soluble antioxidants (tocopherols), water-soluble antioxidant (betalains and ascorbic acid) and natural colorants (betacyanins/betaxanthins).

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