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

Running Title: Profiling of three varieties of *Opuntia* spp. fruit

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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.

Keywords: *Opuntia ficus-indica*; *Opuntia engelmannii*; nutritional properties; betalains; antimicrobial activity

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.

Scientist 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 opt 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.

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

93 coordinates: 41.797344, -6.772735) in early September 2016. Dr. Carlos Aguiar of the
94 School of Agriculture, Polytechnic Institute of Bragança (Trás-os-Montes, Portugal),
95 confirmed the botanical identifications and voucher specimens were deposited.
96 Within 24 hours, the fruit were washed with distilled water in order to remove glochids,
97 and then air-dried on the countertop of the laboratory. Afterwards, all the fruits (3
98 samples of each) were peeled and the resulting pulp was lyophilized (LabConco,
99 Frezone -105 °C, 4.5 L Cascade Benchtop Freeze Dry System, Kansas, MO, USA),
100 crushed in a porcelain mortar, and stored in a cool, dry place until use.
101
102 *2.2. Morphological parameters*
103 Fruit size: the length and width of the entire fruit and pulp were measured with a
104 calliper. The whole fruit, pulp and peel where weighed separately. For colour detection,
105 fresh and lyophilized pulp and peel were measured with a colorimeter (model CR-400;
106 Konica Minolta Sensing Inc., Japan), previously calibrated using the standard white
107 plate. Using illuminant C and a diaphragm opening of 8 mm, the CIE L* a* b* colour
108 space values were registered with a computerized system using the colour data software
109 "Spectra Magic Nx" (version CM-S100W 2.03.0006).
110
111 *2.3. Chemical characterisation*
112 *2.3.1. Proximal nutritional composition*
113 Chemical and nutritional parameters (protein, fat, moisture, ash, carbohydrates and
114 energy) were determined only for the edible part of the fruit (pulp). Samples were
115 analysed according to the AOAC procedures (AOAC, 2016) Crude protein content (N ×
116 6.25) was determined by the macro-Kjeldahl method (AOAC, 991.02). Crude fat
117 (AOAC, 989.05) was estimated by extracting a known weight of powdered sample with

118 petroleum ether, using a Soxhlet apparatus. Moisture content was determined by the
119 weight difference before and after the drying process (oven at 100 ºC); the ash content
120 (AOAC, 935.42) was determined by incineration at 550 ± 15 °C. Total carbohydrates
121 (including fibre) were calculated by difference [total carbohydrates (g/100 g) = 100 − (g
122 fat + g protein + g ash)]. Total energy was calculated according to the following
123 equation: Energy (kcal/100 g) = 4 × (g proteins + g carbohydrates) + 9 × (g fat).
124
125 *2.3.2. Hydrophilic compounds*
126 *Soluble sugars.* Sugars were determined in defatted samples by high performance liquid
127 chromatography coupled to a refraction index detector (HPLC-RI, Knauer, Smartline
128 system 1000, Berlin, Germany) following a procedure described by Pereira, Barros,
129 Carvalho, & Ferreira (2011). Mobile phase consisted of acetonitrile:water mixture
130 (70:30 v/v, acetonitrile HPLC-grade, Lab-Scan, Lisbon, Portugal) and separation was
131 achieved using a Eurospher 100-5 NH_2 column (4.6 × 250 mm, 5 µm, Knauer).
132 melezitose was used as internal standard. The results were recorded and processed using
133 Clarity 2.4 software (DataApex, Prague, Czech Republic).
134 *Organic acids*. Organic acids were determined by an optimised procedure previously
135 described by Barros, Pereira, & Ferreira (2013) and the analysis was performed using a
136 Shimadzu 20A series UFLC (Shimadzu Coperation, Kyoto, Japan) coupled to a diode
137 array detector (DAD), using 215 and 245 nm (for ascorbic acid) as the preferred
138 wavelengths. Results were expressed as g and mg per 100 g of pulp fresh weight (FW),
139 for sugars and organic acids, respectively.
140 The sugars and organic acids were identified by comparing their retention times with
141 standard compounds, and quantification was conducted by comparison with dose–
142 response curves constructed from authentic standards. For sugar determination,

143 melezitose was used as the internal standard. Results were expressed as g and mg per
144 100 g of pulp fresh weight (FW), for sugars and organic acids, respectively.
145 *Betalains.* The profile of these compounds was determined by LC-DAD-ESI/MSn
146 (Dionex Ultimate 3000 UPLC, Thermo Scientific, San Jose, CA, USA). The lyophilized
147 pulps were re-dissolved in water at a concentration of 150 mg/mL. Chromatographic
148 separation was achieved with a Waters Spherisorb S3 ODS-2 C18 (3 μm, 4.6 mm × 150
149 mm, Waters, Milford, MA, USA) column working at 35 °C. The solvents used were:
150 (A) 0.1% trifluoroacetic acid (TFA) in water, and (B) acetonitrile. The gradient elution
151 followed these parameters: from 0% to 10% B for 15 min, from 10% to 15% B for 5
152 min, from 15 to 18% B for 5 min, from 18 to 50% B for 8 min, and from 50 to 0% B for
153 12 min. The resulting total run time was 45 minutes, using a flow rate of 0.5 mL/min.
154 Detection was carried out in the DAD using 480 nm (for betaxanthins) and 530 nm (for
155 betacyanins), as the preferred wavelengths, and in a mass spectrometer (MS). MS
156 detection was performed using positive mode, with a Linear Ion Trap LTQ XL mass
157 spectrometer (ThermoFinnigan, San Jose, CA, USA) equipped with an ESI source.
158 Nitrogen served as the sheath gas (50 psi); the system was operated with a spray voltage
159 of 4.8 kV, a source temperature of 320 °C, and a capillary voltage of 39 V. The tube
160 lens offset was kept at a voltage of 140 V. The full scan covered the mass range from
161 *m/z* 100 to 1500. The collision energy used was 24 (arbitrary units). Data acquisition
162 was carried out with the Xcalibur® data system (ThermoFinnigan, San Jose, CA, USA).
163 Identification of the betalain compounds (betacyanins and betaxanthins) was performed
164 by comparing the obtained information with available data reported in the literature,
165 providing a tentative identification. For quantitative analysis, a calibration curve using
166 an isolated compound gomphrenin III (isolated from *Gomphrena globosa* L.) was
167 constructed based on the UV signal ($y = 14670x - 19725$, $R^2 = 0.9997$). The results for

168 betacyanins were expressed as mg per 100 g of pulp fresh weight (FW), and the results
169 for betaxanthins were expressed as a relative percentage (%) of the areas recorded at
170 480 nm.
171
172 *2.3.2. Lipophilic compounds*
173 *Fatty acids*. Fatty acid determination was achieved via the transesterification procedure
174 described previously by Guimarães et al. (2013). The analysis was performed in gas
175 chromatography (GC DANI 1000; Contone, Switzerland) equipment with flame
176 ionization detection. Results were expressed as relative percentages of each fatty acid.
177 *Tocopherols*. The four isoforms of tocopherols were analysed according to the
178 previously described procedure (Heleno, Barros, Sousa, Martins, & Ferreira, 2010).
179 Analysis was performed using a HPLC system (Knauer, Smartline system 1000, Berlin,
180 Germany), coupled to a fluorescence detector (FP-2020; Jasco, Easton, MD, USA).
181 Quantification was based on the fluorescence signal response of each standard, using
182 the internal standard (tocol) method and using calibration curves obtained from
183 commercial standards of each isoform. The results were expressed in µg per 100 g of
184 pulp fresh weight (FW).
185
186 *2.4. Antimicrobial effect of fruit pulp*
187 Antibacterial activity was performed using the lyophilized pulps re-dissolved in water at
188 a concentration of 10 mg/mL and following a procedure previously reported by Reis et
189 al., (2014). Four Gram-positive bacteria: *Staphylococcus aureus* (ATCC 6538), *Bacillus*
190 *cereus* (clinical isolate), *Micrococcus flavus* (ATCC10240), and *Listeria*
191 *monocytogenes* (NCTC7973) and four Gram-negative bacteria: *Escherichia coli* (ATCC
192 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) was 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 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 varity. 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), direct correlation with betalainic concentration can be clearly observed, higher concentration of betalians in varieties, 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, & Paredes-López (2014).

Conversely, OE was statistically different from OG and OS, moisture in OE was around 17 percent 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, & 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 worth to note, that besides the differences presented here between the two different species, there is also an additional factor that plays an important role. The cultivar location influence also the different species due to environmental factors as soil, precipitation, sun exposure, among others. In the present work we could for instance,

observed the different maturation times, where Portuguese OE got their optimum maturation 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 Diaz et al., 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, & Wesjohann (2017), also identified succinic acid as the more abundant organic acid in Italian OFI varieties. Tretter, Patocs, & Chinopoulos,(2016) described that

317 succinic acid is an important metabolite involved in several signalling processes, not
318 only in the mitochondria were it is generated, but in the cytoplasm as well as the
319 extracellular space and it is also involved in the elimination of reactive oxygen species,
320 this mechanism of action helped to understand part of the antioxidant effect of the OFI
321 fruits performed on this assay. Succinic acid is generally recognized as safe (FDA,
322 2017) and has different applications in the pharmaceutical and food industries, although
323 it is primarily used as an acidity regulator (Ahn, Jang, & Lee, 2016). Organic acid
324 profiles are not reported for *Opuntia* spp. in the literature, although, extensive assays
325 have been performed on ascorbic acid for OFI. Variable values are found ranging from
326 3.5 to 45 mg /100 g edible pulp (Kuti, 2004; Stintzing et al., 2005). OG and OS showed
327 lower levels of ascorbic acid, compared with reported data, while OE was almost 10-
328 fold higher than the previously mentioned varieties.
329 The betalain profiles of OG, OS and OE are shown in **Figure 1**. Data regarding
330 retention time, λmax in the visible region, molecular ion and main fragment ions
331 observed in MS^2, obtained by HPLC-DAD-ESI/MS analysis regarding betalains
332 identification and quantification are presented in **Table 3**. Compounds 1-3 were
333 identified as betaxanthin derivatives, and compounds 4-7 as betacyanin derivatives. All
334 of the identified compounds have been previously described in *Opuntia* spp (Cejudo
335 Bastante, Chaalal, Louaileche, Parrado, & Heredia, 2014; Mata et al., 2016), although,
336 not all the compounds in the same variety like its presented in this assay. Betaxanthins
337 were found in a higher percentage in OG, followed by OS and were not identified in
338 OE. Nevertheless, betacyanidin content was lower in OG (11.32 mg/100 g of FW),
339 followed by OS (215 mg/100 g FW) and the largest amounts were present in OE (sum
340 content of 283 mg/100 g of FW), which presented 25-fold higher quantities then OG
341 and 1.3-fold higher then OS. The hierarchical order observed agrees with the data found

342 by other researchers, were the amount of betalains increased from yellow to red and to
343 purple varieties (Farag et al., 2017). Several works have demonstrated the potent
344 antiradical scavenging activity of betalains in vitro (Azeredo, 2009), which contributes
345 to the prevention of several degenerative diseases. Therefore, betalians could be a great
346 substitutive of artificial dyes with bioactive activity.
347 Saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated
348 fatty acids (PUFA) are presented in **Table 4**. Samples were richer in PUFA with over
349 60% of the total fatty acids, where OE was notably higher than OS and OG. SFA were
350 the second most abundant group followed by MUFA. In all the *Opuntia* species, linoleic
351 palmitic and oleic acids were the main fatty acids present, respectively. Although
352 plethora of information on the seed oils of *Opuntia* spp are available, few publications
353 have analysed the fatty acid content in the fruit, Farag et al., (2017) also report linoleic,
354 palmitic and oleic acid as the major fatty acids present in OFI fruits. The essential fatty
355 acids have given rise to great interest due to the health potential of PUFA. According to
356 Timilsena, Wang, Adhikari, & Adhikari, (2017), PUFA plays a vital role in maintaining
357 health in humans by minimizing the risk of cardiovascular and neurodegenerative
358 disease, arthritis, diabetes and certain types of cancer. Therefore, it is important to stress
359 the possibility of finding richer PUFA matrices in other fruit, vegetables and seeds.
360 As shown in **Table 4**, OS was the variety that displayed the highest tocopherol content,
361 α-tocopherol being the main isoform present, followed by γ- and β-tocopherol. δ-
362 tocopherol was analysed but not found in any variety. The lowest content in tocopherols
363 was found in OE samples, where only α- and γ- isoforms were present, the latter
364 isoform being the one that contributes most to the total content. Regarding OG, α-
365 tocopherol was around 18-fold higher compared with β- and γ-tocopherol. As far as we
366 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 et al. (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 functions 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,*

392 *Escherichia coli, Enterobacter cloacae* and *Salmonella typhimurium)*, but in the other
393 three strains, the difference shown was very small. It is important to stress the strong
394 effect of OS samples against *Micrococcus flavus*, this sample displayed 4-fold stronger
395 inhibition compared to the best antibiotic tested as a positive control (streptomycin).
396 On the other hand, OE showed higher potential than the commercial antibiotic
397 ampicillin against all 8 strains assayed. When this sample was compared to
398 streptomycin, only 2 strains (*Staphylococcus aureus* and *Listeria monocytogenes*) stood
399 out as the species with the highest resistance against the OE sample, otherwise for the
400 remaining 6 strains OE showed a similar or better performance than the mentioned
401 antibiotic.
402 The sample with the least potential was OG, which only had an effect on 5 out of 8
403 strains tested. Nevertheless, for 4 of the strains, the OG sample showed the same or
404 better potential compared to the positive antibiotic controls
405 The fungi positive controls used in this assay were ketoconazole and bifonazole (**Table**
406 **5**), the latter showing overall a stronger effect against the pathogenic fungal strains.
407 Samples of OS, OG and OE exhibit fungistatic and fungicidal effects against all 8
408 strains tested. The minimum inhibitory concentrations (MIC) were similar or better than
409 the concentration of ketoconazole control (except in *Trichoderma viride, Aspergillus*
410 *ochraceus* and *Penicillium ochrochloron*), but none of the samples exhibited better
411 performance than bifonazole. The minimum fungicidal concentrations (MFC) of the
412 samples against bifonazole were always inferior, except for the *T. viride* strain against
413 all the tested samples and in the *P. ochrochloron* strain against OG. The fungicidal
414 power of OE against *T. viride* presented 3-fold lower concentrations (higher potential)
415 than the fungicidal power displayed by bifonazole. Chahdoura et al., (2016), tested the
416 antimicrobial activity from *Opuntia microdasys* flowers and reported lower effects on

417 their samples compared to OS, OG and OE extracts. Polyphenols and other
418 biofunctional molecules, such as betalains, have shown the capacity to induce cellular
419 damage in pathogenical microorganisms (Azeredo, 2009; Sansano, Rivas, Pina-Pérez,
420 Martinez, & Rodrigo, 2017).
421
422 Overall, fruit from OFI var gialla and sanguigna and *Opuntia engelmannii*, revealed
423 interesting results, the importance of the study of the hydrophilic and lipophilic
424 compounds, was to characterize them in order to discover potential additives
425 alternatives to the synthetic ones, that could exert their additive function plus inherent
426 bioactive functions which might act positively on the health and well-being of
427 consumers. *Opuntia* samples have shown strong antimicrobial activity as well as
428 antioxidant potential, providing a wide range of possibilities, from thickener (mucilage),
429 acidity regulators (succinic acid), lipid-soluble antioxidants (tocopherols), water-soluble
430 antioxidant (betalains) and natural colorants (betacyanins/betaxanthins).
431
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