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Phytochemical characterization of different prickly pear (*Opuntia ficus-indica* (L.) Mill.) cultivars and botanical parts: UHPLC-ESI-MSnmetabolomics profiles and their chemometric analysis

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1 **Phytochemical characterization of different prickly pear (*Opuntia ficus-indica* (L.)**
2 **Mill.) cultivars and botanical parts: UHPLC-ESI-MS^a metabolomics profiles and [their](#)**
3 **chemometric analysis**

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20

21 **ABSTRACT**

22 Prickly pear is an [interesting-important](#) source of bioactive compounds. However, a
23 comprehensive characterization of the phytochemical profile of its aerial botanical parts,
24 considering genotypic differences, has not been conducted. This study evaluated the
25 phytochemical composition of four botanical parts (fruit pulp and skin, and young and adult
26 cladodes) of six cultivars. Analysis was carried out by using two non-targeted UHPLC-ESI-
27 MSⁿ experimental conditions and assisted with multivariate analysis to facilitate data
28 interpretation. Up to 41 compounds, mainly (poly)phenolic molecules, were identified and
29 quantified, 23 compounds being reported for the first time in *Opuntia ficus-indica*. Phenolic
30 composition varied significantly depending on the part of the plant. Betalains were detected
31 only in the fruit of a red cultivar. This study provided novel insights in terms of identification
32 of bioactives and thorough characterization of botanical parts [of prickly pears](#). This
33 information may be used for the development of prickly pear-derived products with high
34 levels of bioactive compounds.

35
36 **KEYWORDS**

37 Cactus; phenolic compounds; metabolomics; foodomics; mass spectrometry; multivariate
38 analysis.

39 1. Introduction

40 Cactus prickly pear (*Opuntia ficus-indica* (L.) Mill.) is a plant that could be easily
41 cultivated in arid and semiarid climates (Russell & Felker, 1987). It produces edible fruits
42 (called “tuna”) and cladodes (fleshy flattened stems, commonly called “nopal”), both used as
43 food and as feed. Prickly pear is employed for nutrition, cosmetic, and ethnopharmacological
44 purposes in the forms of tea, jam, juice, and oil -extracted from the seeds- (Stintzing et al.,
45 2005). Recently, some authors have highlighted the prospects of different prickly pear aerial
46 parts as good sources of phytochemicals with proven biological activities and high-added
47 value for the food/nutraceutical industry (Barba et al., 2017; Msaddak et al., 2017; Sánchez-
48 Tapia et al., 2017). This interest in *Opuntia* bioactives becomes even more relevant when
49 ~~being~~ considering the need to cope with climate change challenges. Taking into account
50 the tolerance of cactus species to extreme climatic/soil conditions; (Russell & Felker, 1987),
51 the exploitation of its phytochemical content may contribute to its ~~represent a~~ sustainable
52 production ~~activity~~.

53 The main phytochemical compounds in prickly pear fruits and cladodes are vitamins,
54 carotenoids, betalains, and (poly)phenolic compounds (Barba et al., 2017; Fernández-López,
55 Almela, Obón, & Castellar, 2010; Stintzing et al., 2005). Fruits are good sources of betalains,
56 but the real physiological relevance of these compounds has not been fully unraveled
57 (Moreno, García-Viguera, Gil, & Gil-Izquierdo, 2008). Among the different prickly pear
58 phytochemicals, (poly)phenolic compounds are likely those attracting more attention due to
59 their health-related effects (Del Rio et al., 2013; Rodríguez-Mateos et al., 2014; Zanotti et al.,
60 2015). The (poly)phenolic fingerprint of prickly pear products is characterized mainly by
61 flavonols and phenolic acids (Fernández-López et al., 2010; Kuti, 2004; Mata et al., 2016;
62 Moussa-Ayoub et al., 2014; Serra, Poejo, Matias, Bronze, & Duarte, 2013; Stintzing et al.,
63 2005; Yeddes, Cherif, & Trabelsi Ayadi, 2014). However, despite considerable

64 characterizations have been reported (Guevara-Figueroa et al., 2010; Mata et al., 2016;
65 Moussa-Ayoub et al., 2014; Serra et al., 2013; Yeddes et al., 2014), a detailed profiling of the
66 bioactive compounds of the aerial parts of prickly pear is lacking.

67 The accurate characterization of the phytochemical fingerprinting of any vegetal
68 matrix is key to better understand its biological, technological, and nutritional properties
69 (Mena et al., 2012). The use of mass spectrometric (MS) metabolomics techniques, assisted
70 by chemometric analysis, has been identified as a valuable [asset to evaluate technique in the](#)
71 [evaluation of](#) the phytochemical profile of different plant materials rich in bioactive
72 compounds (Calani et al., 2013; [Eva M^a Sánchez-Salcedo et al., 2016](#)). Analytical approaches
73 allowing easy sample handling and quick, high-throughput chromatographic screening are
74 encouraged to accomplish this task (Filigenzi, Ehrke, Aston, & Poppenga, 2011).
75 Nevertheless, the comprehensive study of bioactive compounds may pose some analytical
76 constraints due to the varying capability of diverse chemical scaffolds to respond to the MS
77 ionization settings. Thus, versatile experimental conditions leading to the identification of
78 different phytochemical classes are required (Mena et al., 2016).

79 The present work aimed at investigating the phytochemical composition of four
80 different botanical parts (young and adult cladodes, fruit pulp, and skin) of six prickly pear
81 cultivars grown in Spain, [extending a preliminary characterization of this plant material](#)
82 [\(Andreu, Nuncio-Jáuregui, Carbonell-Barrachina, Legua, & Hernández, 2018\)](#). The study
83 was performed by using two complementary non-targeted UHPLC-ESI-MSⁿ experimental
84 conditions and paired with multivariate analysis to facilitate a comprehensive screening. The
85 high number of samples and the presence of different matrices and classes of phytochemicals
86 represented a major analytical challenge; however, the insights provided in terms of both
87 identification of bioactive compounds and thorough characterization are of interest.

88

89 **2. Materials and methods**

90 *2.1. Chemicals*

91 Protocatechuic acid, ferulic acid, quercetin-3-*O*-rutinoside (rutin), naringenin-7-*O*-rutinoside
92 (narirutin), secoisolariceresinol, and betanin were purchased from Sigma-Aldrich (Steinheim,
93 Germany). [HPLC-grade s](#)Solvents were also purchased from Sigma-Aldrich. Water for
94 HPLC analysis was purchased from VWR Chemicals (Fontenay-sous-bois, France).

95
96 *2.2. Plant material*

97 Cladodes and fruits of six different cultivars of *Opuntia ficus-indica* were used for this study.
98 Four cultivars, named “NA”, “NT”, “NE”, and “NO”, were collected at the experimental
99 field station of the Miguel Hernandez University in the province of Alicante, Spain
100 (02°03’50’’E, 38°03’50’’N, and 25 m above sea level). The other two cultivars were
101 collected from private farms in Murcia (“Fresa” cultivar) and Alicante (“Nalle” cultivar) (SE
102 Spain) (less than 50 km far from the experimental station).

103 Young (less than a year) and old cladodes (2 years old), as well as the fruits, were
104 manually harvested during spring and summer of 2015. Ten young cladodes, 10 adult
105 cladodes, and 10 fruits from three *Opuntia ficus-indica* plants per cultivar were harvested.
106 After picking, the plant material was immediately transported to the lab. The spines from the
107 cladodes were removed manually, while the fruits were washed under tap water with a brush
108 for 2 minutes. The peels from the fruits [were](#) removed manually. The fresh cladodes
109 (young and old), the pulp plus seeds, and the peel were immediately frozen in liquid nitrogen,
110 to be later freeze-dried in an Alpha 2-4 freeze drier (Christ Alpha 2-4; Braum Biotech,
111 Osterode am Harz, Germany) for 24 hours at a pressure reduction of 0.220 mbar. The
112 temperature in the drying chamber was -25 °C, while the heating plate reached 15 °C.

113 ~~The~~ Thereafter, seeds were removed from the pulp, and all the samples were powdered
114 (particle size < 0.4 mm) and packed under vacuum.

115

116 2.3. Extraction of (poly)phenolic compounds

117 The (poly)phenolic compounds in prickly pear cladodes (young and old) and fruits (pulp and
118 skin) were extracted following a protocol previously reported (Sánchez-Salcedo, Mena,
119 García-Viguera, Martínez, & Hernández, 2015). Briefly, 200 mg of freeze-dried powder were
120 mixed with 1 mL of 80% aqueous methanol acidified with formic acid (1%). This mixture
121 was then sonicated for 25 min, centrifuged at 10,480 g for 5 min at room temperature, and the
122 supernatant was collected. Two additional extractions were performed for each sample with
123 additional 0.5 mL of the extraction solvent, as described above, after which they were
124 centrifuged. The three supernatants were pooled before UHPLC-ESI-MSⁿ analysis. Each
125 sample was extracted in triplicate.

126

127 2.4. Liquid chromatography-mass spectrometry (UHPLC-ESI-MSⁿ) analysis

128 Methanolic extracts of prickly pear parts were analysed using an Accela UHPLC 1250
129 equipped with a linear ion trap-mass spectrometer (MS) (LTQ XL, Thermo Fisher Scientific
130 Inc., San Jose, CA, USA) fitted with a heated-electrospray ionization (ESI) probe (H-ESI-II;
131 Thermo Fisher Scientific Inc., San Jose, CA, USA). Separations were performed using a
132 XSelect HSS T3 (50_x_2.1 mm), 2.5 µm particle size (Waters, Ireland). Volume injected was
133 5 µL and column oven was set to 30°C. Two complementary MS experiments were
134 performed, one in negative mode, for non-coloured phenolics, and one using positive
135 ionization, for betalains, following an analytical approach previously developed for the
136 comprehensive identification of (poly)phenolic compounds (Mena et al., 2012). Each sample
137 was analysed in duplicate for each experimental condition.

138 The experimental condition optimized in negative ionization mode for the analysis of
139 non-coloured phenolics was based on the following conditions. The MS worked with a
140 capillary temperature equal to 275 °C, while the source heater temperature was set to 250 °C.
141 The sheath gas flow was 40 units, while both auxiliary and sweep gas were set to 5 units. The
142 source voltage was 3 kV. The capillary and tube lens voltages were -9 and -53 V,
143 respectively. Elution was performed at a flow rate of 0.2 mL/min. The gradient started with
144 90% of 0.1% aqueous formic acid and 10% of acetonitrile 0.1% formic acid, followed by a
145 13-min linear gradient of 10% to 70% acidified acetonitrile. From 13.5 to 14 min the
146 acidified acetonitrile was increased to 80%, followed to 2.5 min of 80% acetonitrile and then
147 4 min at the start conditions to re-equilibrate the column. Analyses were carried out using full
148 scan mode, data-dependent MS³ scanning from *m/z* 100 to 2000, with collision induced
149 dissociation (CID) equal to 30 (arbitrary units). Pure helium gas was used for CID.

150 For the analysis of betalains, in positive ionization mode, the MS worked with a
151 capillary temperature equal to 275 °C, while the source heater temperature was set to 200
152 °C. The sheath gas flow was 40 units, while auxiliary gas was set to 5 units, without sweep
153 gas. The source voltage was 4 kV. The capillary voltage and tube lens were 39 and 110 V,
154 respectively. The chromatographic conditions were identical to those used for the previous
155 experimental condition.

156 Data processing was performed using Xcalibur software from Thermo Scientific. All
157 compounds were identified by comparing with standards, when available, and mass spectral
158 and chromatographic data reported in literature. For quantification purposes, area calculation
159 was performed in selected ion monitoring mode by selecting the relative base peak at the
160 corresponding mass to charge ratio (*m/z*). The quantification of (poly)phenolics was carried
161 out by comparison with commercial standards, when available. For those compounds that
162 could not be quantified with their corresponding standards, a reference compound was

163 selected based on structural similarity and considering the functional groups that may affect
164 the ionisation properties (i.e., flavonols were quantified as rutin equivalents, lignans as
165 secosolariceresinol, etc.). Finally, the molecules responding to the ESI source in a unique
166 way with respect to the reference compound of choice, or not reaching the limit of
167 quantification of the corresponding reference compound, were not quantified. Details on the
168 identification and quantification of the phytochemicals are presented in the [Supplemental](#)
169 [Supplementary Table S1](#).

170

171 2.5. Statistical analysis

172 Statistical analyses were performed using the IBM SPSS Statistics 23 software package
173 (SPSS Inc., Chicago, IL, USA) and performed at $p < 0.05$ of significance level. Data are
174 presented as mean \pm standard deviation (SD) since the distribution of these variables was
175 normal. A one-way ANOVA with post hoc Tukey HSD test was employed for mean
176 comparisons among cultivars for each botanical part. The assessment of the main effects
177 (botanical part, cultivar, and the interaction of botanical part x cultivar) was also carried out
178 with Bonferroni *post-hoc* tests for multiple comparisons. Principal component analysis
179 (PCA) with varimax was performed to explore the differences in the phytochemical profile of
180 the different cultivars and prickly pear parts.

181

182 3. Results

183 3.1. Identification of phytochemicals in *Opuntia ficus-indica* cladodes and fruits

184 The phytochemical screening of prickly pear cladodes (young and old) and fruits (pulp and
185 skin) belonging to six different cultivars was carried out by using two complementary MS
186 experimental conditions. About 120 mass spectra were evaluated for each botanical part,
187 cultivar, experimental condition, and analytical replicate. This exhaustive analysis of the

188 *Opuntia ficus-indica* phytochemical composition allowed the tentative identification of up to
189 41 compounds (Table 1). Taking into account the number of compounds identified in prickly
190 pear parts, flavonoids were the most relevant class of phytochemicals (16 flavonols, -
191 compounds **6**, **13**, **15**, **16**, **18**, **20-22**, **24**, **26-28**, **31**, **32**, **37**, and **38**- and 2 flavanones, -**30** and
192 **33**-). Phenolic acids (6 hydroxycinnamic acids, -**4**, **7**, **9**, **12**, **14**, and **36**-, 2 phenylpyruvic
193 acids, -**8** and **35**-, 2 hydroxyphenylpropionic acids, -**19** and **23**-, and 2 hydroxybenzoic acids,
194 -**3** and **11**-) and lignans (6 compounds, -**5**, **10**, **17**, **25**, **29**, and **34**-) were also present. In
195 addition, some other compounds such as betalains (compounds **39-41**) and organic acids
196 (compounds **1** and **2**) were detected.

197 Two compounds (**24** and **39**) were identified by comparison with their respective
198 analytical standards. Thirty-nine compounds were identified based on their retention time,
199 fragmentation patterns obtained from mass spectra (MS² and MS³ experiments) (Table 1),
200 and by comparing their mass spectral characteristic with the available literature (see
201 Supplementary[†] Material, Table S1). The interpretation of the mass spectra fragmentation
202 patterns reported in the literature was not discussed unless of special interest. In this sense,
203 compounds **19**, **22**, and **26** were tentatively identified according to their characteristic
204 aglycone fragment ions. Compounds **22** and **26** presented a major MS² fragment ion at *m/z*
205 315 and showed MS³ fragments matching those of other isorhamnetin derivatives
206 (compounds **20**, **31**, **32**, and **37**). Compounds **22** and **26** (*m/z* 755 and 609) also had losses of
207 *m/z* 440 and 294, respectively, which might correspond to sambubioside-rhamnoside and
208 sambubioside moieties; however, the full structure could not be identified and, hence, they
209 were classified simply as isorhamnetin derivatives. Compound **19** presented the same
210 fragmentation pattern of compound **23** and was identified as an isomer of dihydrosinapic
211 acid-hexoside. 23 compounds (**3-6**, **10-19**, **21**, **23**, **25**, **29**, **30**, **33**, **34**, **37** and **38**) were
212 tentatively identified for the first time, as far as we know, in *Opuntia ficus-indica*.

213 Most of the compounds were identified in all the botanical parts analysed, while some
214 compounds were detected only in some of them ([Supplemental-Supplementary Material](#),
215 Table S1). In the case of betalains, they were only detected in the pulp and skin of the
216 “Fresa” cultivar, the only one presenting an intense red colour.

217

218 3.2. Quantification of major (poly)phenolic compounds in *Opuntia ficus-indica*.

219 The total amount of (poly)phenolic compounds for each botanical part and cultivar is
220 reported ~~at~~in Figure 1. There were [significant](#) main effects of botanical part, cultivar, and the
221 interaction of botanical part ~~×~~ cultivar on the content of (poly)phenolic compounds
222 ($p < 0.001$ for all). Regarding the botanical part, the highest (poly)phenolic content was found
223 in young cladodes > old cladodes > skin > pulp ($p < 0.05$). Comparison among cultivars for
224 each botanical part showed statistically significant differences on the content of
225 (poly)phenolic compounds (Figure 1). The concentration of these compounds varied between
226 5.3 (“NE”) and 14.3 (“Fresa”) mg/g dw for young cladodes and from 4.2 (“NO”) to 12.4
227 (“NE”) mg/g dw for old cladodes. The content of (poly)phenolic compounds in fruit skin
228 ranged from 4.3 to 7.1 mg/g dw for “NA” and “NT”, respectively, while it varied from 0.7 to
229 5.1 mg/g dw for “NO” and “Nalle”, respectively, in fruit pulp.

230 The profile of individual (poly)phenolic compounds for each botanical part was
231 dependent on the cultivar (Tables 2-5, [Supplementary Figure S1](#)). Twenty-six phenolic
232 compounds were quantified in young cladodes, [with](#) flavonoids (in particular,
233 flavonols) [being](#) the main (poly)phenolic compounds (Table 2). Individual phenolics in
234 young cladodes varied greatly among prickly pear varieties. Myricetin-hexoside (**6**) was the
235 predominant compound in most of the tested cultivars, except for “NE”, where it was present
236 at a very low amount. Young cladodes were also characterized by the presence of relevant
237 amounts of some isorhamnetin derivatives (**20**, **22**, and **31**), rutin (**24**), and ferulic acid-

238 hexoside (9) (Table 2). In the case of old cladodes, up to 25 compounds were quantified
239 (Table 3). Similarly to what was reported for young cladodes, flavonols were the major group
240 of (poly)phenolic compounds, and several isorhamnetin glycosides (20, 22, 26, and 31),
241 together with myricetin-hexoside (6) and ferulic acid-hexoside (9), were the main individual
242 phenolics (Table 3). Regarding With respect to fruit skin and pulp, a higher prevalence of
243 phenolic acids over flavonols was noted (Tables 4 and 5). Twenty-six (poly)phenolic
244 compounds were quantified in prickly pear skin, with ferulic acid-hexoside (9), sinapic acid-
245 hexoside (12), dihydrosinapic acid-hexoside (23), and isorhamnetin-rutinoside (31) present in
246 high concentrations for most of the cultivars (Table 4). Prickly pear pulp presented a lower
247 number of quantifiable phenolics (21 compounds), the main amount corresponding to a
248 ferulic acid derivative (36) (Table 5).

249 Betalains were not quantified due to the lack of commercially available, pure
250 reference standards (i.e, the purity of the Sigma-Aldrich's betanin and that of other chemical
251 providers is was not enough to use them as reliable analytical standards, to our concern).

253 3.3. Chemometric classification

254 Principal component analysis (PCA) was used to better understand the relationships among
255 different botanical parts and cultivars of the species *Opuntia ficus-indica* in terms of
256 (poly)phenolic composition. Only quantified phenolic compounds (reported in
257 Supplementary Table S1) were taken into account for the PCA. ~~Betalains and organic acids
258 were excluded from this unsupervised multivariate analysis to avoid confounding factors
259 limiting the description of the differences in the (poly)phenolic profile of the samples (i.e.,
260 betalains in the pulp and skin of "Fresa" cultivar conditioned strongly the PCA outcomes
261 according to preliminary tests).~~

262 Two principal components (PCs) were able to explain 61.3% of the total variability.
263 The first PC (PC1), representing 39.3% of the total variance, was positively linked to
264 isorhamnetin derivatives (20, 22, 26, 31, 32, 37), quercetin derivatives (15, 16, 21, 24, 27),
265 kaempferol derivatives (18, 28), and a ferulic acid derivative (7) (Figure 2A), while
266 negatively associated with compounds 10 and 36. PC2 accounted for 22% of the total
267 variance and it was positively correlated with compounds 9, 12, 23, 25, 29, 30, and 38, while
268 it was inversely correlated to compounds 17 and 34 (Figure 2A).

269 Sample scores for each PC accounted mostly for the similarities among cultivars and
270 the differences among botanical parts (Figure 2B). All cultivars presented a similar negative
271 PC1 value for the pulp (low content in flavonoids, rich in lignans), differing only in their
272 scores for PC2: “Fresa”, “NT”, “NA”, “NO”, and “NE” cultivars formed a sub-cluster with
273 negative scores for PC2, while “Nalle” had positive PC2 values (higher content in phenolic
274 acids). For the skin samples, all cultivars displayed neutral scores for PC1 and positive scores
275 for PC2 (medium content in most of the phenolic compounds). “Nalle” cultivar was the skin
276 sample showing a higher value for PC2, characterised by a high content of sinapic acid-
277 hexoside (12), dihydrosinapic acid-hexoside (23) and secoisolariciresinol-hexoside (25).
278 Most of the cladodes presented similar values for both PCs, although old cladodes had
279 slightly lower PC1 and PC2 scores than young ones. In this sense, young cladodes exhibited
280 a higher [flavonol](#) content ~~in flavonols~~ than old cladodes. Nevertheless, some samples showed
281 very high positive scores for PC1, accounting for high concentrations of quercetin and
282 isorhamnetin derivatives, ~~that-which~~ was the case ~~of-for~~ the old cladodes of “NE” cultivar
283 and the young cladodes of “Fresa”.

284

285 4. Discussion

311 investigated (Moussa-Ayoub et al., 2014; Stintzing et al., 2005). However, there is a limited
312 knowledge on the (poly)phenolic composition of both edible and residual parts of *Opuntia*
313 taking into account genotypic characteristics (Moussa-Ayoub et al., 2014). This work
314 provides novel insights ~~on~~in this regard, with data for individual phenolics on the basis of
315 different botanical parts and genotypes grown under the same environmental conditions. This
316 information may be used as starting point for the development of prickly pear-derived
317 products with high levels of (poly)phenolic compounds, as well as for botanical purposes. In
318 addition, the understanding of the phytochemistry of the aerial parts of prickly pear may
319 favour an integrated exploitation of cactus orchards.

320 The importance of assessing the (poly)phenolic content of prickly pear fruit pulp
321 is due to their use as edible plants for humans. Since prickly pear fruits are rich in a series
322 of flavonoids and phenolic acids with proven bioactivities (Del Rio et al., 2013; Rodriguez-
323 Mateos et al., 2014; Zanotti et al., 2015), data on their actual content are key to further
324 explore the biological prospects of prickly pear fruit consumption on human health. The
325 content in (poly)phenolic compounds of the six cultivars was similar in line with previous
326 reports on *Opuntia* fruits (Moussa-Ayoub et al., 2014; Yeddes et al., 2014), but slightly lower
327 than those recently reported for this same plant material by using a colorimetric method
328 (Andreu et al., 2018). In terms of individual phenolics, the presence of phenolic acids in juice
329 made from pulp has been confirmed (Mata et al., 2016). Regarding flavonols, while some
330 authors have identified a few isorhamnetin derivatives in the pulp of *Opuntia ficus-indica*
331 fruits (Kuti, 2004; Yeddes et al., 2014), others have reported a lack of flavonols in pulp
332 (Moussa-Ayoub et al., 2014). The present characterization accounted for the presence of up
333 to 9 flavonols, as well as several other phenolic scaffolds, in the pulp of prickly pear fruits,
334 which represent a step forward in the definition of the bioactives contained in the main edible
335 part of this plant. Although these inconsistencies in the flavonoid profile of prickly pear pulp

336 might be attributed to geographic and genotypic differences, they could likely be due to the
337 sensitivity and accuracy of the methodological approaches used.

338 A higher amount of (poly)phenolic compounds has been reported for fruit skin than
339 for fruit pulp (Moussa-Ayoub et al., 2014; Yeddes et al., 2014), in agreement with our
340 results. Important quantitative differences among cultivars were not found. This similarity
341 among cultivars has also been shown for cultivars grown in different countries in terms of
342 flavonol content (Moussa-Ayoub et al., 2014). So far, the (poly)phenolic profile of fruit skins
343 was restricted mainly to flavonols and some phenolic acids (Fernández-López et al., 2010;
344 Kuti, 2004; Mata et al., 2016; Moussa-Ayoub et al., 2014; Serra et al., 2013; Stintzing et al.,
345 2005; Yeddes et al., 2014), while the present work extends the number of molecules present
346 in this fruit part. Skins, which are usually a waste product, represent a potential source of
347 bioactive compounds that may increase the amounts of (poly)phenolic compounds if used for
348 juice elaboration together with the pulp (Fernández-López et al., 2010; Serra et al., 2013).
349 Considering its phytochemical content not only in phenolics but also in betalains (Stintzing et
350 al., 2005), prickly pear fruit skin may also be industrialized for the development of
351 sustainable alternatives allowing the exploitation of their bioactives as nutraceuticals
352 (Matias et al., 2014; Serra et al., 2013). This would minimize production by-products and
353 might generate profits from a by-product generally lacking economic value.

354 Cladodes were rich in (poly)phenolic compounds. The (poly)phenolic profile of
355 cladodes had been previously reported to comprise flavonols and phenolic acids (Guevara-
356 Figueroa et al., 2010; Msaddak et al., 2017). The newly-described presence of flavanones and
357 lignans increases the number of bioactive compounds in cladodes and, thus, its interest for
358 human health. Young cladodes exhibited a higher content in (poly)phenolic compounds when
359 compared to their older counterparts, which may be explained by changes in the physiology
360 of the cladode as a consequence of the age and maturation stage (El-Mostafá et al., 2014;

361 Rodríguez-García et al., 2007). Young cladodes are frequently consumed as a green
362 vegetable in salads, sauces, soups, stews, snacks, beverages and desserts in Mexico and
363 Southern US (Stintzing et al., 2005). Therefore, considering their (poly)phenolic content,
364 they may contribute to the total intake of (poly)phenolic compounds with the diet. With
365 respect to old cladodes, their use as a valuable source of bioactive compounds or to produce
366 functional products rich in bioactives should be further explored (Msaddak et al., 2017).

367 From a botanical/evolutionary point of view, the assessment of the (poly)phenolic
368 profile of all the aerial parts of different cultivars of prickly pear represents an important
369 advance in the understanding of *Opuntia* plant biology and defence. Multivariate analysis on
370 prickly pear (poly)phenolic composition accounted for the similarity among cultivars instead
371 of among botanical parts, which may indicate the selective synthesis of phenolic scaffolds in
372 each plant part. Among other ecological roles, this fact could be linked to [plant](#) defence ~~plant~~
373 mechanisms, where (poly)phenolic compounds play a key role as antibacterial agents and
374 reducing the palatability and nutrient digestibility for herbivores (Salminen & Karonen,
375 2011).

376 ~~Despite~~ [Even though](#) this work contributes significantly to the identification of
377 bioactive compounds in alternative plant sources, a couple of analytical constraints should be
378 acknowledged. The first one is related to betalains. Although the most representative *Opuntia*
379 betalains were identified [only](#) in the ~~only~~-red coloured cultivar (Cejudo-Bastante et al., 2013),
380 they were not quantified because of the low purity of the ~~standard~~-commercially available
381 [standard](#) (circa 40%, as stated by the provider). Secondly, an accurate quantification of all the
382 phenolic compounds was not possible due to the unavailability of all their respective
383 reference standards. This led to the semi-quantification of most of the phenolics, which,
384 however, did not impair the conclusions drawn from this study.

385

386 **5. Conclusions**

387 In summary, this analytical work allowed the characterization of the phytochemical
388 profiles of four botanical parts from six different prickly pear cultivars. Up to 41
389 compounds, mainly (poly)phenolics, were identified, with 23 of them reported in
390 *Opuntia ficus-indica* for the first time. Moreover, some insights on plant biology with respect
391 to phenolic distribution were provided. This information may also be used as starting point
392 for the development of prickly pear-derived products with high levels of (poly)phenolic
393 compounds. Lastly, this analytical approach could also be used in other plant products,
394 supposedly rich in phytochemicals.

395

396 **SUPPLEMENTAL-SUPPLEMENTARY MATERIAL**

397 **Supplemental-Supplementary Table S1.** References used for the identification of the
398 phytochemicals described in different *Opuntia ficus-indica* botanical parts (reported in Table
399 1), the compounds used for their quantification, and the occurrence of each compound by
400 botanical part regardless of the cultivar.

401 **Supplementary Figure S1.** Representative chromatograms for each botanical part of cultivar
402 “NT”, extracted as base peak chromatogram.

403

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515

516 **FIGURE CAPTIONS**

517 **Figure 1.** Total (poly)phenolic content of the different aerial parts of prickly pear for
518 different cultivars, [obtained as the sum of individual phenolics](#). Letters above bars denote
519 significant differences at $p < 0.05$.

520 **Figure 2.** Principal component analysis of different prickly pear aerial parts for six different
521 Spanish cultivars. A) loading plot of PC1 versus PC2; B) score plot and distribution of the
522 samples in the consensus space. In the loading plot, C# indicates the compound code, as
523 reported in Table 1. [Non-quantified compounds \(1, 2, 4, 5, 8, 11, 13, 14, 19, 35, and 34-41\)](#)
524 [were excluded from the analysis](#). In the score plot, dark green circles correspond to old
525 cladodes, light green ones to young cladodes, red to fruit skin, and orange to fruit pulp.
526 “Fresa” cultivar has been abbreviated as “FR”, while “Nalle” as “NL”.

Table 1. Retention time (RT) and characteristic MS ions of phytochemical compounds identified in different *Opuntia ficus-indica* cultivars and botanical parts.

Id. Compounds	RT (min)	[M-H]⁻ (m/z)	MS² (m/z)^b	MS³ (m/z)^b
1 L-Malic acid	1.32	133^a	115 (100), 87 (10)	71 (100), 115 (20)
2 Citric acid	1.51	191	111 (100), 173 (40)	111 (100), 67 (25)
3 Protocatechuic acid-hexoside ^c	1.92	315	153 (100)	109 (100)
4 Caffeic acid-hexoside ^c	2.69	341	179 (100), 161 (20), 135 (5)	135 (100)
5 Guaiacyl(8- <i>O</i> -4)ferulic acid ^c	2.80	389	343 (100)	139 (100), 283 (50), 223 (45)
6 Myricetin-hexoside ^c	3.97	479	317 (100)	179 (100), 151 (45)
7 Ferulic acid derivative	4.10	517	193 (100), 337 (60), 175 (50)	149 (100), 134 (55), 178 (40)
8 Piscidic acid	4.18	255	165 (100), 193 (30), 221 (20)	135 (100), 107 (60), 147 (40)
9 Ferulic acid-hexoside	4.26	355	193 (100), 217 (30), 175 (20)	134 (100), 149 (90), 178 (40)
10 Guaiacyl(t8- <i>O</i> -4)guaiacyl-hexoside ^c	4.38	537	375 (100)	327 (100), 195 (50), 179 (20)
11 Salicylic acid-hexoside ^c	4.42	299	137 (100)	93 (100), 137 (50)
12 Sinapic acid-hexoside ^c	4.47	385	223 (100)	179 (100), 153 (75), 205 (70), 161 (30)
13 Quercetin-malonyl-hexoside ^c	4.51	549	505 (100), 356 (40), 461 (20)	356 (100), 461 (20)
14 Ferulic acid- <i>C</i> -hexoside ^c	4.78	355	265 (100), 235 (90), 295 (70), 193 (50)	193 (100), 149 (10)
15 Quercetin-rhamnose-hexoside-rhamnose ^c	4.84	755	300 (100), 591 (60), 489 (40)	271 (100), 255 (40), 179 (20), 151 (15)
16 Rutin-pentoside ^c	4.90	741	300 (100), 591 (80), 609 (50), 475 (45)	271 (100), 255 (60), 179 (25), 151 (20)
17 Syringyl(t8- <i>O</i> -4)guaiacyl ^c	5.03	613	405 (100), 567 (20)	357 (100), 195 (70), 209 (60)
18 Kaempferol-di-rhamnose-hexoside ^c	5.18	739	575 (100), 285(60), 393 (20)	339 (100)
19 Dihydroxynapic acid-hexoside isomer ^c	5.20	387	225 (100)	151 (100)
20 Isorhamnetin- rhamnose-rutinoside	5.25	769	315 (100), 605 (80)	300 (100)
21 Quercetin-hexoside-pentoside ^c	5.30	595	300 (100), 445 (20), 475 (15)	271 (100), 255 (70), 179 (30), 151 (20)
22 Isorhamnetin derivative	5.35	755	315 (100), 605 (90), 300 (35), 623 (25)	300 (100)
23 Dihydroxynapic acid hexoside ^c	5.68	387	255 (100)	
24 Quercetin-3- <i>O</i> -rutinoside (rutin)	5.70	609	301 (100)	179 (100), 151 (60)
25 Secoisolariciresinol-hexoside ^c	5.71	523	388 (100), 243 (15)	361 (100)
26 Isorhamnetin derivative	5.75	609	315 (100), 459 (20), 300 (15)	300 (100)
27 Quercetin-hexoside	5.80	463	301 (100)	179 (100), 151 (60), 257 (20)
28 Kaempferol-rutinoside	5.98	593	285 (100)	257 (100), 267 (80), 229 (59), 241 (50)
29 Syringaresinol ^c	6.00	417	181 (100), 402 (40), 166 (35)	166 (100)
30 Naringenin-hexoside ^c	6.02	433	415 (100)	271 (100)
31 Isorhamnetin-rutinoside	6.09	623	315 (100), 300 (20)	300 (100)
32 Isorhamnetin- <i>C</i> -hexoside	6.31	477	314 (100), 315 (70), 357 (20), 449 (10)	300 (100), 285 (80), 271 (50)
33 Naringin ^c	6.33	579	459 (100), 271 (30)	357 (100), 235 (80), 271 (75), 441 (60)
34 Guaiacyl(8- <i>O</i> -4)syringyl(8-8)guaiacyl-hexoside ^c	6.38	745	583 (100)	535 (100), 369 (50), 357 (30)
35 Eucomic acid	7.09	239	179 (100), 149 (80), 221 (20)	107 (100), 151 (20)
36 Feruloyl derivative	7.15	562	337 (100), 386 (80)	193 (100), 175 (90)
37 Isorhamnetin pentoside ^c	7.47	447	315 (100)	161 (100)
38 Trihydroxy-methoxy-flavonol ^c	8.55	315	300 (100)	271 (100), 255 (50)
Id. Compounds	RT (min)	[M]⁺ (m/z)	MS² (m/z)	MS³ (m/z)
39 Betanin	8.22	551	389 (100)	345 (100), 150 (50), 194 (40)
40 Proline-betaxanthin	8.37	309	265 (100), 263 (90)	221 (100), 152 (40)
41 Isobetainin	8.66	551	389 (100)	345 (100), 150 (50), 194 (40)

^a MS ions in bold were those subjected to further MS fragmentation. ^b Abundance relative of each fragment ions is reported in brackets. Compounds 1-38 were identified in negative ionization mode, while compounds 39-41 were detected in positive mode. RT, retention time. ^c Compounds (tentatively) identified for the first time in *Opuntia ficus-indica*.

Table 2. Concentration (mg/g dw) of (poly)phenolic compounds in young cladodes of six cultivars of *Opuntia ficus-indica*.

Id.	Compounds	FRESA	NA	NALLE	NE	NO	NT
3	Protocatechuic acid-hexoside	0.09 ± 0.03 a	0.03 ± 0.01 b	0.05 ± 0.02 ab	0.02 ± 0.00 b	0.07 ± 0.01 ab	0.03 ± 0.00 b
6	Myricetin-hexoside	4.27 ± 0.43 a	2.66 ± 0.33 b	4.71 ± 0.26 a	0.03 ± 0.00 c	3.38 ± 0.23 b	3.21 ± 0.18 b
7	Ferulic acid derivative	0.36 ± 0.04 ab	0.36 ± 0.03 ab	0.37 ± 0.01 a	0.13 ± 0.03 c	0.27 ± 0.03 b	0.29 ± 0.02 ab
9	Ferulic acid-hexoside	0.86 ± 0.10 ab	1.19 ± 0.13 a	0.65 ± 0.06 bc	0.31 ± 0.16 c	0.81 ± 0.10 b	0.96 ± 0.11 ab
12	Sinapic acid-hexoside	0.17 ± 0.01 b	0.06 ± 0.02 cd	0.02 ± 0.01 d	0.47 ± 0.03 a	0.02 ± 0.02 d	0.10 ± 0.02 c
15	Quercetin-rhamnose-hexoside-rhamnose	0.15 ± 0.01 a	0.09 ± 0.01 b	0.04 ± 0.00 c	0.05 ± 0.01 c	0.04 ± 0.01 c	0.08 ± 0.01 b
16	Rutin-pentoside	0.10 ± 0.02 a	0.06 ± 0.03 ab	0.03 ± 0.01 b	0.08 ± 0.02 ab	0.04 ± 0.01 b	0.09 ± 0.00 ab
17	Syringyl(t8-O-4)guaiacyl	0.15 ± 0.02 a	0.06 ± 0.01 cd	0.10 ± 0.03 bc	0.03 ± 0.00 d	0.12 ± 0.02 ab	0.03 ± 0.01 d
18	Kaempferol-di-rhamnose-hexoside	0.47 ± 0.13 ab	0.34 ± 0.02 ab	0.49 ± 0.08 ab	0.08 ± 0.02 c	0.53 ± 0.07 a	0.31 ± 0.05 b
20	Isorhamnetin-rhamnose-rutinoside	0.82 ± 0.06 a	0.58 ± 0.07 b	0.20 ± 0.02 c	0.58 ± 0.10 b	0.29 ± 0.06 c	1.00 ± 0.12 a
21	Quercetin-hexoside-pentoside	0.12 ± 0.02 a	0.06 ± 0.01 b	0.03 ± 0.01 b	0.05 ± 0.00 b	0.03 ± 0.01 b	0.04 ± 0.00 b
22	Isorhamnetin derivative	0.62 ± 0.04 ab	0.39 ± 0.07 bc	0.20 ± 0.02 c	0.75 ± 0.19 a	0.29 ± 0.06 c	0.84 ± 0.08 a
23	Dihydrosinapic acid hexoside	0.11 ± 0.01 b	0.06 ± 0.01 c	0.04 ± 0.00 cd	0.16 ± 0.01 a	0.02 ± 0.00 d	0.07 ± 0.01 c
24	Quercetin-3-O-rutinoside (rutin)	1.80 ± 0.29 a	0.61 ± 0.23 b	0.41 ± 0.09 b	0.21 ± 0.04 b	0.46 ± 0.06 b	0.40 ± 0.03 b
25	Secoisolariciresinol-hexoside	-	-	0.02 ± 0.00 a	-	-	0.01 ± 0.00 b
26	Isorhamnetin derivative	0.43 ± 0.06 b	0.31 ± 0.04 bc	0.17 ± 0.03 c	0.62 ± 0.01 a	0.23 ± 0.02 c	0.64 ± 0.09 a
27	Quercetin-hexoside	1.02 ± 0.62 a	0.57 ± 0.15 ab	0.28 ± 0.06 ab	0.06 ± 0.01 b	0.29 ± 0.03 ab	0.23 ± 0.03 b
28	Kaempferol-rutinoside	0.77 ± 0.07 a	0.23 ± 0.03 c	0.46 ± 0.01 b	0.22 ± 0.03 c	0.41 ± 0.03 b	0.43 ± 0.00 b
29	Syringaresinol	0.17 ± 0.02 a	0.03 ± 0.01 b	0.05 ± 0.01 b	0.03 ± 0.01 b	0.04 ± 0.00 b	0.04 ± 0.01 b
30	Naringenin-hexoside	0.05 ± 0.01 a	0.05 ± 0.01 ab	0.03 ± 0.01 c	0.05 ± 0.01 ab	0.03 ± 0.01 bc	0.03 ± 0.00 bc
31	Isorhamnetin-rutinoside	0.94 ± 0.05 b	0.56 ± 0.11 c	0.31 ± 0.01 d	1.22 ± 0.10 a	0.40 ± 0.08 cd	0.93 ± 0.08 b
32	Isorhamnetin-C-hexoside	0.61 ± 0.08 a	0.46 ± 0.06 b	0.24 ± 0.03 c	0.07 ± 0.01 d	0.25 ± 0.06 c	0.19 ± 0.02 cd
33	Naringin	0.04 ± 0.01 ab	0.03 ± 0.01 b	0.05 ± 0.00 a	0.03 ± 0.00 b	0.01 ± 0.00 c	0.03 ± 0.01 ab
34	Guaiacyl(8-O-4)syringyl(8-8)guaiacyl-hexoside	0.05 ± 0.01 a	0.03 ± 0.00 ab	0.03 ± 0.00 ab	0.02 ± 0.00 b	0.02 ± 0.01 ab	0.01 ± 0.00 b
37	Isorhamnetin pentoside	0.08 ± 0.01 a	-	-	-	-	0.05 ± 0.00 b
38	Trihydroxy-methoxy-flavonol	0.05 ± 0.01 a	0.02 ± 0.00 bcd	0.02 ± 0.01 bc	0.01 ± 0.00 cd	0.01 ± 0.00 d	0.03 ± 0.01 ab

Values are presented as means ± SD (n=3). Different letters within a row indicate significant differences at $p < 0.05$ according to Tukey's test.

Table 3. Concentration (mg/g dw) of (poly)phenolic compounds in old cladodes of six cultivars of *Opuntia ficus-indica*.

Id.	Compounds	FRESA	NA	NALLE	NE	NO	NT
3	Protocatechuic acid-hexoside	0.02 ± 0.01 b	0.02 ± 0.00 b	0.06 ± 0.01 a	0.03 ± 0.01 b	0.03 ± 0.00 b	0.01 ± 0.02 b
6	Myricetin-hexoside	0.76 ± 0.13 b	0.03 ± 0.00 d	0.61 ± 0.12 bc	2.43 ± 0.06 a	0.39 ± 0.09 c	0.79 ± 0.10 b
7	Ferulic acid derivative	0.33 ± 0.01 a	0.20 ± 0.09 b	0.37 ± 0.04 a	0.33 ± 0.03 a	0.28 ± 0.04 b	0.37 ± 0.02 a
9	Ferulic acid-hexoside	1.82 ± 0.16 a	1.13 ± 0.21 bc	1.27 ± 0.16 b	0.81 ± 0.12 cd	0.41 ± 0.05 e	0.50 ± 0.04 de
12	Sinapic acid-hexoside	0.30 ± 0.05 ab	0.32 ± 0.07 a	0.11 ± 0.02 cd	0.06 ± 0.01 d	0.30 ± 0.04 ab	0.19 ± 0.02 bc
15	Quercetin-rhamnose-hexoside	0.07 ± 0.01 ab	0.04 ± 0.01 bc	0.02 ± 0.01 c	0.07 ± 0.01 a	0.03 ± 0.01 c	0.07 ± 0.01 ab
16	Rutin-pentoside	0.07 ± 0.01 a	0.03 ± 0.01 b	0.02 ± 0.01 b	0.06 ± 0.01 a	0.03 ± 0.00 b	0.08 ± 0.01 a
17	Syringyl(8-O-4)guaiacyl	0.03 ± 0.00 b	0.02 ± 0.01 b	0.02 ± 0.00 b	0.21 ± 0.06 a	0.02 ± 0.00 b	0.02 ± 0.00 b
18	Kaempferol-di-rhamnose-hexoside	0.10 ± 0.02 bc	0.05 ± 0.01 bc	0.04 ± 0.02 c	0.38 ± 0.08 a	0.15 ± 0.02 b	0.10 ± 0.01 bc
20	Isorhamnetin-rhamnose-rutinoside	1.08 ± 0.18 a	0.54 ± 0.10 b	0.38 ± 0.07 b	0.35 ± 0.04 b	0.48 ± 0.06 b	1.16 ± 0.10 a
21	Quercetin-hexoside-pentoside	0.04 ± 0.01 bc	0.03 ± 0.00 bc	0.02 ± 0.01 c	0.06 ± 0.00 a	0.02 ± 0.00 c	0.04 ± 0.00 ab
22	Isorhamnetin derivative	0.76 ± 0.12 b	0.43 ± 0.07 c	0.40 ± 0.26 c	0.38 ± 0.08 a	0.40 ± 0.09 c	0.89 ± 0.06 bc
23	Dihydrosinapic acid hexoside	0.16 ± 0.03 bc	0.11 ± 0.01 b	0.28 ± 0.07 a	-	0.21 ± 0.04 ab	0.11 ± 0.01 b
24	Quercetin-3-O-rutinoside (rutin)	0.34 ± 0.05 b	0.09 ± 0.02 c	0.05 ± 0.05 c	1.66 ± 0.16 a	0.10 ± 0.01 c	0.15 ± 0.00 bc
25	Secoisolariciresinol-hexoside	-	-	0.01 ± 0.00 a	-	0.01 ± 0.00 a	0.01 ± 0.00 a
26	Isorhamnetin derivative	0.62 ± 0.07 b	0.42 ± 0.02 b	0.30 ± 0.04 b	1.88 ± 0.45 a	0.29 ± 0.04 b	0.74 ± 0.09 b
27	Quercetin-hexoside	0.22 ± 0.04 b	0.04 ± 0.02 b	0.01 ± 0.00 b	1.61 ± 0.29 a	0.04 ± 0.00 b	0.05 ± 0.00 b
28	Kaempferol-rutinoside	0.15 ± 0.04 bc	0.05 ± 0.01 c	0.07 ± 0.01 c	0.75 ± 0.08 a	0.20 ± 0.02 b	0.23 ± 0.01 b
29	Syringaresinol	0.06 ± 0.02 a	-	0.04 ± 0.01 ab	-	0.03 ± 0.01 b	-
30	Naringenin-hexoside	0.06 ± 0.02 a	0.02 ± 0.03 abc	-	0.01 ± 0.00 c	0.01 ± 0.00 bc	0.04 ± 0.01 ab
31	Isorhamnetin-rutinoside	1.19 ± 0.13 a	0.66 ± 0.08 c	0.16 ± 0.03 c	0.73 ± 0.06 b	0.72 ± 0.13 b	1.27 ± 0.12 a
32	Isorhamnetin-C-hexoside	0.09 ± 0.03 b	0.03 ± 0.01 bc	0.01 ± 0.00 c	0.50 ± 0.07 a	0.01 ± 0.00 bc	0.08 ± 0.02 bc
33	Naringin	0.03 ± 0.01 a	0.03 ± 0.00 ab	0.02 ± 0.01 ab	0.01 ± 0.00 ab	0.02 ± 0.00 ab	0.03 ± 0.00 a
34	Guaiacyl(8-O-4)syringyl(8-8)guaiacyl-hexoside	0.03 ± 0.00 a	0.02 ± 0.01 a	0.04 ± 0.02 a	0.03 ± 0.01 a	0.03 ± 0.01 a	0.02 ± 0.00 a
38	Trihydroxy-methoxy-flavonol	0.02 ± 0.00 a	0.01 ± 0.00 a	0.02 ± 0.02 a	0.03 ± 0.01 a	0.02 ± 0.01 a	0.03 ± 0.01 a

Values are presented as means ± SD (n=3). Different letters within a row indicate significant differences at $p < 0.05$ according to Tukey's test.

Table 4. Concentration (mg/g dw) of (poly)phenolic compounds in fruit skin of six cultivars of *Opuntia ficus-indica*.

Id.	Compounds	FRESA	NA	NALLE	NE	NO	NT
3	Protocatechuic acid-hexoside	0.01 ± 0.00 b	0.03 ± 0.01 ab	0.08 ± 0.04 a	0.02 ± 0.00 ab	0.07 ± 0.02 a	0.02 ± 0.001 ab
6	Myricetin-hexoside	0.02 ± 0.00 c	0.01 ± 0.00 c	0.03 ± 0.01 c	0.01 ± 0.00 c	0.08 ± 0.02 b	0.56 ± 0.04 a
7	Ferulic acid derivative	0.23 ± 0.06 b	0.15 ± 0.03 b	0.37 ± 0.07 a	0.23 ± 0.02 b	0.23 ± 0.03 b	0.39 ± 0.02 a
9	Ferulic acid-hexoside	1.55 ± 0.22 ab	1.03 ± 0.15 bc	1.03 ± 0.32 bc	0.82 ± 0.20 c	1.16 ± 0.15 bc	1.81 ± 0.28 a
10	Guaiacyl(8-O-4)guaiacyl-hexoside	-	-	-	-	-	0.02 ± 0.00 a
12	Sinapic acid-hexoside	0.47 ± 0.08 b	0.62 ± 0.13 b	1.72 ± 0.41 a	0.81 ± 0.11 b	0.64 ± 0.08 b	0.47 ± 0.09 b
15	Quercetin-rhamnose-hexoside-rhamnose	0.03 ± 0.01 ab	0.02 ± 0.00 ab	0.01 ± 0.00 b	0.02 ± 0.00 ab	0.01 ± 0.00 b	0.03 ± 0.01 a
16	Rutin-pentoside	0.04 ± 0.02 abc	0.02 ± 0.01 c	0.03 ± 0.00 bc	0.06 ± 0.01 a	0.02 ± 0.00 c	0.05 ± 0.01 ab
17	Syringyl(t8-O-4)guaiacyl	0.03 ± 0.01 a	0.01 ± 0.00 bc	0.03 ± 0.00 ab	0.03 ± 0.00 a	-	0.03 ± 0.00 a
18	Kaempferol-di-rhamnose-hexoside	0.01 ± 0.00 ab	0.01 ± 0.00 b	0.02 ± 0.00 a	0.02 ± 0.00 ab	0.03 ± 0.00 a	0.02 ± 0.00 ab
20	Isorhamnetin-rhamnose-rutinoside	0.45 ± 0.08 ab	0.28 ± 0.05 bc	0.26 ± 0.04 bc	0.34 ± 0.03 bc	0.23 ± 0.01 c	0.61 ± 0.15 a
21	Quercetin-hexoside-pentoside	0.02 ± 0.01 b	0.02 ± 0.00 ab	0.02 ± 0.00 b	0.04 ± 0.01 a	0.01 ± 0.00 b	0.02 ± 0.01 b
22	Isorhamnetin derivative	0.42 ± 0.07 bc	0.31 ± 0.08 b	0.44 ± 0.03 bc	0.72 ± 0.07 a	0.38 ± 0.02 b	0.65 ± 0.15 ab
23	Dihydroxysinapic acid hexoside	0.35 ± 0.08 c	0.55 ± 0.09 cd	1.16 ± 0.16 a	0.93 ± 0.11 ab	0.66 ± 0.08 bc	0.54 ± 0.13 cd
24	Quercetin-3-O-rutinoside (rutin)	0.10 ± 0.01 bc	0.10 ± 0.02 bc	0.06 ± 0.01 c	0.16 ± 0.04 ab	0.08 ± 0.01 c	0.18 ± 0.03 a
25	Secoisolariciresinol-hexoside	-	0.03 ± 0.00 bc	0.13 ± 0.04 a	0.02 ± 0.00 c	0.08 ± 0.01 b	-
26	Isorhamnetin derivative	0.30 ± 0.06 abc	0.27 ± 0.05 bc	0.33 ± 0.04 abc	0.49 ± 0.09 a	0.21 ± 0.03 c	0.44 ± 0.11 ab
27	Quercetin-hexoside	0.07 ± 0.02 a	0.04 ± 0.01 bc	0.02 ± 0.00 c	0.06 ± 0.00 ab	0.02 ± 0.01 c	0.08 ± 0.01 a
28	Kaempferol-rutinoside	0.04 ± 0.01 bc	0.02 ± 0.00 c	0.06 ± 0.01 ab	0.05 ± 0.00 b	0.07 ± 0.01 a	0.06 ± 0.01 ab
29	Syringaresinol	0.20 ± 0.03 a	0.11 ± 0.02 b	0.13 ± 0.04 b	0.13 ± 0.00 b	0.12 ± 0.01 b	0.24 ± 0.02 a
30	Naringenin-hexoside	0.06 ± 0.02 ab	0.02 ± 0.01 b	0.18 ± 0.05 a	0.12 ± 0.01 ab	0.07 ± 0.01 ab	0.07 ± 0.01 ab
31	Isorhamnetin-rutinoside	0.53 ± 0.12 b	0.53 ± 0.10 b	0.61 ± 0.04 ab	0.85 ± 0.19 a	0.58 ± 0.03 ab	0.75 ± 0.11 ab
32	Isorhamnetin-C-hexoside	0.03 ± 0.01 a	0.01 ± 0.00 b	-	0.04 ± 0.01 a	0.02 ± 0.00 b	0.01 ± 0.00 bc
33	Naringin	0.02 ± 0.00 c	0.03 ± 0.01 abc	0.04 ± 0.01 a	0.03 ± 0.00 abc	0.01 ± 0.00 bc	0.03 ± 0.00 ab
34	Guaiacyl(8-O-4)syringyl(8-8)guaiacyl-hexoside	0.01 ± 0.00	0.03 ± 0.02 a	0.01 ± 0.00 b	0.02 ± 0.01 ab	0.03 ± 0.00 a	0.01 ± 0.01 b
38	Trihydroxy-methoxy-flavonol	0.05 ± 0.01 b	0.06 ± 0.01 b	0.11 ± 0.02 a	0.11 ± 0.01 a	0.05 ± 0.02 b	0.05 ± 0.01 b

Values are presented as means ± SD (n=3). Different letters within a row indicate significant differences at $p < 0.05$ according to Tukey's test.

Table 5. Concentration (mg/g dw) of (poly)phenolic compounds in fruit pulp of six cultivars of *Opuntia ficus-indica*.

Id.	Compounds	FRESA	NA	NALLE	NE	NO	NT
3	Protocatechuic acid-hexoside	0.02 ± 0.00 bc	0.01 ± 0.00 c	0.08 ± 0.02 a	0.02 ± 0.00 bc	0.02 ± 0.01 bc	0.03 ± 0.00 b
6	Myricetin-hexoside	-	-	-	-	-	0.01 ± 0.00 a
7	Ferulic acid derivative	0.08 ± 0.02	-	-	-	-	-
9	Ferulic acid-hexoside	0.14 ± 0.03 a	0.02 ± 0.02 b	0.06 ± 0.00 b	0.02 ± 0.00 b	0.05 ± 0.01 b	0.15 ± 0.03 a
10	Guaiacyl(8-O-4)guaiacyl-hexoside	0.19 ± 0.02 b	0.19 ± 0.01 b	0.10 ± 0.00 d	0.18 ± 0.02 bc	0.14 ± 0.03 cd	0.33 ± 0.02 a
12	Sinapic acid-hexoside	0.10 ± 0.01 b	0.21 ± 0.05 b	1.71 ± 0.36 a	0.06 ± 0.01 b	0.06 ± 0.01 b	0.10 ± 0.02 b
17	Syringyl(8-O-4)guaiacyl	0.13 ± 0.04 ab	0.12 ± 0.01 b	0.08 ± 0.01 c	0.07 ± 0.02 c	0.06 ± 0.01 c	0.17 ± 0.01 a
20	Isorhamnetin-rhamnose-rutinoside	0.01 ± 0.00 a	-	-	-	-	0.01 ± 0.00 a
21	Quercetin-hexoside-pentoside	0.01 ± 0.00 a	-	-	0.01 ± 0.00 a	-	-
22	Isorhamnetin derivative	-	-	0.01 ± 0.00 a	-	-	0.01 ± 0.00 b
23	Dihydrosinapic acid hexoside	-	-	2.39 ± 0.28 a	-	0.12 ± 0.01 b	-
25	Secoisolariciresinol-hexoside	-	-	0.10 ± 0.02	-	-	-
26	Isorhamnetin derivative	0.02 ± 0.00 a	0.01 ± 0.00 a	0.02 ± 0.00 a	0.02 ± 0.00	0.01 ± 0.00 a	0.02 ± 0.00 a
27	Quercetin-hexoside	0.01 ± 0.01 a	-	-	0.01 ± 0.00 a	-	-
29	Syringaresinol	0.07 ± 0.01 b	0.02 ± 0.00 cd	0.13 ± 0.03 a	0.02 ± 0.01 d	0.06 ± 0.01 bc	0.06 ± 0.01 bcd
30	Naringenin-hexoside	-	-	0.21 ± 0.04	-	-	-
31	Isorhamnetin-rutinoside	0.02 ± 0.00 a	0.01 ± 0.00 a	0.01 ± 0.01 a	0.01 ± 0.00 a	0.01 ± 0.00 a	0.01 ± 0.00 a
33	Naringin	0.04 ± 0.01 ab	0.03 ± 0.00 bc	0.05 ± 0.01 a	0.01 ± 0.00 c	0.02 ± 0.00 bc	0.03 ± 0.00 ab
34	Guaiacyl(8-O-4)syringyl(8-8)guaiacyl-hexoside	0.16 ± 0.03 a	0.12 ± 0.01 ab	0.05 ± 0.01 b	0.13 ± 0.03 ab	0.04 ± 0.01 b	0.08 ± 0.08 ab
36	Feruloyl derivative	0.96 ± 0.07 a	0.7 ± 0.14 b	0.08 ± 0.01 c	0.28 ± 0.03 c	0.11 ± 0.01 c	1.06 ± 0.19 a
38	Trihydroxy-methoxy-flavonol	-	-	0.01 ± 0.00	-	-	-

Values are presented as means ± SD (n=3). Different letters within a row indicate significant differences at $p < 0.05$ according to Tukey's test.