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*Original*
Phytochemical characterization of different prickly pear (Opuntia ficus-indica (L.) Mill.) cultivars and botanical parts: UHPLC-ESI-MSnmetabolomics profiles and their chemometric analysis / Mena, P., Tassotti, M., Andreu, L., Nuncio-Jáuregui, N., Legua, P., Del Rio, D., Hernández, F.. - In: FOOD RESEARCH INTERNATIONAL. - ISSN 0963-9969. - 108:(2018), pp. 301-308. [10.1016/j.foodres.2018.03.062]

*Availability:*
This version is available at: 11381/2842338 since: 2021-10-13T10:25:22Z

*Publisher:*
Elsevier Ltd

*Published*
DOI:10.1016/j.foodres.2018.03.062

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07 October 2026

# Phytochemical characterization of different prickly pear (*Opuntia ficus-indica* (L.) Mill.) cultivars and botanical parts: UHPLC-ESI-MS$^n$ metabolomics profiles and their chemometric analysis

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## ABSTRACT

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



## KEYWORDS

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

## 1. Introduction

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

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

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

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

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

## 2. Materials and methods

### *2.1. Chemicals*

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

### *2.2. Plant material*

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

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

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

*2.3. Extraction of (poly)phenolic compounds*

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

*2.4. Liquid chromatography-mass spectrometry (UHPLC-ESI-$MS^n$) analysis*

Methanolic extracts of prickly pear parts were analysed using an Accela UHPLC 1250 equipped with a linear ion trap-mass spectrometer (MS) (LTQ XL, Thermo Fisher Scientific Inc., San Jose, CA, USA) fitted with a heated-electrospray ionization (ESI) probe (H-ESI-II; Thermo Fisher Scientific Inc., San Jose, CA, USA). Separations were performed using a XSelect HSS T3 (50 x 2.1 mm), 2.5 µm particle size (Waters, Ireland). Volume injected was 5 µL and column oven was set to 30°C. Two complementary MS experiments were performed, one in negative mode, for non-coloured phenolics, and one using positive ionization, for betalains, following an analytical approach previously developed for the comprehensive identification of (poly)phenolic compounds (Mena et al., 2012). Each sample was analysed in duplicate for each experimental condition.

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

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

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

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

*2.5. Statistical analysis*

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

## 3. Results

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

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

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

Two compounds (**24** and **39**) were identified by comparison with their respective analytical standards. Thirty-nine compounds were identified based on their retention time, fragmentation patterns obtained from mass spectra ($MS^2$ and $MS^3$ experiments) (Table 1), and by comparing their mass spectral characteristic with the available literature (see Supplementary Material, Table S1). The interpretation of the mass spectra fragmentation patterns reported in the literature was not discussed unless of special interest. In this sense, compounds **19**, **22**, and **26** were tentatively identified according to their characteristic aglycone fragment ions. Compounds **22** and **26** presented a major $MS^2$ fragment ion at *m/z* 315 and showed $MS^3$ fragments matching those of other isorhamnetin derivatives (compounds **20**, **31**, **32**, and **37**). Compounds **22** and **26** (*m/z* 755 and 609) also had losses of *m/z* 440 and 294, respectively, which might correspond to sambubioside-rhamnoside and sambubioside moieties; however, the full structure could not be identified and, hence, they were classified simply as isorhamnetin derivatives. Compound **19** presented the same fragmentation pattern of compound **23** and was identified as an isomer of dihydrosinapic acid-hexoside. 23 compounds (**3-6, 10-19, 21, 23, 25, 29, 30, 33, 34, 37** and **38**) were tentatively identified for the first time, as far as we know, in *Opuntia ficus-indica.*

Most of the compounds were identified in all the botanical parts analysed, while some compounds were detected only in some of them (~~Supplemental~~ Supplementary Material, Table S1). In the case of betalains, they were only detected in the pulp and skin of the "Fresa" cultivar, the only one presenting an intense red colour.

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

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

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

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

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

*3.3. Chemometric classification*

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

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

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

## 4. Discussion

This work investigated the phytochemical profile of four different botanical parts of six prickly pear cultivars by using two complementary MS experimental conditions. ~~To the best of our knowledge, this is the first time that so many classes of phytochemicals (betalains, flavonols, flavanones, phenolic acids, lignans, and organic acids) are described in *Opuntia ficus-indica*,~~ Although~~despite~~ some accurate works have been ~~found~~ reported in the literature (Guevara-Figueroa et al., 2010; Mata et al., 2016; Moussa-Ayoub et al., 2014; Serra et al., 2013; Yeddes et al., 2014), t~~. T~~his challenging study provide~~d~~s an exhaustive characterization of the phytochemical profile (betalains, flavonols, flavanones, phenolic acids, lignans, and organic acids) of the aerial parts of *Opuntia ficus-indica*. Obviously, the range of molecules present in prickly pear phytochemical pool comprises way more than 41 structures, but these may be considered those contributing to a better extent to the definition of its phytochemical fingerprinting, regardless of genotypic differences. From a methodological point of view, this work also reinforces the need for versatile, high-throughput experimental conditions allowing the identification of several groups of bioactives (Filigenzi et al., 2011; Mena et al., 2012; Mena et al., 2016; Rak, Fodor, & Abrankó, 2010).

While the role of betalains as some of the most interesting phytochemicals in *Opuntia* genera has been widely discussed for pigmented cultivars during the latest years (Cejudo-Bastante, Chaalal, Louaileche, Parrado, & Heredia, 2014; Mata et al., 2016; Stintzing et al., 2005), the (poly)phenolic profile of prickly pear has been scarcely assessed. It is known that the concentration of (poly)phenolic compounds in prickly pear depends on genetic and environmental conditions, as well as the part of the cactus plant taken into consideration (Khatabi, Hanine, Elothmani, & Hasib, 2016; Moussa-Ayoub, et al., 2014; Stintzing, et al., 2005). The study of the (poly)phenolic composition of different parts of *Opuntia ficus-indica* had been previously addressed (Moussa-Ayoub, et al., 2014; Yeddes, et al., 2014). The effect of genotypic differences on the (poly)phenolic profile of prickly pear fruits had also been

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

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

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

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

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

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

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

## 5. Conclusions

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

## ~~SUPPLEMENTAL~~ SUPPLEMENTARY MATERIAL

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

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

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515

## FIGURE CAPTIONS

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

**Figure 2.** Principal component analysis of different prickly pear aerial parts for six different Spanish cultivars. A) loading plot of PC1 versus PC2; B) score plot and distribution of the samples in the consensus space. In the loading plot, C# indicates the compound code, as reported in Table 1. Non-quantified compounds (1, 2, 4, 5, 8, 11, 13, 14, 19, 35, and 34-41) were excluded from the analysis. In the score plot, dark green circles correspond to old cladodes, light green ones to young cladodes, red to fruit skin, and orange to fruit pulp. "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*) | $MS^2$ (*m/z*) [b] | $MS^3$ (*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-*O*-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-*O*-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-*C*-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 | Syrinigyl(t8-*O*-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 | Dihydrosinapic 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 | Dihydrosinapic acid hexoside[c] | 5.68 | **387** | **255** (100) | |
| 24 | Quercetin-3-*O*-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-*C*-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-*O*-4)syrinigyl(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*) | $MS^2$ (*m/z*) | $MS^3$ (*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 | Isobetanin | 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 | Syrinigyl(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)syrinigyl(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 raw 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-rhamnose | 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 | Syrinigyl(t8-*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)syrinigyl(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 raw 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(t8-*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 | Syrinigyl(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 | Dihydrosinapic 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)syrinigyl(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 raw 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(t8-*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 | Syrinigyl(t8-*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)syrinigyl(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 raw indicate significant differences at $p < 0.05$ according to Tukey's test.