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# BIOACTIVE COMPOUNDS AND MORPHOLOGY IN *OPUNTIA* SPP. FRUITS FROM PORTUGUESE ECOTYPES

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## Abstract

Reis, C. M. G., C. Gouveia, M. C. Vitorino, L. C. Gazarini, M. M. Ribeiro and F. Peres, 2017. Bioactive compounds and morphology in *Opuntia* spp. fruits from Portuguese ecotypes. *Bulg. J. Agric. Sci.*, 23 (6): 929–938

The *Opuntia* spp. has minimal soil and water requirements, and the O. *ficus-indica*, in particular is sought to be an alternative for the Mediterranean region agricultural economy. The morphology, bioactive compounds and antioxidant properties of fruits were studied in twenty Portuguese ecotypes belonging to four *Opuntia* species (*O. ficus-indica*, *O. robusta*, *O. dillenii* and *O. elata*). The ecotypes were compared with the *O. ficus-indica* cultivars 'Bianca', 'Gialla' and 'Rossa'. The fruits from *Opuntia* spp. ecotypes displayed variability in morphological and bioactive characteristics. Among *O. ficus-indica* ecotypes, the orange pulp fruits were larger, heavier and had a higher percentage of pulp as well as a lower percentage of seeds compared to the white pulp fruits. However, the weight of 100 seeds was lower in the white pulp ecotypes. The OFI-04 ecotype contrasted the other OFI ecotypes due to its pale yellow pulp, ovoid shape, and low seed weight per fruit as well as the amount of seeds as a percentage of pulp weight. The *O. dillenii* ecotypes had the highest betalain content, total phenolic compounds, and antioxidant activity, while *O. elata* had the highest ascorbic acid content. Both *O. dillenii* and *O. elata* had the highest acidity values. The red pulp cv. Rossa had the highest betalain content among the *O. ficus-indica* populations, followed by the orange and white pulp ecotypes. The highest amount of total phenolic compounds was found in the white pulp *O. ficus-indica* ecotypes. The hierarchical clustering analysis revealed that the ecotypes could be grouped into four major groups, and geographical origin was unrelated to the clustering pattern. This study provides original data on the morphology and bioactive compounds of *Opuntia* spp. fruits from Portuguese ecotypes.

*Key words:* ascorbic acid; betalains; cactus pear; fruit morphology; phenolic compounds

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## Introduction

The genus *Opuntia* (Cactaceae) is native to Central America and was likely introduced in the Iberian Peninsula after the discovery of the Americas between the end of the 15th and beginning of the 16th centuries, later spreading throughout the Mediterranean basin (Barbera et al., 1992; Anderson, 2001). The Mediterranean region, particularly inland areas, is currently suffering from severe drought during extensive summers, and global climate change is expected to deeply affect this area in the near future with climatic models predicting drier climates with shorter and wetter rainy seasons followed by long dry summers (Schröter et al., 2005). *Opuntia* spp. has minimal soil and water requirements, and *Opuntia ficus-indica* (OFI), in particular, is thought to be an alternative species for the agricultural economy of the Mediterranean region (Barbera et al., 1992).

In Portugal, several *Opuntia* species were naturalized (*O. ficus-indica* (L.) Miller, *O. dillenii* (Ker-Gael) Haw., *O. robusta* Wendl and *O. elata* Salm-Dick), and the most widespread and economically relevant sp. is both forms of OFI, including the inermis typical form, *Opuntia ficus-indica* f. *ficus indica*, and the spiny form, *Opuntia ficus-indica* f. *amyclaea* (Ten.) Schell (Kiesling, 1998). The OFI local ecotypes have variable plant vigour and biomass production (Reis et al., 2016) as well as differences in the shape of the cladodes, presence or absence of spines, spine length, corolla colour, pulp colour, and fruit ripening time (our unpublished results). Traditionally, OFI is cultivated for edible fresh fruit production and hedge establishment under non-irrigated conditions, but recently some farmers have been focusing on OFI orchards that are drip irrigated for fresh fruit production along with a plant layout and spacing design. Cactus pear fruit is a fleshy berry derived from an inferior ovary (acrosarcum) that varies in shape, size, and number of hard seeds, with high total soluble solids content (TSS) (12 to 17%), and low acidity content (0.03 to 0.12% citric acid) (Yahia and Mondragon-Jacobo, 2011). The *O. dillenii* fruits are a source of betacyanin pigments that can be used as a red-purple food colorant and an alternative to beetroot red (Cejudo-Bastante et al., 2015). The *O. elata* is cultivated as ornamental plant because the cladodes have few spines, the purple fruits are small, and they can be found growing sub-spontaneously in some places in the centre and north inland regions of Portugal, especially the Douro valley region. Recently, significant attention has been given to the cactus pear due its nutritional and health benefits as well as its high bioactive antioxidant compound content, including betalains, phenolic compounds, ascorbic acid and carotenoids (Sumaya-Martínez et al., 2011; Yahia and Mondragon-Jacobo, 2011; El-Mostafa et al., 2014; Albano et al., 2015). Additionally, health benefits, such as antioxidant, neuroprotective, anti-inflammatory, hypoglycaemic and anticancer effects were reported (Tesoriere et al., 2004; Chavez-Santoscoy et al., 2009; Serra et al., 2013; Jiménez-Aguilar et al., 2014).

The information about bioactive antioxidant compounds in Portuguese ecotypes of OFI, *O. robusta* and *O. dillenii* is scarce, and to the best of our knowledge, there are no studies on the composition of *O elata* fruit. The main objectives of this study were i) to characterize the morphology, bioactive compounds and antioxidant properties of *Opuntia* spp. fruits to determine their nutraceutical potential, ii) to classify different species and ecotypes into distinct groups according to their morphology and fruit chemical characteristics using a multivariate analysis approach, and iii) to understand the overall pattern of genetic diversity and relationships among germplasm accessions.

## Materials and Methods

***Plant material and provenance trial***

Cladodes from OFI, *O. elata* and *O. robusta* were planted in a provenance trial at the Castelo Branco School of Agriculture, Portugal (39°49′17″N; 7°27′41″W, elev. 365 m), in 2012. Sixteen OFI Portuguese ecotypes and two improved Italian cultivars (cv.), Bianca and Gialla, which were included for comparison purposes, were studied. The *O. robusta* and *O. elata* were represented by only one ecotype. The locations of the mother plants from which the cladodes were taken, propagated and used in the experiment are indicated in Table 1. The experimental design was a randomized complete block design with three replicates per ecotype, and each replicate had five plants in a row. The provenance trial was planted in a granitic soil with pH 5.9 and low organic matter content, which is a marginal soil with a reduced overall soil profile depth and low water holding capacity. Fertilizers with nitrogen, phosphorus, and potassium were applied at 40 kg $ha^{-1}$ for each element annually to reduce the possible differences in soil fertility. Irrigation (60 mm) was applied in the second and third year of cultivation. No tilling was used, and the weeds were controlled by mechanical mowing. Pruning was performed in spring to lighten the canopy. In the third year after planting, thinning was carried out after flowering to achieve a maximum of 6 fruits per cladode. Afterwards, three samples of 10 full mature fruits were collected from each replicate for each of the different *Opuntia* species established in the provenance trial (Table 1). Additionally, two ecotypes of *O. dillenii* were studied, and 30 fruits from each ecotype were collected in fifteen plants from local origin (Table 1). Commercially mature fruits from the OFI cv. Rossa were acquired from a local producer. In both cases, the fruits were divided into three samples of 10 fruits each.

**Table 1**
**Identification, fruit shape, pulp colour and origin of the studied *Opuntia* spp. populations**

| Population | Fruit Shape | Pulp colour | Origin | Altitude (m) | Geographic coordinates | |
|---|---|---|---|---|---|---|
| | | | | | Latitude | Longitude |
| OFI-01 | Ell | White | Alcochete | 25 | 38°43′32.14″N | 8°57′58.22″W |
| OFI-03 | Ell | White | Cascais, Guincho | 185 | 38°45′23.18″N | 9°27′38.48″W |
| OFI-04 | Ovd | Pale yellow | Portalegre | 372 | 39°16′22.45″N | 7°26′13.12″W |
| OFI-05 | Ovd | Orange | Arronches | 293 | 39° 5′21.06″N | 7°12′7.05″W |
| OFI-08 | Ell | White | Melides | 29 | 38° 8′28.91″N | 8°44′14.28″W |
| OFI-09 | Ell | White | Santo André | 25 | 38° 4′38.13″N | 8°46′38.08″W |
| OFI-11 | Ell | White | Albufeira | 61 | 37° 5′23.33″N | 8°17′27.03″W |
| OFI-12 | Ovd | Orange | Cacela-a-Velha | 20 | 37° 9′22.50″N | 7°32′47.98″W |
| OFI-13 | Ovd | Orange | Monforte da Beira | 260 | 39°45′8.34″N | 7°16′54.83″W |
| OFI-14 | Ovd | Orange | Idanha-a-Velha | 275 | 39°59′57.30″N | 7° 9′3.51″W |
| OFI-15 | Ell | White | Ponte de Sor | 125 | 39°16′15.45″N | 8° 0′44.72″W |
| OFI-16 | Ell | White | Biscainho, Coruche | 76 | 38°54′40.93″N | 8°37′17.00″W |
| OFI-17 | Ell | White | Castelo Branco | 402 | 39°48′58.84″N | 7°29′37.85″W |
| OFI-18 | Ell | White | Reg. Monsaraz | 223 | 38°27′27.04″N | 7°39′21.77″W |
| OFI-19 | Ell | White | Alvega | 105 | 39°27′15.96″N | 8° 3′51.88″W |
| OFI-20 | Ovd | Orange | Madeira | 116 | 32°38′54.18″N | 16°57′46.38″W |
| OFI, cv. Bianca | Ell | White | Italy | – | – | – |
| OFI, cv. Gialla | Ovd | Orange | Italy | – | – | – |
| OFI, cv. Rossa | Ell | Red | Italy | – | – | – |
| *O. robusta* | Rou | Red | Castelo Branco | 365 | 39°49′17.00″N | 7°27′41.00″W |
| *O. dillenii*, OD-1 | Ell | Purple | Lagos | 48 | 37°8′42.24″N | 8°40′33.42″W |
| *O. dillenii*, OD-2 | Ell | Purple | Cacela-a-Velha | 20 | 37°9′22.50″N | 7°32′47.98″W |
| *O. elata* | Obl | Purple | S. J. Pesqueira | 450 | 41°9′5.83″N | 7°22′5.43″W |

OFI – *Opuntia ficus-indica*; OD – *Opuntia dillenii*. Ell – elliptic; Obl – oblong; Ovd – ovoid; Rou – round

***Morphological characterization***

The following morphological characteristics were evaluated in three replicates of 10 fruits each: weight (g), length (cm), diameter (cm), shape (measured by the ratio diameter/length), receptacle scar diameter and depth (mm), peel thickness (mm), peel weight (g), pulp weight (g) and pulp as fruit weight percentage. The seed weight per fruit (g), seed as pulp weight percentage and 100 seed weight (g) were measured in triplicate from pooled samples of ten fruits.

***Fruit sample preparation and chemical reagents***

The peel was manually separated from the pulp, followed by weighing the pulp and briefly homogenizing it in a kitchen-type blender. Afterwards, the pulp was separated from the seeds, portioned and stored at −80°C until analysis. For *O. elata* fruits, due to the low pulp yield, the whole fruit was used after seed removal. After defrosting, the juice was centrifuged at 14000 rpm for 10 min, and the supernatant was used for pH, acidity, and total soluble solid (TSS, %) determination. The remaining supernatant was filtered through Whatman® filter paper, Grade 42, and the filtrate was used to estimate the total phenolic compound (TPC), the ascorbic acid (AA) and the betalain content (betaxanthins and betacyanins). The antioxidant activity was quantified after an additional filtration through a 45 μm syringe filter. The readings were collected in triplicate for each sample. All reagents were ACS grade and were purchased from Sigma-Aldrich Company.

***TSS, pH, acidity and colour determination***

The TSS concentration (%) was determined in the juice using a digital refractometer (Hanna, HI 96800). Total acidity was determined using a pH metre (Radiometer PHM 61) after titration of 10 mL of seedless pulp-juice against 0.01 N NaOH to the end point (pH 8.2), and the results were expressed as a percentage of citric acid. The chromatic characteristics, which were defined by the colorimetric or chromaticity coordinates, the lightness ($L^*$, ranging from 0, black, to 100, white), the $a^*$ (which takes positive values for reddish colours and negative values for greenish ones), and the $b^*$

(positive for yellowish colours and negative for bluish ones), were determined using a Minolta CR-300 colorimeter.

***Ascorbic acid determination***

The AA content was determined by UV/Vis spectrophotometry as described in previous studies (Dürüst et al., 1997). Briefly, 0.25 mL of the juice (a different dilution factor in acid oxalic at 0.4% was used from each sample) was added into 0.25 mL of acetate buffer, and 2.0 mL of DCPI (2,6-dichloroindophenol sodium) was added. The absorbance of the mixture was measured immediately at 520 nm using a Biochrom Libra S21 single beam spectrophotometer. Ascorbic acid was used as a reference standard, and the results were expressed as mg ascorbic acid $kg^{-1}$ fresh weight (FW).

***Betalain determination***

The aqueous pigment extracts were diluted in water to obtain absorption values of $0.9 < A < 1.0$ at their respective maximum absorption. The betalain content (BC) was calculated by spectrophotometry as described in previous studies (Stintzing et al., 2005): BC (mg $L^{-1}$) = ($A$ x $DF$ x MW x 1000)/(ε x $1$), where $A$ is the absorption value at the absorption maximum, DF is the dilution factor and $1$ is the path length (1 cm) of the cuvette. For the quantification of betacyanins and betaxanthins, the molecular weights (MW) and the molar extinction coefficients (ε) of betanin (MW = 550 g $mol^{-1}$; ε = 60 000 L $mol^{-1}$ $cm^{-1}$ in $H_2O$; λ = 538 nm) and indicaxanthin (MW = 308 g $mol^{-1}$; ε = 48 000 L $mol^{-1}$ $cm^{-1}$ in $H_2O$; λ = 480 nm) were applied, respectively. The determination was performed using a Biochrom Libra S21 single beam spectrophotometer.

***Total phenolic compounds***

The TPC was determined using the Folin-Ciocalteau VIS spectrophotometric method (Singleton et al., 1999), and the absorbance measurement was performed in a Jasco 7800 spectrophotometer. Gallic acid was used as a standard to produce the calibration curve, and TPC were estimated using three average readings and expressed in mg of Gallic acid equivalents (GAE), mg $kg^{-1}$ FW.

***DPPH radical scavenging activity assay***

The antioxidant capacity of the filtered juices was tested using the DPPH (1,1-diphenyl-2-picrylhyydrazyl) approach (Yen and Duh, 1994). The juice was centrifuged and filtered through a 45 µm syringe filter. A methanol DPPH solution (0.06 mM) was mixed with the juice at different concentrations and then solutions with different concentrations were made. The mixtures were vortexed, incubated for 30 min in the dark and placed in a UV/Vis spectrophotometer (Jasco 7800) where the absorbance was read at 517 nm. The inhibition of free radical DPPH ($I$ %) was calculated as $I\% = [(A_0 - A_1)/A_0] \times 100$, where $A_0$ and $A_1$ are the absorbance values of the blank (all reagents except the test compounds) and the tested samples, respectively. The $I$% was plotted against the respective concentrations that were used. The linear equation of each graph was used to calculate $IC_{50,}$ which is the antioxidant concentration required to inhibit the DPPH absorbance by half.

***Data analysis***

The data was analysed using one-way ANOVA or, in absence of variance homogeneity, Welch ANOVA was performed, followed by pairwise comparisons using the Tukey or the Games-Howell post-hoc tests, respectively. Statistical significance was accepted with 5% as the probability of type I error for both the omnibus test and the multiple comparisons test. A principal component analysis (PCA) was conducted using morphological and chemical data, and a hierarchical cluster analysis was performed with standardized data ($Z$ scores) using the Euclidean distances following the between-groups (average) linkage method. The decision about the number of clusters to retain was made by plotting the number of clusters on the x-axis against the distance at which objects or clusters were combined on the y-axis (Sarstedt and Moi, 2014). The statistical analyses were performed using IBM SPSS Statistics software v.21 (IBM Corp., NY.).

## Results and Discussion

***Fruit morphological characterization***

The shapes of the fruits were oblong (*O. elata*), elliptic (*O. dillenii* and in the white pulp fruits from OFI), ovoid (in orange pulp fruits from OFI) and round (*O. robusta*) (Figure 1 and Table 1).

The fruit weight varied between 19.5 (*O. elata*) and 132.5 g (OFI-13) (Figure 2A). In *O. dillenii* (the mean value of the two populations) and *O. robusta*, the mean weights of the fruit were 50.1 and 124.9 g, respectively. The mean weights of the fruits were 90.6 g in the OFI populations with white pulp fruits and 121.4 g in the populations with orange pulp fruits, and significant differences were found, Welch's $F$ (18, 203.9) = 165.2, $p < 0.05$. The diameter ($R^2$ = 0.91) and the pulp weight ($R^2$ = 0.96) had the highest correlation coefficients with the weight of the fruit. The pulp yield varied between 22.6 (*O. elata*) and 67% (OFI cv. Rossa) (Figure 2A). The OFI mean pulp yield was 53.8 and 64.30% for the white and orange pulp fruits, respectively, Welch's $F$ (18, 203.9) = 136.5, $p < 0.05$. *O. elata* had the smallest fruits, while in the OFI ecotypes the lengths varied

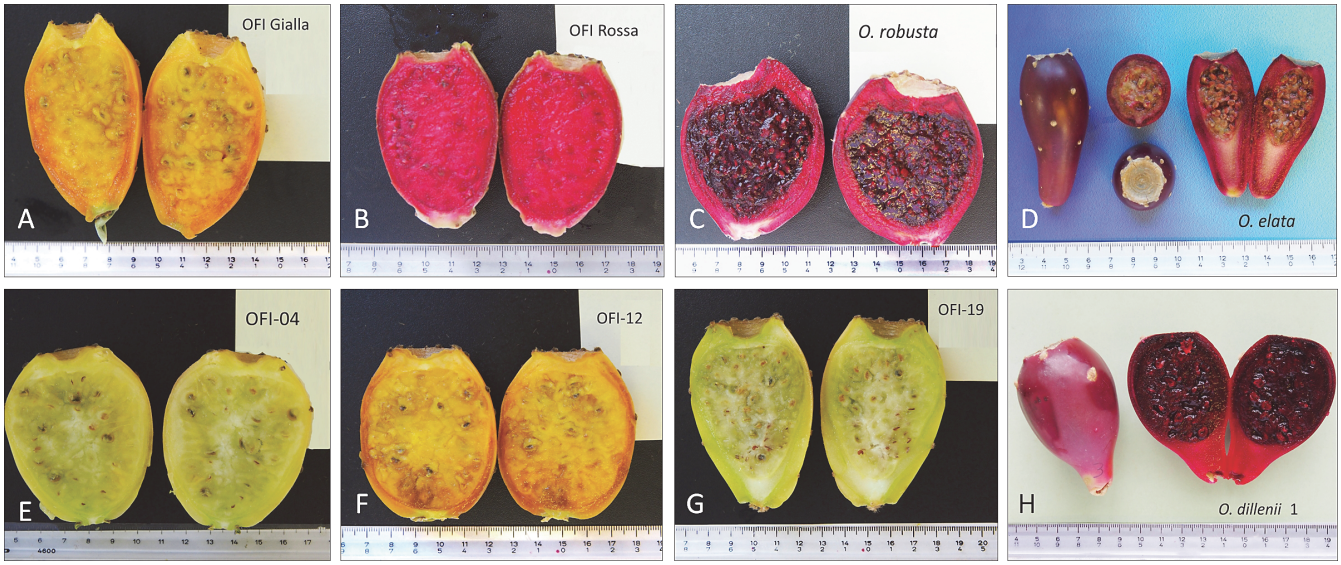


**Fig. 1. Cactus pear fruit from some populations studied. A – *Opuntia ficus-indica* (OFI) cv. Gialla; B – OFI cv. Rossa; C – *O. robusta*; D – *O. elata*; E – OFI-04; F – OFI-12; G – OFI-19; H – *O. dillenii* (OD-1)**

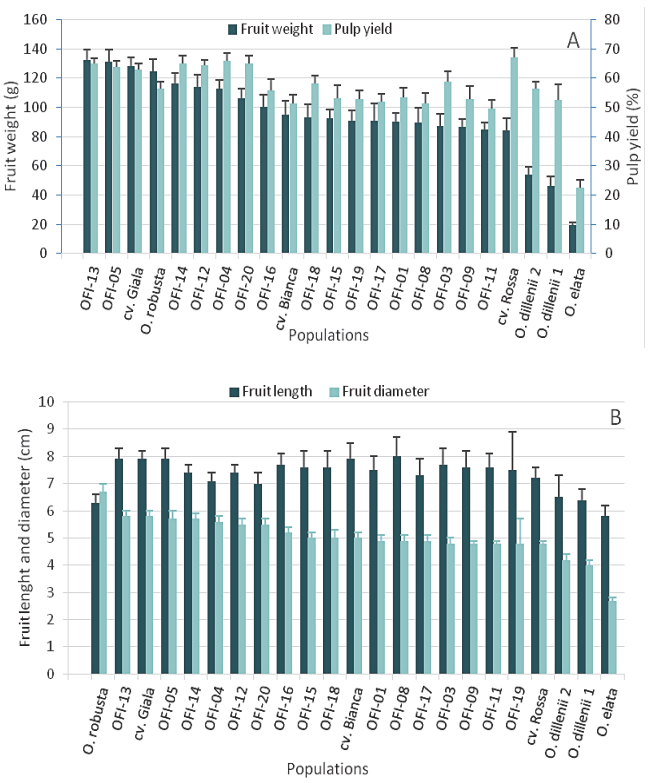


**Fig. 2. A – Fruit weight (g) and pulp yield (%); B – Fruit length (cm) and diameter (cm). Values are the means from the *Opuntia* spp. populations studied (n = 30 fruits per population)**

from 7.0 (OFI-20) to 8.0 cm (OFI-08), and the diameters varied from 4.8 (OFI-03, OFI-09, OFI-11 and cv. Rossa) to 5.8 cm (OFI-13 and cv. Gialla) (Figure 2B). The seed weight per fruit varied between 5.8 (*O. robusta*) and 1.1 g (*O. elata*) (Figure 3A). The amount of seeds as a percentage of pulp weight ranged between 22.8 (*O. elata*) and 3.7% (OFI-04), and the weight of 100 seeds varied between 0.8 (*O. elata*) and 3.1 g (*O. dillenii*) (Fig. 3A). In OFI ecotypes, the following average values were found in the white pulp and orange pulp fruits: seed weights per fruit were 3.0 and 3.7 g, the amount of seeds as pulp weight percentage were 5.9 and 4.6%, and the weights of 100 seeds were 1.7 and 2.1 g, respectively (Figure 3B). The OFI-04 ecotype contrasted the other OFI ecotypes due to its pale yellow pulp, ovoid shape, and low seed weight per fruit as well as the amount of seeds as a percentage of pulp weight. The OFI orange pulp fruits were larger, heavier and had a higher percentage of pulp as well as a lower percentage of seeds compared to the white pulp fruits. However, the weight of 100 seeds was lower in the white pulp ecotypes.

The presence of seeds is a deterrent factor to those who consume the fruits of the cactus pear for the first time (Felker et al., 2002). All of the ecotypes in our study had hard seeds, but the percentage of seeds as pulp weight was variable in the OFI ecotypes. The fruit fresh weight (128 g), pulp yield (62.9%) and seed percentage (4.6%) values found in cv. Gialla were comparable to the measurements reported in studies made from Argentina (Felker et al., 2002) and in Sicily (Barbera et al., 1992) using the same cv.

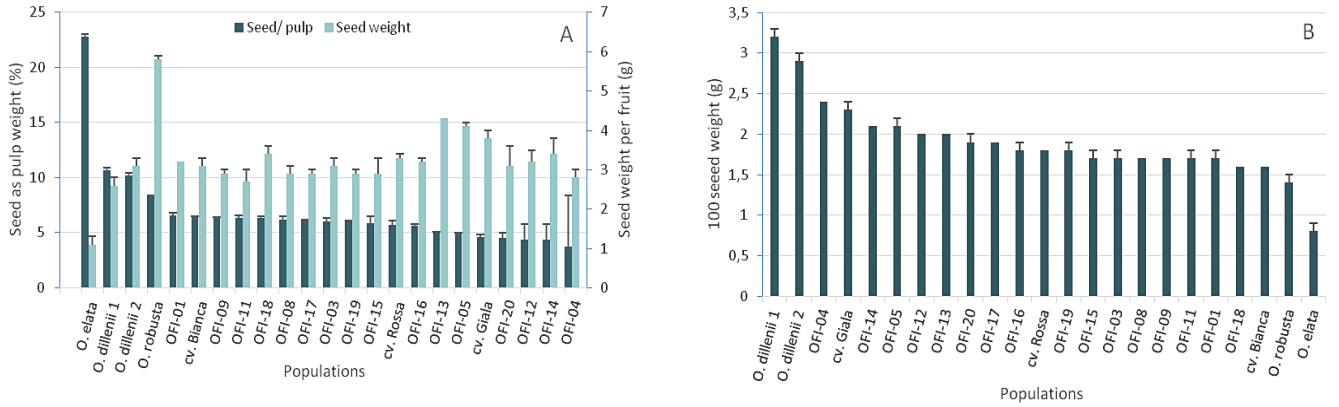


**Fig. 3. A – Seed as pulp weight percentage and seed weight per fruit (g); B – The 100 seed weight (g). Values are the means from the *Opuntia* spp. populations studied (n = 30 fruits per population)**

Fruit pulp percentages of 50–60% and a minimum of 120 g of fruit fresh weight are required for the export market (Inglese and Gugliuzza, 2002). This minimum weight value was achieved only in a few ecotypes, which may be partially explained by both the low soil water retention capacity and the age of the plants (3 years old).

***Total soluble solids, pH, acidity and colour***

The cactus pear TSS content varied from 10.6 (*O. dillenii*, the mean value of the two populations was considered) to 15.6% (OFI-03) (Table 2). In the OFI, the values ranged from 12.2 (cv. Rossa) to 15.6% (OFI-03) and statistically significant differences were found among ecotypes, $F(18, 48) = 39.6$, $p < 0.05$.

**Table 2**

**Colorimetric coordinates (*L**, *a**, and *b**), pH, acidity (% citric acid) and total soluble solids (TSS %) from the juice of the different cactus pear populations (n=30, each sample analysed in triplicate)**

| Population | *L** | *a** | *b** | pH | Acidity (% citric ac.) | TSS (%) |
|---|---|---|---|---|---|---|
| OFI-01 | $27.44^{abc}$ | $-1.07^{h}$ | $8.09^{c}$ | $6.30^{bcd}$ | $0.05^{d}$ | $14.25^{bcdef}$ |
| OFI-03 | $27.14^{abc}$ | $-0.84^{h}$ | $7.29^{cde}$ | $6.30^{bcd}$ | $0.05^{d}$ | $15.63^{gh}$ |
| OFI-04 | $26.16^{bc}$ | $-1.28^{h}$ | $9.24^{c}$ | $6.10^{fg}$ | $0.05^{d}$ | $15.10^{a}$ |
| OFI-05 | $23.02^{de}$ | $5.18^{ef}$ | $18.55^{a}$ | $6.10^{fg}$ | $0.06^{d}$ | $15.12^{a}$ |
| OFI-08 | $27.73^{abc}$ | $-1.03^{h}$ | $8.19^{c}$ | $6.20^{def}$ | $0.07^{d}$ | $13.70^{defg}$ |
| OFI-09 | $28.41^{a}$ | $-1.07^{h}$ | $6.87^{cde}$ | $6.27^{cde}$ | $0.06^{d}$ | $14.10^{cdef}$ |
| OFI-11 | $27.17^{abc}$ | $-0.80^{h}$ | $5.86^{cdef}$ | $6.20^{def}$ | $0.06^{d}$ | $13.55^{efg}$ |
| OFI-12 | $23.10^{d}$ | $5.47^{de}$ | $19.45^{a}$ | $6.17^{ef}$ | $0.05^{d}$ | $15.07^{ab}$ |
| OFI-13 | $21.84^{de}$ | $8.03^{bc}$ | $16.57^{ab}$ | $6.03^{g}$ | $0.06^{d}$ | $15.05^{ab}$ |
| OFI-14 | $21.00^{e}$ | $7.49^{c}$ | $14.81^{b}$ | $6.20^{def}$ | $0.06^{d}$ | $14.65^{abc}$ |
| OFI-15 | $27.98^{ab}$ | $-1.12^{h}$ | $8.06^{cd}$ | $6.27^{cde}$ | $0.05^{d}$ | $13.47^{fgh}$ |
| OFI-16 | $26.61^{abc}$ | $-1.13^{h}$ | $8.32^{c}$ | $6.47^{a}$ | $0.05^{d}$ | $15.10^{a}$ |
| OFI-17 | $25.74^{c}$ | $-0.89^{h}$ | $7.32^{cde}$ | $6.33^{bc}$ | $0.05^{d}$ | $14.35^{abcde}$ |
| OFI-18 | $26.40^{abc}$ | $-0.93^{h}$ | $7.48^{cde}$ | $6.20^{def}$ | $0.06^{d}$ | $13.23^{gh}$ |
| OFI-19 | $27.11^{abc}$ | $-0.97^{h}$ | $7.13^{cde}$ | $6.30^{bcd}$ | $0.06^{d}$ | $14.37^{abcde}$ |
| OFI-20 | 22.19 | $6.79^{cd}$ | $17.36^{ab}$ | $6.17^{ef}$ | $0.06^{d}$ | $13.05^{gh}$ |
| OFI, cv. Bianca | $26.60^{abc}$ | $-1.01^{h}$ | $7.06^{cde}$ | $6.40^{ab}$ | $0.07^{d}$ | $13.72^{defg}$ |
| OFI, cv. Gialla | $22.43^{de}$ | $6.79^{cde}$ | $17.53^{ab}$ | $6.10^{fg}$ | $0.06^{d}$ | $14.67^{abc}$ |
| OFI, cv. Rossa | $14.84^{f}$ | $9.78^{ab}$ | $4.60^{defg}$ | $5.90^{h}$ | $0.06^{d}$ | $12.22^{i}$ |
| *O. robusta* | $13.42^{fg}$ | $5.45^{de}$ | $2.75^{fg}$ | $5.83^{h}$ | $0.07^{d}$ | $14.42^{abcd}$ |
| *O. dillenii,* OD-1 | $12.54^{g}$ | $3.20^{gf}$ | $2.07^{g}$ | $3.27^{j}$ | $0.62^{b}$ | $10.37^{j}$ |
| *O. dillenii*, OD-2 | $12.62^{g}$ | $2.47^{g}$ | $2.02^{g}$ | $3.22^{j}$ | $0.73^{a}$ | $10.75^{j}$ |
| *O. elata* | $14.87^{f}$ | $11.66^{a}$ | $4.57^{efg}$ | $4.20^{i}$ | $0.36^{c}$ | $12.70^{hi}$ |

OFI – *Opuntia ficus-indica*; OD – *Opuntia dillenii*. For a given variable, means with different alphabetic superscripts differ significantly ($P < 0.05$)

In the case of the cv. Rossa, the lowest value we obtained for the TSS could partially be explained by the fact that the fruits were harvested earlier (at commercial maturity) compared to the other ecotypes of OFI, which were harvested at physiological maturity. Indeed, TSS is a variable parameter that depends on the maturity stage and fruit metabolism (Albano et al., 2015). Apart from that difference, we can assume that the observed variations in the different parameters reflected differences at the genotype level since the studied ecotypes were grown in the same soil and the climate conditions and the fruits were harvested at approximately the same physiological state.

The pH values found in *O. dillenii* agreed with the values reported in previous studies (Medina et al., 2007). Considering the OFI populations, the pH varies from 5.90 (cv. Rossa) to 6.47 (OFI-16) (Table 2). In this species, the pH of the fruit usually increases from 5 to a range of approximately 5.5–6.5 during ripening (Albano et al., 2015). In our data, *O. dillenii* and *O. elata* showed the highest acidity values (0.68 and 0.36, respectively), while in OFI ecotypes, the acidity values ranged between 0.05 and 0.07% citric acid (Table 2). In the populations we studied, the pulp fruit colour varied from white to purple (Table 2). The highest $L^*$ values were observed in the white pulp populations. The populations with purple, red and orange coloured fruits had the highest $a^*$ values, while the populations with white pulp fruits had the lowest ones. The red and purple fruits had the lowest $L^*$ and $b^*$ values.

***Ascorbic acid***

The highest AA content was observed in *O. elata* followed by *O. dillenii* (Table 3). The AA content of OFI ecotypes ranged from 180 (OFI-20) to 344 mg $kg^{-1}$ FW (OFI-16), and significant differences among the populations were found, $F$ (18, 38) = 19.3, $p < 0.05$. The cv. Bianca had a higher AA content than the cvs. 'Gialla' and 'Rossa'. The

**Table 3**

**Ascorbic acid (mg $kg^{-1}$ FW), total phenolic compounds (mg GAE $kg^{-1}$ FW), DPPH Antioxidant Scavenging Capacity ($IC_{50}$, g $L^{-1}$) and betalains content (mg $L^{-1}$), from the juice of the different cactus pear populations studied (n=30, each sample analysed in triplicate)**

| Population | Ascorbic acid (mg $kg^{-1}$ FW) | Total Phenolic compounds (mg GAE $kg^{-1}$ FW) | DPPH ASC $IC_{50}$ (g $L^{-1}$) | Betalains | |
|---|---|---|---|---|---|
| | | | | Betaxanthins (mg $L^{-1}$) | Betacyanins (mg $L^{-1}$) |
| OFI-01 | $204.2^{gh}$ | $827.0^{def}$ | $0.88^{fghi}$ | $5.83^{e}$ | $6.82^{d}$ |
| OFI-03 | $204.2^{gh}$ | $672.7^{def}$ | $0.88^{fghi}$ | $5.86^{e}$ | $6.86^{d}$ |
| OFI-04 | $242.7^{efgh}$ | $650.0^{def}$ | $1.04^{bcde}$ | $6.00^{e}$ | $6.48^{d}$ |
| OFI-05 | $201.8^{gh}$ | $630.7^{f}$ | $0.97^{defg}$ | $44.72^{de}$ | $9.87^{d}$ |
| OFI-08 | $198.8^{gh}$ | $863.3^{def}$ | $0.81^{i}$ | $5.14^{e}$ | $6.14^{d}$ |
| OFI-09 | $252.7^{efg}$ | $833.8^{def}$ | $1.04^{bcde}$ | $5.58^{e}$ | $6.54^{d}$ |
| OFI-11 | $224.3^{fgh}$ | $822.1^{def}$ | $0.98^{cdef}$ | $5.46^{e}$ | $6.50^{d}$ |
| OFI-12 | $206.3^{gh}$ | $633.9^{ef}$ | $0.65^{j}$ | $50.99^{de}$ | $8.27^{d}$ |
| OFI-13 | $201.6^{gh}$ | $641.9^{def}$ | $0.85^{hi}$ | $46.39^{de}$ | $6.63^{d}$ |
| OFI-14 | $241.8^{efgh}$ | $829.0^{def}$ | $1.09^{abc}$ | $63.25^{d}$ | $12.38^{d}$ |
| OFI-15 | $230.7^{fgh}$ | $890.9^{def}$ | $1.06^{bcd}$ | $6.11^{e}$ | $7.19^{d}$ |
| OFI-16 | $344.1^{d}$ | $886.9^{def}$ | $0.99^{cde}$ | $6.67^{e}$ | $7.82^{d}$ |
| OFI-17 | $247.9^{efg}$ | $795.7^{def}$ | $0.83^{hi}$ | $6.37^{e}$ | $7.35^{d}$ |
| OFI-18 | $278.3^{ef}$ | $850.4^{def}$ | $0.99^{cdef}$ | $6.26^{e}$ | $7.63^{d}$ |
| OFI-19 | $223.8^{fgh}$ | $981.0^{de}$ | $0.93^{efgh}$ | $5.50^{e}$ | $6.47^{d}$ |
| OFI-20 | $180.3^{h}$ | $734.4^{def}$ | $1.12^{ab}$ | $41.25^{de}$ | $5.06^{d}$ |
| OFI, cv. Bianca | $299.9^{de}$ | $870.2^{def}$ | $0.78^{i}$ | $5.87^{e}$ | $6.77^{d}$ |
| OFI, cv. Gialla | $219.5^{fgh}$ | $617.0^{f}$ | $0.87^{ghi}$ | $40.97^{de}$ | $9.79^{d}$ |
| OFI, cv. Rossa | $206.9^{gh}$ | $785.6^{def}$ | $0.80^{i}$ | $51.05^{de}$ | $84.17^{d}$ |
| O. robusta | $222.2^{fgh}$ | $982.8^{d}$ | $1.18^{a}$ | $211.25^{c}$ | $434.81^{c}$ |
| *O. dillenii,* OD-1 | $541.6^{a}$ | $3790.3^{a}$ | $0.06^{l}$ | $575.93^{b}$ | $1516.98^{b}$ |
| *O. dillenii*, OD-2 | $456.3^{c}$ | $3403.9^{b}$ | $0.07^{l}$ | $778.56^{a}$ | $1675.36^{a}$ |
| O. elata | $896.9^{a}$ | $2879.3^{c}$ | $0.47^{k}$ | $35.51^{de}$ | $117.99^{d}$ |

DPPH ASC $IC_{50}$ – DPPH Antioxidant Scavenging Capacity $IC_{50}$; FW – Fresh weight; GAE – Gallic acid equivalents; OFI – *Opuntia ficus-indica*; OD – *Opuntia dillenii*. For a given variable, means with different alphabetic superscripts differ significantly ($P < 0.05$)

values for the AA content that were found in the *O. dillenii* and the OFI ecotypes (Table 3) were higher than the content found in previous studies for both species (Medina et al., 2007). A similar value for AA content in the cv. Gialla and a higher value for a purple OFI cv. compared to the cv. Rossa were reported (Albano et al., 2015). The differences found among the OFI populations in AA content could be attributed to differences at the genotype level. In our study, an underestimation of the cv. Rossa AA content cannot to be excluded since the fruits were purchased from a local producer and a few days passed between harvest and the juice analysis. Indeed, the AA content has been shown to decline slightly a few days after harvesting in fresh cut summer fruits (Allegra et al., 2015).

***Total phenolic compounds***

*O. dillenii* had the highest values of TPC followed by *O. elata* (Table 3). In the OFI ecotypes, significant differences were found among ecotypes, $F$ (18, 38) = 20.8, $p < 0.05$, and the TPC ranged from 617 (cv. Gialla) to 981 mg GAE $kg^{-1}$ FW (OFI-19). The TPC in the cv. Rossa and in the cv. Bianca were 788 and 870 mg GAE $kg^{-1}$ FW, respectively. The TPC found in the *O. dillenii* and the *O. robusta* ecotypes were higher than the values reported in previous studies for the same species (Medina et al., 2007; Serra et al., 2013). A moderate variation in TPC was found among the OFI ecotypes that we studied. Nevertheless, the TPC values were similar to the values previously reported by other authors (Stintzing et al., 2005; Saénz et al., 2009) in OFI fruits. The fruits from cv. Rossa were purchased at a local producer, and a decrease in TPC after harvest could explain the low TPC values that were obtained. As stated previously (Allegra et al., 2015), polyphenol content significantly decreases after 3 days of storage. In *O. elata*, the highest TPC values that were found may have resulted from whole fruit processing, since in *Opuntia* spp. fruits, the TPC is higher in the peel than in the pulp (Yeddes et al., 2013). Our results showed that the TPC was higher in *O. dillenii* and *O. elata* compared to the OFI ecotypes, and the differences in TPC values found in the OFI populations could be attributed to differences at the genotype level. A positive correlation was found between total phenols and ascorbic acid content ($R^2 = 0.81$). In general, cultivars that contained the highest vitamin C levels had the highest phenol and β-carotene contents (Yahia and Mondragon-Jacobo, 2011).

***Betalains***

Variations in BC were found among the different populations that were studied. *O. dillenii* ecotypes had the highest values of BC, followed by *O. robusta*, *O. elata*, and the lowest contents were found in the OFI populations with white pulp fruits (Table 3). Significant differences for yellow-orange betaxanthins and red-violet betacyanins contents were found among the OFI populations, $F$ = (18, 38) = 105.74, $p < 0.05$ and $F$ = (18, 38) = 173.79, $p < 0.05$, respectively. In the OFI fruits with orange pulp, a higher content of betaxanthins was found compared to white pulp fruits, and the cv. Rossa had a higher betacyanin content compared to other ecotypes. In the three cultivars 'Bianca', 'Gialla' and 'Rossa', the betaxanthin contents were 5.9, 40.9 and 51.1 mg $L^{-1}$, and the betacyanin contents were 6.8, 9.8, and 84.2 mg $L^{-1}$, respectively. In the group of orange pulp fruits, the ecotype OFI-14 had the highest BC content followed by the ecotype OFI-12. The sequence of BC content in decreasing order was *O. dillenii*, *O. robusta*, *O. elata*, cv. Rossa, orange pulp populations of OFI and, finally, white pulp populations of OFI. This ranking was similar to one published by Stintzing et al. (2005). The BC of *O. robusta* and orange pulp populations (mean value of 56.6 mg $L^{-1}$), were lower than the values reported in previous studies for similar populations (Stintzing et al., 2005). Nevertheless, the BC of cv. Rossa was slightly higher than the value reported by the same authors for a red pulp clone. The betalain values found in *O. dillenii* populations were comparable to those reported for *O. stricta* (Castellar et al., 2012). The betalain content was affected by factors such as variety (Stintzing et al., 2005), stage of maturity (Castellar et al., 2012), and climate or geographic site of production (Sumaya-Martínez et al., 2011).

***DPPH radical scavenging activity***

The antioxidant activity of *Opuntia* spp. juice extracts is shown in Table 3. The purple juice extract from *O. dillenii* had the highest antioxidant activity (lower value of $IC_{50}$) followed by the extract from *O. elata*. In the OFI ecotypes, significant differences were found among populations, $F$ = (18, 38) = 30.3, $p < 0.05$. The lowest value of $IC_{50}$ was found in the OFI-12 ecotype, and the highest value was found in the OFI-20 ecotype. Interestingly, the extract from the red juice of *O. robusta* had higher values of $IC_{50}$ (lower antioxidant activity) compared to juice extracts from the OFI ecotypes.

The antioxidant activity of the fruit extracts was stronger in the purple-skinned fruits (*O. dillenii*) compared to other populations. This result was consistent with the total phenol and betalain contents of purple-skinned fruits. Positive correlations between 1-$IC_{50}$ and the TPC ($R^2 = 0.87$), betacyanin ($R^2 = 0.81$), betaxanthin ($R^2 = 0.78$) and ascorbic acid ($R^2 = 0.64$) were found. Negative correlations between 1-$IC_{50}$ and both the $L^*$ ($R^2 = -0.60$) and $a^*$ ($R^2 = -0.54$) colorimetric coordinates were found.

***Multivariate analysis***

The PCA correlation matrix showed that all variables had at least one correlation coefficient greater than 0.3. The overall Kaiser-Meyer-Olkin (KMO) measure was 0.83 with individual KMO measures all greater than 0.7 and classifications of 'middling' to 'meritorious' according to the Kaiser method (1974). Bartlett's test of sphericity was statistically significant ($p < 0.05$), which indicated that the data were likely factorable. The PCA revealed two components with eigenvalues greater than one, which explained 85.2% of the total variance. The screen plot indicated that two components should be retained, and Varimax orthogonal rotation was employed to help interpret these results. The first component explaining 67.8% of variation was represented by the betaxanthin and betacyanin contents, acidity, pH, TPC, DPPH ASC ($IC_{50}$), TSS, and fruit length. The second component (with 17.4% of the total variation) was explained by the diameter, weight of the fruit, pulp weight, seed weight, amount of seeds as pulp percentage and AA content.

The hierarchical cluster analysis highlighted an overall pattern of genetic diversity and the relationship between germplasm accessions. The clustering analysis (Figure 4) revealed that the 23 populations could be classified into four major groups. Cluster 1 comprised all OFI populations, cluster 2 comprised *O. robusta*, cluster 3 included the two populations from the species *O. dillenii* and finally the *O. elata* population was included in cluster 4. Four subgroups were found in cluster 1, which were the white pulp fruits (including cv. Bianca), the orange pulp fruits (including cv. Gialla), the cv. Rossa, and the ecotype OFI-04. The latter ecotype was distinguishable from the others due to the quantitative and qualitative characteristics of its fruit. The distribution of populations in the dendrogram indicates that geographical origin was unrelated to the clustering pattern.

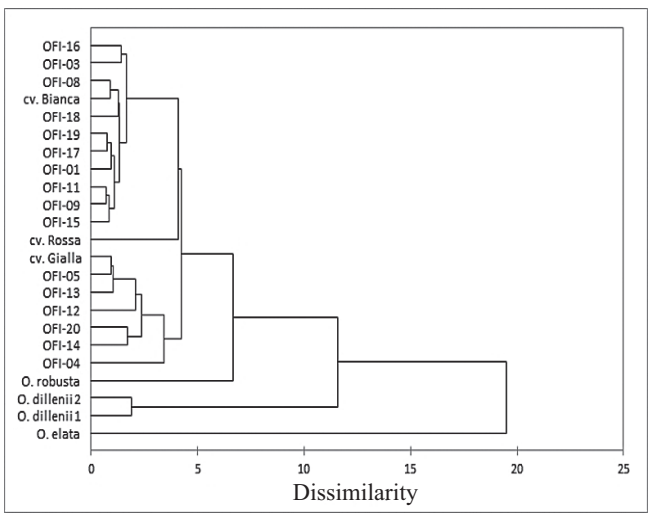


**Fig. 4. Hierarchical clustering analysis for 20 *Opuntia* spp. Portuguese ecotypes and three *O. ficus-indica* cultivars (Bianca, Gialla and Rossa) based on fruit morphological and chemical characteristics pairwise Euclidian distances**

## Conclusion

The Mediterranean region is prone to global climate change, which is expected to deeply affect the agricultural systems in this area in the near future. *Opuntia ficus-indica* and related species, which are characterized by their minimal water requirements, rustic durability and adaptability to high temperatures, could play an important role in the land use in the marginal areas of this region. A considerable genetic variation in the concentration of bioactive compounds and morphological characteristics of the fruits was observed among different *Opuntia* spp. and among different OFI ecotypes. *Opuntia*. spp. are an interesting source of phenolic compounds, betalains, and ascorbic acid. Additionally, moderate consumption of cactus pear fruit can provide important antioxidant intake. The Iberian Peninsula is likely a source of additional morphological and genetic variability inside this genus, and further germplasm collection as well as harvest and subsequent characterization of ecotypes (particularly OFI) should be undertaken to better understand the *Opuntia* spp. in this area.

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