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Morphological and nutraceutical characterization of six pomegranate cultivars of global commercial interest

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ABSTRACT

Pomegranate tree is diffused worldwide, and several ancient varieties and novel cultivars are adopted for producing fruits. This study aimed at investigating the morphological and nutraceutical characteristics of six pomegranate cultivars of worldwide interest. The cultivars were chosen based on their economic relevance and commercial traits. Fresh fruits were characterized through morphological parameters, while seeds and juices were analysed for sugars, organic acids, phenolic compounds, and mineral content. The cultivars showed big or medium-size fruits, and, among them, Acco, 29-101 and Purple Queen showed the highest aryl yields. The highest juice pH value, maturity index and seed moisture content were observed on ME17, while 29-101 had the highest presence of crude fiber. Purple Queen juice was characterized by a red intense color. Kingdom and Wonderful juices exhibited the highest concentrations of organic acids and total anthocyanins. Acco seeds were the richest in macro and micro-mineral. Hierarchical cluster analysis allowed to cluster Wonderful-Kingdom, Acco-29-101 and Purple Queen-ME17 as pairs of cultivars showing the highest similarity, additionally the heatmap showed several quite highly correlated parameters. The results indicated different qualitative profiles and attributes of pomegranates to comply with consumer expectations.

1. Introduction

Pomegranate (*Punica granatum* L.) is a very ancient domesticated species and its geographic origin was identified by Nikolai Vavilov in the primary Center IV which corresponds to the area of Asia Minor, Transcaucasia, Iran and the land of Turkmenistan (Melgarejo and Salazar, 2003). Thanks to its popular medical and therapeutic properties, pomegranate reached an important economic value in the agricultural sector, covering at present an area of cultivation of about 300.000 ha among India, Iran, China, Turkey and USA (Melgarejo-Sánchez et al., 2015a,b). Pomegranate adaptability to different climatic conditions and soils facilitated its widespread diffusion. However, this fruit tree reaches high productive yields in environments characterized by high temperatures during fruit ripening period (August-October). Pomegranate is largely diffused in Spain, which represents the first producer in Europe. In 2018, Spanish cultivation covered an area of about 5.741 ha with a production accounted for 76,165 t (MAPA, 2018) mainly located in the southern regions. In these areas, pomegranate cultivation allowed to economically enhance unproductive territories characterized by poor and saline soils and water scarcity.

Pomegranate fruits are very appreciated by consumers, due to their unique organoleptic characteristics. Fruit quality in pomegranate is a balance between taste-related attributes (e.g. sugars and organic acids), and nutraceutical compounds (e.g. polyphenols) (Legua et al., 2016; Viuda-Martos et al., 2010). These compounds are generally present at high concentrations in the pulp membrane of the seed, also named sarcotesta (Melgarejo et al., 2020), which can be consumed fresh and/or squeezed for obtaining juice. Sugars and organic acids can affect the final sensorial characteristics such as flavour and taste, thus influencing the consumer preference towards sweet, sour-sweet and sour genotypes. Moreover, pomegranate contains a very broad array of polyphenolic compounds such as phenolic acids, flavonoids, and

Abbreviations: Ay, arils yield; CF, crude fiber; Cm, carpellary membranes weight; D1, equatorial diameter; D2, calyx diameter; FW, fruit weight; L1, fruit length without calyx; L2, total fruit length; MC, moisture content; MI, maturity index; Nc, number of carpels; PQ, Purple Queen; Rt, rind thickness; RW, rind weight; TA, titratable acidity; TSS, total soluble solids

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hydrolyzable tannins (Mena et al., 2012), exhibiting several beneficial health properties such as antioxidant, anti-cancer, anti-mutagenic, anti-inflammatory activities (Asgary et al., 2014; Cano-Lamadrid et al., 2016; Jaganathan et al., 2014).

Besides, pomegranate fruit is considered healthy also for the presence of other phytochemical compounds. The woody seed part (cotyledons and embryo) is relatively rich in lipids, fiber and minerals (Hernández et al., 2011; Mirdehghan and Rahemi, 2007). Previous researches showed that the oil content ranged between 12–20 % of total seed weight, with unsaturated fatty acids being the predominant lipids (Fernandes et al., 2015; Khoddami et al., 2014). Seed crude fiber refers to the quantity of woody material compared the total seed weight and gives indication about the whole seed unpalatability (Hernández et al., 2011). Moreover, several studies demonstrated that pomegranate seed contained a great mineral amount, in particular K, P, Ca, Mg and Na (Alcaraz-Mármol et al., 2017; Mirdehghan and Rahemi, 2007).

Consumers are increasingly willing to buy healthy foods and, therefore, the market is facing high demand for products rich in bioactive compounds. The qualitative characterization of different fruit varieties can be a tool to discriminate high-quality products. Accordingly, given that pomegranate plays an important role both in the fresh and processing market, fruit quality characterization pretends to meet the current commercial demand for quality fruits (Hernández et al., 2014).

In this context, the aim of this study was to investigate and characterize the qualitative composition of the edible part (complete seeds) of six pomegranate cultivars of global commercial interest. Thus, two old varieties (Wonderful and ME17), one recent variety (Acco) and three modern cultivars (Kingdom, 29-101 and Purple Queen) were chosen. Fruit morphological and physicochemical parameters, as well as nutritional quality parameters (sugars, organic acids, phenolic compounds and minerals), were assessed.

2. Materials and methods

2.1. Plant material and sample preparation

The trees of the selected cultivars were grown on an experimental farm located in the southeast of Spain (38° 04´33´´N, 1° 21´39´´W 259) during 2018. The farm was located in an historically consolidated area for pomegranate cultivation, which represents more than 90 % of the total Spanish production. Six cultivars were chosen because of their significant economic and commercial interest related to the fruit maturation period and to their appropriate characteristics according to the consumer preferences (large and intense colored fruits). Acco (soft seed semi-acid variety), Purple Queen (soft seed sweet variety) and 29-101 (soft seed sweet variety) are early varieties (from August to September), while Wonderful (hard seed acid variety) and ME17 (soft seed sweet variety) are medium season traditional varieties. Kingdom is a very productive new variety, considered semi-acid and presents a better color and conformation than Wonderful with a similar collection period.

For each cultivar, fruits were collected from three trees. Fruits collection was performed considering all levels of the trees in order to be representative. Fully mature fruits were manually collected and immediately transported to the laboratories. For each cultivar, 15 fruits were randomly chosen and used for morphological characterization. Then, fruits were randomly separated in three replicates (5 fruits each one) for analysis. Pomegranate fruits were cut in halves and seeds were manually extracted. An aliquot of approximately 50 g of seeds for each fruit, was used for pH, total soluble solids (TSS), titratable acidity (TA), sugars, organic acids and phenolic compounds determination after juice extraction by pressuring seeds with a nylon mesh. The remaining seed material was freeze-dried to assess moisture content (MC), crude fiber (CF) and mineral concentrations.

2.2. Fruit morphological characterization

Fruit morphology was evaluated measuring the following parameters: fruit weight (FW), equatorial diameter (D1), calyx diameter (D2), fruit length without calyx (L1), total fruit length (L2), number of carpels (Nc) counted in the equatorial section, rind and carpellary membranes weight (Rw + Cm) and rind thickness (Rt). The arils yield (Ay) was calculated using Equation 1, according to Hernandez et al. (2014):

$$(Ay) = [FW - (Rw + Cm)]/FW \times 100. \quad (1)$$

Color was determined on fruit mesocarp, fresh seeds and juices using the CIEL*a*b*system and a Minolta colorimeter C-300 model with D65 illuminant and 10 observer (Minolta Corp., Osaka, Japan) coupled to a Minolta DP 301 data processor. Hue angle (H*) was calculated from $\tan^{-1}$ (b*/a*) and chroma (C*) from $(a^{*2} + b^{*2})^{1/2}$.

2.3. Juice and seed quality parameters

The pH, titratable acidity (TA) and total soluble solids (TSS) were assessed on the arils juice. pH values were measured using a pH-meter (GLP 21; Crison Ltd, Barcelona, Spain). TA was determined by automatic titration (877 Titrino plus, Metrohm) with 0.1 N NaOH up to pH 8.1, using 1 mL diluted juice in 25 mL distilled H_2O, and the results were expressed as g of citric acid L^{-1}. TSS was determined using an Atago N1 digital refractometer (Atago Co., Ltd., Tokyo, Japan) and expressed as a percentage (Brix°). Maturity index (MI) was calculated as the ratio of TSS/TA. Seed moisture content (MC) was measured after freeze-drying until constant weight. Finally, crude fiber (CF) was determined by a digester (Ankon fiber analyzer model A220), according to the official methodology established by the Spanish Ministry of Agriculture, Fisheries and Food (MAPA, 1993).

2.4. Analysis of sugars and organic acids in pomegranate juice

Sugar and organic acids analysis were performed using ^{1}H NMR according to Choi et al. (2004) with few modifications. Briefly, an aliquot of pomegranate juice (130 µL) was mixed to 70 µL of D_2O (deuterium oxide) containing 0.1 % of TSP (trimethylsilylpropionic acid sodium salt, w/v) and to 350 µL of CD_3OD (tetradeuteromethanol). The sample was vortexed and filtered and then, was introduced into a 5 mm of NMR tube. All spectra were recorded on a Bruker Avance III HD NMR operating at a proton NMR frequency of 500.16 MHz. Each ^{1}H NMR spectrum consisted of 64 scans with the following parameters: 0.191 Hz/point, pulse width (PW) = 4.0 µs (90°), and relaxation delay (RD) = 2.0 s. FIDs were Fourier transformed with LB = 1 Hz, GB = 0, and PC = 1.0. The spectra were referenced to the residual solvent signal of D_2O (4.78 ppm) for the CD_3OD extract and TSP at 0.00 ppm for the water extract. The ^{1}H-NMR spectra were analysed with Chenomx Profiler (v. 8.0., Edmonton, Canada). Spectral intensities were scaled to TSP for the water extract and reduced to integrated regions of equal width (0.03 ppm) corresponding to the region δ 0.30 − 12.00. The region δ 4.6–4.8 was excluded from the analysis due to the residual water signal.

2.5. Juice phenolic compounds characterization

Phenolic compound analysis was performed according to Legua et al. (2016) with few modifications. Briefly, pomegranate juice (5 mL) was mixed with 5 mL of MeOH, vortexed for 1 min, and then, the extraction was performed in an ultrasonic bath (2.7 L Ultrasonic cleaner, Toctech) for 10 min at room temperature. Before injection, the extract was centrifuged at 4000 rpm for 4 min and the supernatant was filtered using a 0.45 µm nylon membrane. Juice analysis was carried out using an Agilent 1200series HPLC-DAD-ESI-MS^n Ion Trap (Agilent

Technologies, Waldbronn, Germany). The separation was performed using a Luna Omega Polar C18 (250 × 4.6 mm i.d. and particle size 5 µm, Macclesfield, UK Phenomenex). The mobile phase consisted of two solvents: (1) water formic acid (95:1, v/v) and (2) acetonitrile, with a flow rate of 1 mL min^{-1}. The gradient started with 5% of solvent B, reaching 80 % solvent B at 25 min, and 99 % at 35 min, which was maintained up to 2 min. The injection volume was 20 µL. The identification of the compounds was carried out by their fragmentation patterns obtained from mass spectra. The quantification of the phenolic compounds was performed by comparing chromatography with pure standards (Chlorogenic Acid, Routine and Cyanidine-3-Glucoside, Sigma-Aldrich) and their maximum absorbance spectrum at an emitted wavelength at 290 nm, 320 nm and 520 nm respectively through a diode UV detector (DAD) integrated in the HPLC and connected in line to the mass spectrometer.

2.6. Seed mineral content determination

Elemental analysis (K, Ca, P, Mg, Na, Fe, Zn, Cu, Mn and Ni) was performed on the freeze-dried seed samples. Seed aliquots were finely grinded and mineralized by microwave (model Ethos 1, Milestone, Bergamo, Italy). After mineralization, the extracts were analysed by Inductively Coupled Plasma (ICP) (iCAP 7400 DUO ICP-OES; Thermo Fisher Scientific, Waltham, MA, USA).

2.7. Statistical analysis

The Shapiro–Wilk test was used to evaluate data normality. Data were processed with a one-way analysis of variance (ANOVA) followed by Fisher's Least Significant Difference (LSD) procedure at 95 % confidence level (Minitab®17.1.0, Minitab Inc., State College, PA, USA). The whole set of mean values of each cultivar resulted from all measurements and analysis was used to visualize in a heatmap the correlation distance among variables and average linkage among objects by adopting the CLUSTVIS (2020)on line tool (https://biit.cs.ut.ee/clustvis/).

3. Results

3.1. Fruit morphological characterization

The cultivars showed statistically significant differences in fruit morphological characterization (Table 1). Kingdom and Wonderful were the cultivars with the highest mean fruit weight of 507.6 and 499.4 g, respectively, followed by ME17 (403.4 g), 29-101 (382.1 g) and Acco (349.7 g), while the lowest fruit weight was found in Purple Queen cultivar (286.6 g). According to the classification of Zaouay et al. (2012), Kingdom, Wonderful and ME17 fruits were classified as big-sized fruits (> 400 g), while the remaining cultivars were distinguished as medium-sized fruits (200 − 400 g). Considering the external membranes (presented in Table 1 as Rw + Cm), Wonderful and Kingdom displayed the highest values with an average of 277.4 g, Purple Queen showed the lowest (124.3 g), while the other cultivars ranged from 153.1 to 204.3 g. Taking into account the edible part of the fruit, Acco, 29-101 and Purple Queen exhibited significantly higher Ay values than those observed in Wonderful, Kingdom and ME17. Small differences were observed on the rest of the studied morphological parameters (Table 1).

3.2. Juice and seed quality parameters

Juice and seed quality parameters are shown in Table 2. Wonderful juice was characterized by the highest TSS (18.13) followed by Kingdom (17.43), Acco (15.83), 29-101 (15.73), Purple Queen (15.50) and ME17 (14.07). ME17, 29-101 and Purple Queen showed significantly higher pH values than those found in Wonderful, Acco and Kingdom. Kingdom juice exhibited the highest TA (17.13 g L^{-1}), followed by Wonderful (14.83 g L^{-1}), whereas the other cultivars displayed lower juice acidity ranging from 1.88 to 5.10 g L^{-1}. ME17 showed the highest MI and MC, while Kingdom and Wonderful displayed the lowest values. Considering CF, 29-101 showed significantly higher value (2.53 %), followed by Acco (1.90 %) and Kingdom (1.89 %), while Wonderful and ME17 displayed the lowest values of about 1.33 %.

3.3. Fruit color characterization

Color coordinates found in pomegranate peel, seed and juice are shown in Table 3. Regarding the peel, the highest and lowest L* values were found for ME17 (63.7) and Purple Queen (37.8), respectively, while the other cultivars showed similar indices ranging between 40.8 − 44.1. The highest a* index was found in Acco and 29-101 peels, while ME17 showed the highest b* value. Considering chrome (C*), Acco peel displayed the highest intensity (52.6), whereas the lowest value was found in Purple Queen (32.1). Purple Queen showed the highest hue angle (H*) (94.6), followed by ME17 (50.5), while the remaining cultivars had low and similar values. Considering seed and juice, the highest values for L*, a*, b* and C* was found in Purple Queen. Wonderful, ME17 and 29-101 juices exhibited the high H* values (351.4, 344.1 and 350.2, respectively) due to the negative b* values which denoted their dark blue color.

3.4. Sugars and organic acids profile in fruit juice

Individual and total sugar concentrations are shown as Fig. 1. Wonderful resulted the richest cultivar in both individual and total sugar (Fig. 1A–D), followed by Kingdom which showed comparable glucose concentration to Wonderful (Fig. 1A), but significantly lower values of fructose and sucrose (Fig. 1B and C). Compared to Wonderful and Kingdom, Acco, 29-101 and Purple Queen showed similar and

Table 1
Morphological parameters found in pomegranate fruits. Values are the means (n = 15) and standard deviations. Within a same row, different letters mean statistically significant differences ($P < 0.05$).

Parameter	Wonderful	ME17	Acco	Kingdom	29-101	Purple Queen
FW (g)	499.4 (78.6) a	403.4 (54.2) bc	349.7 (53.4) c	507.6 (90.0) a	382.1 (45.8) c	286.6 (40.5) d
D1 (mm)	99.3 (9.2) a	95.7 (4.4) a	88.6 (3.5) b	100.1 (7.2) a	94.4 (4.5) a	85.3 (3.8) b
D2 (mm)	21.7 (2.2) a	23.2 (3.8) a	24.8 (3.7) a	22.7 (2.9) a	23.1 (1.9) a	23.1 (2.1) a
L1 (mm)	90.7 (6.2) a	79.1 (7.2) bc	81.9 (5.0) b	90.7 (6.4) a	81.3 (4.6) b	75.4 (3.8) c
L2 (mm)	110.8 (7.3) a	94.6 (9.4) b	105.4 (5.9) a	109.9 (6.0) a	109.4 (5.4) a	97.4 (3.4) b
Nc	6.1 (1.2) b	7.0 (0.5) a	5.8 (0.6) b	6.2 (0.8) b	6.4 (0.5) ab	6.3 (0.5) b
Rw + Cm (g)	275.3 (71.3) a	204.3 (23.2) b	153.1 (19.6) c	279.5 (54.9) a	175.4 (27.2) bc	124.3 (17.3) d
Rt (mm)	5.4 (0.9) a	5.2 (0.6) a	4.4 (0.9) ab	5.2 (0.8) a	4.3 (0.7) b	3.6 (0.7) b
Ay (%)	44.75 (4.2) b	49.0 (5.3) b	56.0 (3.9) a	45.0 (2.6) b	54.7 (3.8) a	56.5 (2.4) a

FW: fruit weight; D1: equatorial diameter; D2: calyx diameter; L1: fruit length without calyx; L2: total fruit length; Nc: number of carpels; Rw + Cm: rind and carpellary membranes weight; Rt: rind thickness; Ay: aril yield.

Table 2
Qualitative parameters found in pomegranate juices and seeds. Values are the means (n = 3) and standard deviations. Within a same column, different letters mean statistically significant differences (P < 0.05).

	Juice				Seed	
Cultivar	pH	TSS (°Brix)	TA (g L^{-1})	MI	MC (%)	CF (%)
Wonderful	3.34 (0.22) c	18.13 (0.06) a	14.83 (0.68) b	12.24 (0.06) e	75.79 (0.31) e	1.32 (0.06) c
ME17	4.78 (0.13) a	14.07 (0.23) d	1.88 (0.04) d	74.79 (0.64) a	82.71 (0.12) a	1.33 (0.18) c
Acco	3.56 (0.24) c	15.83 (0.12) c	5.10 (0.40) c	31.11 (2.42) d	79.81 (0.34) c	1.90 (0.21) b
Kingdom	3.44 (0.14) c	17.43 (0.23) b	17.13 (2.01) a	10.17 (1.18) e	76.47 (0.51) d	1.89 (0.06) b
29-101	4.71 (0.31) ab	15.73 (0.50) c	3.27 (0.12) d	48.10 (1.15) c	79.80 (0.42) c	2.53 (0.49) a
Purple Queen	4.58 (0.67) bc	15.50 (0.10) c	2.82 (0.10) d	54.96 (1.61) b	80.33 (0.52) b	1.72 (0.17) b

TSS: total soluble solids; TA: titratable acidity; MI: maturity index; MC: moisture content: CF: crude fiber.

lower concentrations for glucose, fructose, sucrose and total sugar (Fig. 1A–D). ME17 cultivar exhibited the lowest glucose (51.3 g L^{-1}), sucrose (0.20 g L^{-1}) and total sugar (85.2 g L^{-1}), concentrations (Fig. 1A, C and D).

A total of 8 organic acids were detected (citric, malic, tartaric, succinic, acetic, fumaric, formic and pyruvic) (Table 4). Kingdom showed the highest total organic acids concentration of 22.05 g L^{-1}, followed by Wonderful (17.98 g L^{-1}), Acco (7.86 g L^{-1}), whereas ME17, 29-101 and Purple Queen showed the lowest total concentrations of 5.31, 5.09 and 3.62 g L^{-1}, respectively. Kingdom presented the highest citric acid concentration (19.53 g L^{-1}), followed by Wonderful (15.31 g L^{-1}) and Acco (5.26 g L^{-1}), while the remaining cultivars ranged from 0.71 to 2.48 g L^{-1}. The highest malic acid concentration was found in ME17 (4.38 g L^{-1}). Purple Queen showed the highest tartaric concentration (324.86 mg L^{-1}), whereas Kingdom and Wonderful showed the lowest values of about 67.59 mg L^{-1}. Succinic acid ranged from 8.57 mg L^{-1} (Acco) to 15.66 mg L^{-1} (Purple Queen), acetic acid from 3.46 mg L^{-1} (29-101) to 8.51 mg L^{-1} (Acco), fumaric acid from 1.53 mg L^{-1} (29-101) to 6.98 mg L^{-1} (Wonderful), formic acid from 0.56 mg L^{-1} (Kingdom) to 2.31 mg L^{-1} (Purple Queen), and pyruvic from 4.81 mg L^{-1} (Acco) to 17.15 mg L^{-1} (Purple Queen) (Table 4).

3.5. Phenolic compound in fruit juice

Phenolic compound concentrations are reported in Table 5. The highest total anthocyanins concentration was found in Wonderful (585.82 mg L^{-1}), followed by Kingdom (529.43 mg L^{-1}), Purple Queen (480.58 mg L^{-1}), 29-101 (392.68 mg L^{-1}), Acco (304.71 mg L^{-1}) and ME17 (213.11 mg L^{-1}). Cyanidin-3,5-diglucoside was the predominant anthocyanin representing between the 37–53 % of the total anthocyanins for the five cultivars except ME17. ME17 reported delphinidin-3,5-diglucoside as the first component (36 % of the total), although its concentration was significantly lower (77.66 mg L^{-1}) than those found in Wonderful (188.78 mg L^{-1}), Kingdom (173.77 mg L^{-1}), 29-101(100.39 mg L^{-1}) and Purple Queen (130.94 mg L^{-1}) reporting delphinidin-3,5-diglucoside as the second abundant component. Cyanidin-3-glucoside was the third compound in Wonderful, Kingdom, 29-101 and Purple Queen showing concentrations of 121.98, 117.08, 63.04 and 108.09 mg L^{-1}, respectively, while it was the second component for ME17 and Acco (62.53 and 100.76 mg L^{-1}, respectively). Delphinidin-3-glucoside ranked fourth in all cultivars, with values varying from 21.9 to 23.76 mg L^{-1}. Small differences concerning the other phenolic compounds were observed among the cultivars (Table 5). Seed mineral content

3.6. Seed Mineral Content

Macro and micro-minerals of pomegranate seeds are presented in Table 6. Acco, Kingdom and Purple Queen showed significantly higher K concentration of about 9.72 g kg^{-1}, than that found for the other cultivars showing an average of about 8.54 g kg^{-1}. Phosphorus varied from 2.10 to 4.07 g kg^{-1} (for Wonderful and Acco, respectively), Ca from 0.32 to 0.56 g kg^{-1} (for Wonderful and 29-101, respectively), Mg from 0.39 to 0.58 g kg^{-1} (for ME17 and Acco, respectively) and Na from 19.40 to 71.93 mg kg^{-1} (for ME17 and Purple Queen, respectively).

Table 3
Color coordinates of pomegranate fruit peels, seeds and juices. Values are the means (n = 15) and standard deviations. Within a same row, different letters mean statistically significant differences (P < 0.05).

Parameter	Wonderful	ME17	Acco	Kingdom	29-101	Purple Queen
Peel						
L*	41.7 (4.0) bc	63.7 (10.4) a	44.1 (4.2) b	43.3 (4.2) bc	40.8 (4.1) c	37.8 (12.7) d
a*	32.7 (9.2) c	24.2 (8.3) e	48.1 (2.7) a	38.9 (6.7) b	44.7 (5.1) a	29.1 (8.0) d
b*	16.8 (3.8) d	27.9 (5.7) a	21.2 (2.2) b	19.4 (2.8) c	18.8 (2.4) c	12.7 (4.0) e
C*	37.0 (9.1) d	38.8 (4.3) d	52.6 (2.8) a	43.4 (6.5) c	48.6 (5.4) b	32.0 (11.0) e
h*	27.9 (7.4) c	50.5 (15.0) b	23.8 (2.3) c	26.9 (4.8) c	22.8 (2.1) c	94.6 (31) a
Seed						
L*	28.5 (7.8) bc	33.7 (8.4) ab	23.0 (5.2) c	27.3 (4.0) bc	24.2 (5.5) c	38.8 (12.3) a
a*	13.3 (3.8) c	14.6 (4.0) bc	9.1 (3.0) c	14.9 (3.3) bc	24.7 (7.0) a	22.6 (6.0) ab
b*	4.9 (1.1) bc	8.0 (2.7) abc	1.9 (0.52) c	5.1 (1.2) bc	9.3 (2.7) ab	14.2 (5.0) a
C*	14.2 (3.5) c	16.9 (5.0) bc	9.6 (3.0) c	15.7 (3.5) c	26.4 (7.8) ab	27.6 (7.0) a
h*	19.1 (3.5) b	28.8 (9.3) b	201.2 (63.0) a	19.0 (1.8) b	20.5 (3.0) b	176.1 (54.0) a
Juice						
L*	30.9 (0.1) c	35.2 (0.6) b	22.4 (7.1) e	30.2 (0.2) cd	26.5 (0.7) d	42.5 (6.8) a
a*	2.2 (0.8) c	3.9 (0.4) c	13.5 (4.2) b	2.5 (0.4) c	5.2 (1.3) c	39.6 (8.7) a
b*	−0.3 (0.1) c	−1.1 (0.1) c	4.4 (1.1) b	0.1 (0.02) c	−0.8 (0.2) c	22.0 (4.1) a
C*	2.3 (0.7) c	4.1 (0.1) c	15.4 (4.9) b	2.4 (0.4) c	5.3 (1.3) c	46.0 (6.4) a
h*	351.4 (5.1) a	344.1 (0.9) a	166.7 (51.0) b	81.1 (27.0) bc	350.2 (3.5) a	30.3 (9.8) c

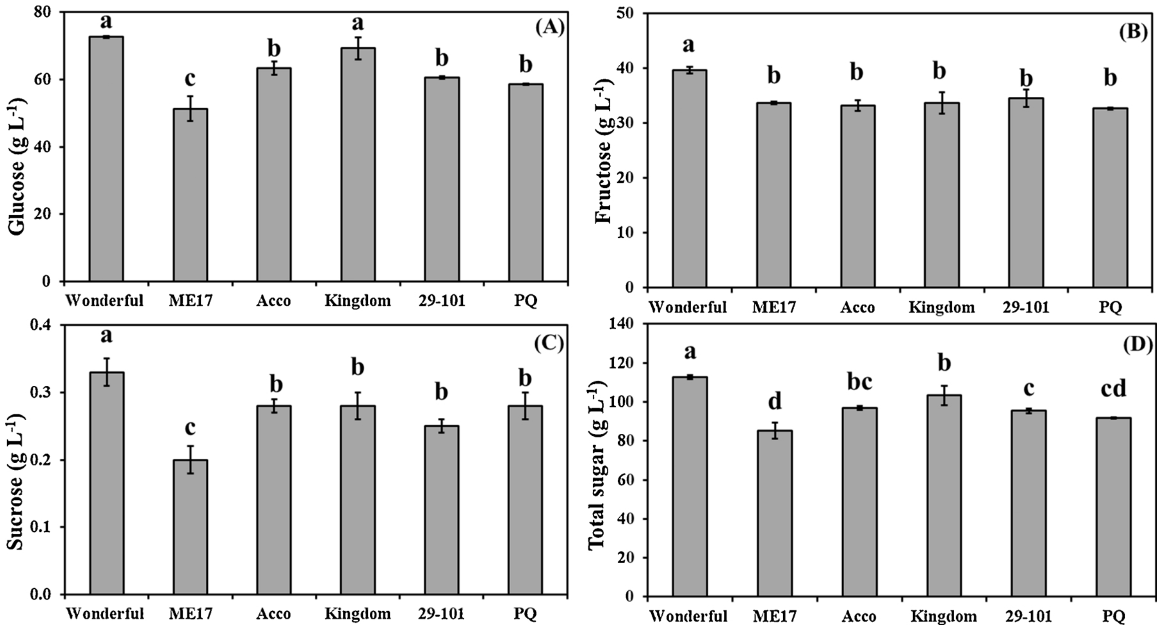

Fig. 1. Mean values (n = 3) of glucose (A), fructose (B), sucrose (C) and total sugar (D), expressed as g L^{-1} found in pomegranate juices. Error bars represent standard deviations. Different letters mean statistically significant differences ($P < 0.05$).

Table 4
Individual (citric and malic acids expressed as g L^{-1}, tartaric, succinic, acetic, fumaric, formic and pyruvic acids expressed as mg L^{-1}) and total organic (g L^{-1}) acids concentrations found in pomegranate juices. Values are the means (n = 3) and standard deviations. Within a same row, different letters mean statistically significant differences ($P < 0.05$).

Paramter	Wonderful	ME17	Acco	Kingdom	29-101	PQ
Citric	15.31 (0.03) b	0.71 (0.03) e	5.26 (0.68) c	19.53 (0.65) a	2.48 (0.33) d	0.84 (0.01) e
Malic	2.61 (0.08) b	4.38 (0.70) a	2.32 (0.16) b	2.42 (0.07) b	2.38 (0.07) b	2.42 (0.04) b
Tartaric	68.41 (1.36) e	180.01 (25.23) d	254.23 (13.68) b	66.76 (5.62) e	203.41 (12.89) c	324.86 (1.52) a
Succinic	11.84 (0.95) b	12.19 (0.34) b	8.57 (1.36) d	10.69 (0.38) bc	9.39 (1.50) cd	15.66 (1.28) a
Acetic	5.42 (0.73) d	4.93 (0.41) cd	8.51 (0.36) a	4.56 (0.37) d	3.46 (0.34) e	6.22 (0.15) c
Fumaric	6.98 (1.11) a	2.12 (0.27) d	3.31 (0.53) c	4.59 (0.02) b	1.53 (0.29) d	4.44 (0.76) b
Formic	0.99 (0.12) c	1.50 (0.06) b	1.51 (0.17) b	0.56 (0.07) d	1.18 (0.13) c	2.31 (0.11) a
Pyruvic	7.51 (0.09) b	16.39 (2.83) a	4.81 (0.74) c	8.73 (0.61) b	7.02 (0.69) bc	17.15 (0.20) a
Total	17.98 (0.05) b	5.31 (0.65) d	7.86 (0.50) c	22.05 (0.72) a	5.09 (0.25) d	3.62 (0.02) e

Table 5
Concentrations of phenolic compounds (mg L^{-1}) found in pomegranate juices. Values are the means (n = 3) and standard deviations. Within a same row, different letters mean statistically significant differences ($P < 0.05$). n.d. = not detected.

Phenolic compound	Wonderful	ME17	Acco	Kingdom	29-101	Purple Queen
Anthocyanins						
Delphinidin-3,5-diglucoside	188.78 (24.89) a	77.66 (2.32) cd	68.43 (9.82) d	173.77 (20.86) a	100.39 (16.06) c	130.94 (9.39) b
Cyanidin-3,5-diglucoside	252.76 (23.99) a	50.99 (1.18) d	112.40 (21.82) c	216.68 (3.18) b	205.94 (16.79) b	217.79 (8.31) b
Delphinidin-3-glucoside	22.30 (0.09) b	21.93 (1.62) b	23.12 (0.58) a	21.90 (0.18) b	23.31 (1.21) a	23.76 (1.13) a
Cyanidin-3-glucoside	121.98 (14.20) a	62.53 (2.82) c	100.76 (12.08) b	117.08 (12.74) ab	63.04 (7.14) c	108.09 (6.63) ab
Total anthocyanins	585.82 (63.06) a	213.11 (15.14) e	304.71 (43.99) d	529.43 (33.27) ab	392.68 (39.52) c	480.58 (24.16) b
Other compounds						
Chlorogenic acid	11.99.0 (0.66) b	13.32 (0.88) ab	13.40 (2.26) ab	11.59 (0.18) b	14.60 (2.03) a	14.85 (0.17) a
p-Coumaric acid	7.22 (0.24) cd	10.06 (1.34) b	9.07 (1.42) bc	6.30 (0.10) d	16.17 (1.53) a	10.69 (0.54) b
Coumaric acid derivative	7.40 (0.36) a	n.d.	4.75 (0.52) b	n.d.	2.61 (0.15) c	8.18 (0.57) a
Galloylhexoside	10.67 (1.30) b	5.98 (0.64) c	12.24 (1.01) a	5.06 (0.40) c	10.97 (0.86) ab	9.92 (0.77) b
Ellagic acid glucoside	19.26 (1.85) a	11.42 (1.99) c	7.72 (1.28) d	17.64 (2.08) ab	5.22 (0.52) d	14.87 (1.69) b
Ellagic acid deoxyhexose	9.02 (1.51) c	7.90 (0.23) cd	11.50 (1.15) b	8.36 (0.70) c	17.87 (0.96) a	6.51 (0.78) d
Vanillic acid hexoside	6.83 (0.87) a	1.95 (0.15) d	3.07 (0.49) c	2.60 (0.07) cd	2.86 (0.25) cd	5.22 (0.79) b
Dihydrokaempferol-hexoside	n.d.	3.35 (0.32) a	n.d.	3.37 (0.03) a	n.d.	n.d.
Ferulic acid hexoside	n.d.	n.d.	4.32 (0.99) a	2.41 (0.30) a	4.58 (0.40) b	n.d.
Guaiacyl-8,5-ferulic acid hexoside	2.94 (0.31) c	3.14 (0.06) c	5.87 (0.45) b	2.76 (0.26) c	7.04 (0.79) a	5.41 (0.71) b

Table 6
Concentrations of K, P, Ca, Mg (g kg^{-1} on a dry weight basis), Na, Fe, Zn, Cu, Mn and Ni (mg kg^{-1} on a dry weight basis) found in pomegranate seeds. Values are the means (n = 3) and standard deviations. Within a same column, different letters mean statistically significant differences ($P < 0.05$).

Cultivar	K	P	Ca	Mg	Na	Fe	Zn	Cu	Mn	Ni
Wonderful	8.54 (0.43) bc	2.10 (0.08) d	0.32 (0.01) c	0.45 (0.04) c	35.23 (4.49) c	14.57 (2.48) c	12.04 (0.73) bc	6.03 (0.46) c	6.15 (0.44) bc	0.21 (0.02) d
ME17	8.25 (0.01) c	3.24 (0.02) c	0.41 (0.04) b	0.39 (0.01) d	19.40 (2.78) d	28.12 (3.57) a	9.97 (0.28) c	4.30 (0.08) d	4.22 (0.11) d	0.31 (0.01) c
Acco	9.75 (0.18) a	4.07 (0.49) a	0.41 (0.01) b	0.58 (0.02) a	37.25 (7.05) c	16.66 (1.15) c	17.29 (0.60) a	9.13 (0.11) a	7.03 (0.54) a	0.48 (0.06) b
Kingdom	9.56 (0.39) a	3.71 (0.33) ab	0.21 (0.01) d	0.45 (0.02) c	57.92 (2.64) b	14.92 (0.30) c	13.66 (1.68) b	6.87 (0.47) b	5.65 (0.33) c	0.17 (0.03) d
29-101	8.84 (0.12) b	3.39 (0.05) bc	0.56 (0.02) a	0.40 (0.01) d	28.54 (1.40) cd	23.09 (3.48) b	17.16 (2.12) a	6.96 (0.05) b	6.45 (0.13) ab	0.81 (0.02) a
PQ	9.86 (0.23) a	2.95 (0.12) c	0.38 (0.01) b	0.52 (0.02) b	71.93 (8.57) a	16.11 (0.76) c	11.76 (0.99) bc	6.48 (0.13) bc	6.30 (0.13) b	0.23 (0.04) d

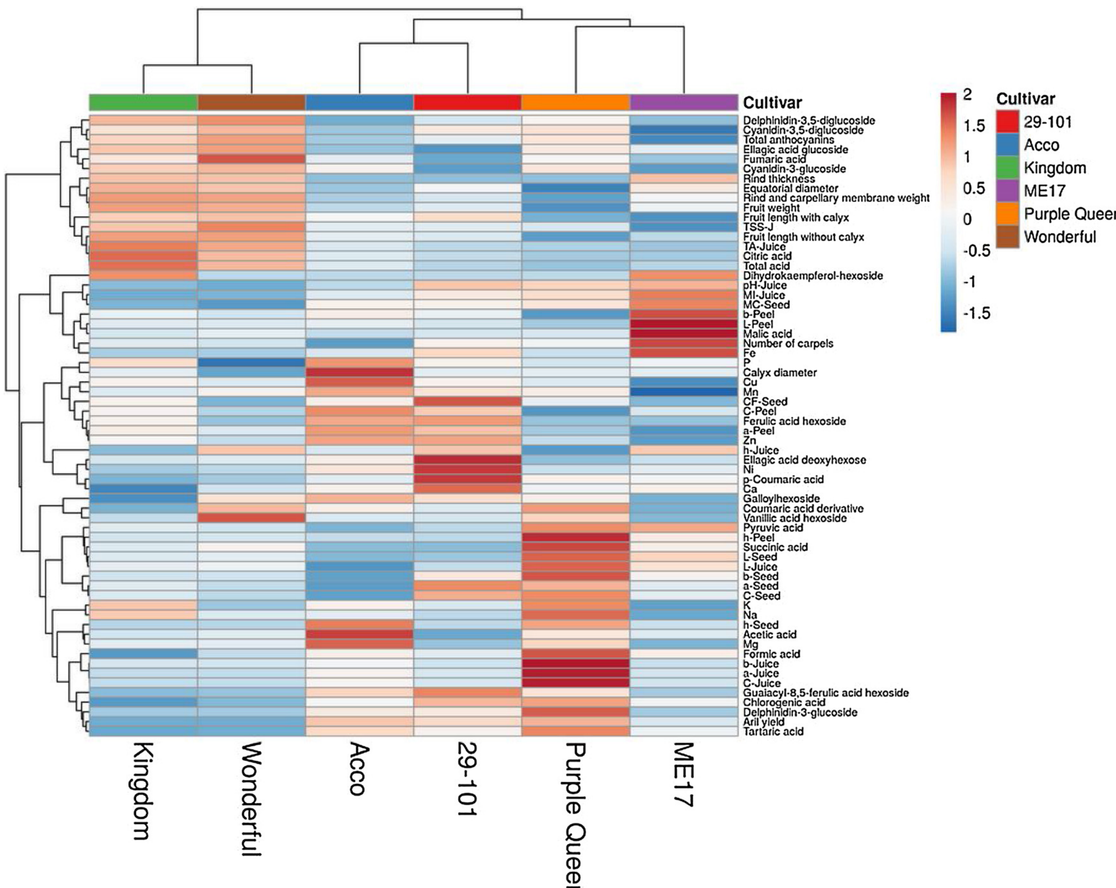

Fig. 2. Heatmap based on the average values of the morphological and biochemical parameters studied for the six cultivars under evaluation.

Iron and Zn were the most abundant microminerals with range between 14.57–28.12 mg kg^{-1} (for Fe in Wonderful and ME17, respectively) and 9.97–17.29 mg kg^{-1} (for Zn in ME17 and Acco, respectively), followed by Cu and Mn, ranging 4.30–9.13 mg kg^{-1} (for Cu in ME17 and Acco, respectively) and 4.22–7.03 mg kg^{-1} (for Mn in ME17 and Acco, respectively). Nickel was the less abundant element, ranging from 0.17 to 0.81 mg kg^{-1} for Kingdom and 29-101, respectively (Table 6).

3.7. Multivariate analysis

The heatmap shown in Fig. 2 illustrates the couples of highest similarities among the cultivars. Namely, Wonderful and Kingdom were the pair with the reciprocal lowest distance and separately clustered with the other two couples (Acco – 29-101, and Purple Queen – ME17). The correlation between the variables showed a quite complex clustering pattern. One main group of relatively highly correlated variables included the concentration of some phenolic compounds (e.g. delphinidin-3,5-diglucoside, cyanidin-3,5-diglucoside, ellagic acid glucoside, cyanidin-3-glucoside) with organic acids (e.g. fumaric, citric acid and titratable acidity), some morphological traits (e.g. rind thickness, equatorial diameter, rind and carpellary membranes weight, fruit weight, fruit length with and without calyx), total anthocyanins and total soluble solid content of juice. Surprisingly, other phenolic compounds (e.g. chlorogenic acid, delphinidin-3-glucoside, gauacyl-8,5-ferulic acid hexoside) were clustered closely to formic and tartaric acid, juice color parameters (a*, b* and C*) and aryl yield. Among mineral elements, K and Na concentrations were well correlated, while Fe, P, Cu, Mn content were clustered with peel color parameters and ferulic acid hexoside.

4. Discussion

Fruit weight is a variable parameter, greatly affected by the genotype. Wide variability across fruit weights of Italian (Ferrara et al., 2014), Spanish (Martínez et al., 2012) and Tunisian (Zaouay et al., 2012) cultivars was reported. Moreover, fruits belonging to the same variety, usually show different fruit weights in response to external factors such as climate, soil nutritional status and orchard management.

In this study, since all fruits were harvested from the same geographic area, differences in fruit weight were more associated with the genotype effect. Accordingly, as expected, Wonderful, Kingdom and ME17, being a medium season varieties, were characterized by a prolonged fruit development period which allowed to increase the fleshy part (i.e. the sarcotesta) and external membranes, reaching, at fruit ripening, higher fruit weights compared with early varieties (i.e. Acco, PQ and 29-101) (Table 1).

Interestingly, cultivars showed different proportions between edible and non-edible tissues. More in details, Purple Queen, 29-101 and Acco displayed the highest Ay of about 56 %, indicating that these fruits were mostly composed by arils. Conversely, the external membranes (Rw + Cm) were the major contributors to the total fruit weight in Kingdom and Wonderful cultivars, as also confirmed by their Ay values (45 % for both cultivars), significantly lower when compared with the other varieties, with the exception of ME17 which showed a fair contribution between edible and non-edible fruit tissues. These findings underlined that the final fruit size and weight is not always a direct consequence of an increased aril development and enlargement (Lal et al., 2013) and the proportion between external membranes and arils can greatly vary depending on the genotype. These morphometric parameters, characterizing fruit pomological shape, offer important information to the juice industry since allow to discriminate genotypes with the most suitable characteristic to the food processing and transforming.

As regard the quality, juices extracted from the fruit of the examined cultivars were found suitable for commercial use since the TSS values were greater than 12 (Vázquez-Araújo et al., 2014) (Table 2). However, the parameter TSS is not used to distinguish and classify varieties since it does not undergo important variations, as highlighted by several authors (Alcaraz-Mármol et al., 2017; Vázquez-Araújo et al., 2014). In pomegranate, important qualitative parameters are represented by pH and TA. Varieties such as Wonderful, Kingdom and Acco, showing low pH and high TA values, were connotated by a more pronounced juice acidity. However, none of the examined cultivars can be classified as sour since all the MI values were higher than 7, according to the classification reported in Martínez et al. (2006). In details, Wonderful and Kingdom, with MI of 12.24 and 10.17 respectively, were between the sour and the sour-sweet cultivar and can be identified as a semi-acid. Differently, Acco, ME17, 29-101 and Purple Queen were included in the sweet group, since they showed MI values higher than 31 (Martínez et al., 2006). Regarding seed quality parameters, ME17 had the highest arils MC (82.71 %), and this result agreed with the value presented by other types of Mollar de Elche cultivar (i.e. ME14, ME15 and ME16) (Hernández et al., 2014; Martínez et al., 2006). On the other hand, Wonderful showed a significantly lower aril MC (75.79 %) as previously observed (Alcaraz-Mármol et al., 2017; Ferrara et al., 2014). The pulpy seed humidity of the remaining cultivars varied between 76.47 and 80.33 %, confirming the range elsewhere reported (75–80 % by Melgarejo-Sánchez et al. (2015a,b); 78–82 % by Ferrara et al. (2014)). CF is a parameter related to the seed unpalatability, referring to the seed woody materials (Hernández et al., 2014) and the cultivars showed moderate variations with the exception of 29-10 cultivar that presented the highest CF (Table 2).

The color is an important and useful parameter for determining consumer propensities. Moreover, the peel color is recognized as an efficient criterion that farmers used for deciding the optimal time to harvest. The L* parameter denotes the lightness which can be 0, indicating black or 100, indicating diffuse white, and the range values of L* observed in this study were in line with those elsewhere reported (Melgarejo-Sánchez et al., 2015a, 2015b). Acco and 29-101 cultivars were connoted for their reddish peel, whereas ME17 peel was found the most yellowish as indicated by the higher b* index (Table 3).

Fruit seed and juice color coordinates showed different trends respect the peel. In fact, it should be pointed out the colors of the edible and non-edible fruit tissues are independent since there are no correlation between these parts (Holland et al., 2009). In fact, among the cultivars, Purple Queen was characterized by the higher L*, a*, b* and C* values in seed and juice, suggesting that this cultivar can be highly appreciated by consumers, given its bright intense red color. However, in the last decade, the market trend has been mainly orientated towards pomegranate juices characterized by a dark purple color, since these products have been associated with higher content of bioactive compounds (i.e. anthocyanins) and, therefore, greater antioxidant capacity and health benefits. For this reason, Wonderful, which in this study was reconfirmed as a dark blue juice variety (Table 3), is commercially cultivated worldwide (Mena et al., 2011).

Generally, glucose and fructose are the most abundant sugars present in pomegranate juice (Bar-Ya'akov et al., 2019), while sucrose represents a small concentration, and sometimes it is not even detected in pomegranate juice (Chater et al., 2019; Zhang et al., 2009). The low sucrose content was ascribable to the enzymatic process occurring during fruit ripening which converts hydrolyse sucrose into glucose and fructose (Conidi et al., 2017). For all the cultivars, glucose was the predominant sugar followed by fructose, and this finding is in accordance with those elsewhere reported (Hasnaoui et al., 2011; Sayyari et al., 2011). However, several authors reported a different trend, observing a greater fructose concentration than glucose (Alcaraz-Mármol et al., 2017; Mena et al., 2011). Therefore, the contribution of glucose and fructose to the total sugar can greatly vary, indicating that these parameters are affected by the agroclimatic condition as well as genetic background. In this study, higher sugar concentrations were found in Kingdom and Wonderful cultivars, with the latter showing the highest values (Fig. 1A–D). This finding can be ascribable to the fruit development stage and, thus, to the fruit ripening period (Bar-Ya'akov et al., 2019). Generally, medium late varieties synthesize higher sugar concentrations and total soluble solids respect early varieties (Borochov-Neori et al., 2009). This behaviour did not occur in ME17, although it was also part of the medium ripening cultivars. Nevertheless, it should be pointed out that ME17 was considered a sweet variety due to its low acidity content which lead to high MI value (Table 2).

The most abundant acids were citric and malic, while the other organic acids were present in smaller amounts (Table 4). Kingdom presented the highest citric concentration, followed by Wonderful and Acco. This trend was consistent with the results observed for the TA and MI (Table 2). In fact, it is well known that citric is the major acid of the semi-acid and acid cultivars (Legua et al., 2016), providing the overall acidic taste in pomegranate (Bar-Ya'akov et al., 2019). Accordingly, total organic acid concentration followed the trend found for citric acid. As also observed by Mena et al. (2011), compared to the citric acid, malic acid was not subjected to great fluctuations, remaining almost constant, with the only exception of ME17 cultivar which exhibited a malic concentration about 1.8-fold higher than that found in the other varieties (Table 4). This result was in accordance with the literature since ME17 belong to the Mollar de Elche group, well-known as a sweet cultivar, characterized by relatively high malic content (Alcaraz-Mármol et al., 2017). Taking into account tartaric acid, the sweet cultivars (i.e. Acco, 29-101, ME17 and Purple Queen) exhibited concentrations of about 3.5-fold higher than those observed in semi-acid varieties. This finding confirmed the observation of Hasnaoui et al. (2011) which noted that sweet varieties were connotated by high tartaric acid concentration. Hence, it should be pointed out that tartaric acid may be negatively correlated with sour taste.

The investigated cultivars exhibited different phenolic profiles (Table 5). Anthocyanins are secondary metabolites, representing the most abundant phenolic compound (Bar-Ya'akov et al., 2019). Wonderful juice displayed the highest anthocyanins concentration, indicating that this cultivar contained a great source of antioxidant compounds. On the contrary, the lowest anthocyanins concentration was found in ME17, confirming that Mollar de Elche cultivar produced slightly colored fruit, not suitable for juice production due to browning and color alteration problems (Mena et al., 2013). The low values of cyanidin-3-glucoside measured in ME17 disagreed with previous study reporting this individual anthocyanin as the predominant anthocyanin of the cultivar Mollar de Elche (Mena et al., 2011; Pérez-Vicente et al., 2004). Generally, cyanidin-3,5-diglucoside, delphinidin-3,5-diglucoside and cyanidin-3-glucoside are the most relevant and abundant anthocyanins, although their proportions can greatly change with genotype and accession (Legua et al., 2016; Mena et al., 2011). Accordingly, the cultivars presented differentiated profiles, showing important variations, with exception of delphinidin-3-glucoside displaying concentrations fairly constant among cultivars.

Other minor phenolic compounds were identified and quantified in pomegranate juices. Chlorogenic acid, p-coumaric acid, coumaric acid derivate and ferulic acid hexoside belong to the group of hydroxybenzoic acid (Fischer et al., 2011). Chlorogenic acid and p-coumaric acid varied between 11.59–14.85 and 6.30–16.17 $mg\,L^{-1}$, respectively, confirming the ranges observed by Herceg et al. (2016). Coumaric acid derivative was absent in ME17 and Kingdom juices, while ferulic acid hexoside was only detected in Acco, Kingdom and 29-101. Ellagitannins were represented by ellagic acid glucoside and ellagic acid deoxyhexose with similar values to those observed by Gil et al. (2000), but slightly higher than those found by Fischer et al. (2011). Vanillic acid hexoside is a hydrolysable tannin ranging from 1.95 to 6.83 $mg\,L^{-1}$. Dihydrokaempferol-hexoside was the only hydroccinamic acid detected in ME17 and Kingdom at similar concentration of about 3.36 $mg\,L^{-1}$. Guaiacyl-8,5-ferulic acid hexoside is a lignan (Mena et al., 2012), and its presence was probably due to small residues of exocarp left after squeezing the arils.

Taking into account macronutrients, the pattern of concentration was K > P > Mg > Ca > Na for Wonderful, Purple Queen, Kingdom, and Acco and K > P > Ca > Mg > Na for 29-101 and ME17 (Table 6). These trends were similar to those reported in other pomegranate fruits grown in Spain and in other parts of the world (Alcaraz-Mármol et al., 2017; Fawole and Opara, 2012). These data confirmed again that K is the most abundant element characterizing seed tissue (Alcaraz-Mármol et al., 2017; Gozlekci et al., 2011; Mirdehghan and Rahemi, 2007). Similarly, several authors reported that K was also predominant in peel (Gozlekci et al., 2011; Mirdehghan and Rahemi, 2007). Comparing with other potassium-rich fruits, the cultivars showed higher K concentrations than those reported for banana (Yap et al., 2017), but lower compared kiwi fruit (Park et al., 2011). Other important minerals in pomegranate are P, Ca, Mg and Na, which were showed slightly higher values than those reported in seven cultivars previously analyzed by Fawole and Opara (2012). Interestingly, the semi-acid cultivars (i.e. Wonderful and Kingdom) had the lowest Ca and Mg concentrations as also observed by Nuncio-Jáuregui et al. (2015). However, no clear trend was observed for other macro-minerals. As regard micronutrient, Fe and Zn was found in similar concentrations, followed by Cu and Mn, whereas Ni was the less abundand element. Same trends were reported by Mirdehghan and Rahemi (2007).

5. Conclusions

In this study, the fruits morphological and nutraceutical characterization remarked a great variability among the most important pomegranate cultivars. The results can be functional for understating the most suitable productive uses. Among the cultivars, Purple Queen, 29-101 and ME17 showed a great aptitude for fresh consumption as they showed high sweetness, while Wonderful, Kingdom and Acco, being characterized by acidic juices were more suitable for juice processing. Besides, these cultivars exhibited a relevant source of nutraceutical compounds such as anthocyanins (i.e. Wonderful, Kingdom and Purple Queen), crude fiber (i.e. 29-101) and minerals (i.e. Acco) which could be used for the formulation of functional products in the pharmaceutical, medical and cosmetic field. The multivariate statistical approach revealed interesting associations between several biochemical and morphological traits, which could be useful for cultivar breeding and selection, as well as to better understand the relationship between phenotypic traits and nutraceutical value of pomegranate.

Author contributions

All the authors contributed in the drafting of the manuscript. FT, DN, PL, JJM, EG performed the analysis. FT, DN, PM carried out the statistical analysis and interpreted the results.

CRediT authorship contribution statement

Francesca Tozzi: Writing - original draft, Data curation, Formal analysis, Validation, Writing - review & editing. **Pilar Legua:** Data curation, Formal analysis. **Juan J. Martínez-Nicolás:** Validation, Writing - review & editing, Methodology, Formal analysis. **Dámaris Núñez-Gómez:** Writing - original draft, Formal analysis, Validation, Writing - review & editing. **Edgardo Giordani:** Methodology, Formal analysis, Supervision. **Pablo Melgarejo:** Conceptualization, Resources, Supervision.

Declaration of Competing Interest

None.

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