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ORIGINAL PAPER

Physicochemical composition and antioxidant activity of several pomegranate (*Punica granatum* L.) cultivars grown in Spain

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Abstract Nine pomegranate cultivars grown in Spain were selected, and their physicochemical (total soluble solids, pH, titratable acidity, maturity index, monomeric anthocyanin pigment, flavonoids, hydrolyzable tannins, and vitamin C) and antioxidant properties and polyphenolic composition of the juices were compared. A total of 53 polyphenols were identified, showing cultivars different profiles. Of all nine cultivars, Katirbasi had the highest contents of flavonoids, hydrolyzable tannins and vitamin C, as well as gallic acid and ellagic acid contents, explaining its high total reducing capacity. Principal component analysis allowed Katirbasi to be differentiated clearly from the others. Other cultivars presented also interesting characteristics such as high monomeric anthocyanin pigment content (CG8 cultivar) and interesting antioxidant activity (Wonderful 2 and CG8 cultivars). CG8 was the cultivar with the highest value of cyanidin-3,5-di-*O*-glucoside. Thus, this study will assist pomegranate producers in choosing the most suitable cultivar according to its ultimate use.

Keywords *Punica granatum* L. · Cultivars · Juice · Physicochemical properties · Antioxidant activity · Polyphenolic composition

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Introduction

Spain is the greatest European producer of pomegranate, being its production mainly located in Valencia provinces, namely: Alicante, Valencia and Castellón [1]. Forty Spanish cultivars have been reported in the literature so far [2]. Mollar de Elche and Valenciana are the most widely spread cultivars in Spain, displaying very attractive sensorial characteristics [3].

The edible parts of pomegranate fruit (about 50% of total fruit weight) comprise 80% juice and 20% seeds. Generally, fresh juice contains 85% water, 10% total sugars, and 1.5% pectin, ascorbic acid, and polyphenolic flavonoids [4]. Pomegranate juice have market potential [5] because it is a source of many valuable substances such as hydrolyzable tannins (punicalagins and punicalins), condensed tannins, anthocyanins, phenolic (gallic acid and ellagic acid) and organic acids (malic acid), as well as, numerous minerals, particularly iron [3, 4, 6–12]. Some of these compounds may contribute to health-promoting effects, namely protection against cancer, cardiovascular disease, diabetes, obesity, inflammations, erectile dysfunction, bacterial infections, antibiotic resistance, UV-induced skin damage, infant brain ischemia, male infertility, Alzheimer's disease, and arthritis[4, 8, 13–20]. Pomegranate juice can be used in beverage formulations, such as juices, carbonated drinks, syrups, liqueurs, and fermented products [21–23], as well as, in jellies, as flavoring and coloring agents and in dietetic and prophylactic treatments [24]. Moreover, many pomegranate supplements have been produced and analyzed [25]. Some studies have been performed in pomegranate juices, concerning their physicochemical characterization, antioxidant activity, and/or polyphenolic composition. Those studies have focused in Iranian [26, 27], Turkish

[28, 29], Croatian [30, 31], Moroccan [32], and Italian [33] pomegranate cultivars. Regarding Spanish cultivars, some works have also been made, being the cultivars studied until now the following: Mollar de Elche, Piñón Tierno de Ojós, Casta del Reino de Ojós, Borde de Albatera, Borde de Orihuela, Borde de Beniel, Valenciana and Wonderful [10–12, 32–37]. Nevertheless, much more pomegranate cultivars are grown in Spain whose potential is not well known.

So, the present study aims to physicochemically characterize, evaluate the antioxidant activity, identify and quantify the main polyphenolic compounds (HPLC-DAD-MS/ESI) present in the juices of nine cultivars grown in Spain. Five of these cultivars had not been studied until now. Thus, this study will allow the comparison between these cultivars and to increase the available information on the less known pomegranate cultivars grown in Spain for further valorisation and development of new pomegranate based products.

Materials and methods

Solvents and reagents

All reagents and solvents were of analytical or HPLC grade. The Folin–Ciocalteu reagent, sodium hydroxide, potassium chloride, and sodium acetate were purchased from Panreac AppliChem (Barcelona, Spain). Potassium ferricyanide (III), potassium iodate, quercetin, tannic acid, gallic acid, 2,2-diphenyl-1-picrylhydrazyl, and iron (III) chloride were obtained from Sigma-Aldrich (St. Louis, MO, USA). Trichloroacetic acid, methanol, hydrochloric acid, aluminum chloride, and sodium nitrate were purchased from Fisher Scientific (Leicestershire, UK), and sodium dihydrogen phosphate monohydrate and di-sodium hydrogen phosphate dehydrate from Scharlau (Sentmenat, Spain). Solvents used for HPLC-DAD-ESI/MS separation and identification were purchased from Sigma-Aldrich (St. Louis, MO, USA). A Milli-Q water purification system (Millipore, Molsheim, France) was used to obtain ultra-pure water (resistivity of 18.2 MΩ cm) for quantitative analysis.

Pomegranate samples

The fruits were collected from adult trees (≅16 year-old) located in Elche-Alicante (Spain) in an area of 2.5 ha. The trees are planted in a 3.5 × 3 plantation frame with a controlled deficit irrigation (33% of the crop evapotranspiration (ETc)), with water conductivity of 5.1 mS. The soil is white loamy, limestone soil (22.2% active limestone) and 0.9% organic matter, with a cation exchange capacity of 10 meq/100 g. The cultivation practice on orchard was based on organic production of agricultural products according Council Regulation (EEC) No 2092/91 of 24 June 1991 [38]. The pomegranates studied in this work were harvested in Valencia, Spain, at their high ripening stage (included pomegranates with reddish skin color). The selected ripening stage corresponded to the Biologische Bundesantalt Bundessortenamt and Chemical (BBCH) 85 stage, according to Meier [39] and Melgarejo and Salazar [40]. Nine cultivars were selected, namely: Mollar de Elche, Valenciana, White, CG8, Cis 127, Parfianka, Katirbasi, Wonderful 1 and Wonderful 2 (Fig. 1a). Wonderful 1 and 2 are clones, namely: Wonderful 1 is the clone 100-1 whose origin is Israel and Wonderful 2 is the clone WG from Davis (California).Three lots of samples were made, each containing three fruits, collected from different trees in the same experimental field. Each lot was analyzed in duplicate. The fruits were transported to the laboratory under refrigeration conditions. Then, each pomegranate was manually separated into its components, namely arils, skin, and pellicle. The juice was extracted by squeezing the seeds without crushing them, and stored frozen (−23 °C) for further analysis.

Physical characteristics, total soluble solids, pH, total titratable acidity, and maturity index

The following parameters were evaluated in the nine pomegranate cultivars: weights of the fruits, skin, pellicles, and arils. According to Codex Alimentarius Commission [41], pomegranate may be sized by weight (individual weight of each fruit) and classified from A (≥501 g) to E (125–200 g) size codes. The content of total soluble solids (TSS) and pH of pomegranate juices were obtained by measuring the ºBrix of juices in a Abbe refractometer (Optic Ivymen System, Madrid, Spain) and the pH value in a potentiometer (370 pH meter of Jenway, Essex, England), respectively. Total titratable acidity (TA) was determined in 2 ml juice mixed with 10 ml ultra-pure water and titrated with 0.1 N NaOH to pH 8.2 [42, 43]. TA was expressed as percentage of citric acid. For each sample the measurements were made in duplicate. The maturity index (MI) was determined by the ratio TSS/TA, as suggested by Martínez et al. [36]. Melgarejo et al. [44] proposed that Spanish pomegranate cultivars could be classified according to their MI values as follow: sweet: MI = 31–98; sour–sweet: MI = 17–24; and sour: MI = 5–8.

Total monomeric anthocyanin

The total monomeric anthocyanin contents in the pomegranate juices were estimated by the pH differential method, following the methodologies used by Bchir et al.

Fig. 1 Pomegranate fruits (**a**) and juices (**b**) of nine cultivars grown in Spain: *1*—Mollar de Elche, *2*—Valenciana, *3*—White, *4*—CG8, *5*—Cis 127, *6*—Katirbasi, *7*—Parfianka, *8*—Wonderful 1, *9*—Wonderful 2

[45] and Rajasekar et al. [46]. The method consisted in using two buffer systems: potassium chloride buffer at pH 1.0 (0.025 M) and sodium acetate at pH 4.5 (0.4 M). 250 µl of juice was diluted with pH 1.0 and pH 4.5 buffers in 25 ml flasks and allowed to stand for 30 min at room temperature. Subsequently, the absorbance readings were made on a UV–visible spectrophotometer (Thermo, Genesys 10 UV, Waltham, USA) at the wavelengths of 510 and 700 nm, being A determined by the equation: $A = (A_{510\ nm}–A_{700\ nm})_{\ pH\ 1.0}—(A_{510\ nm}–A_{700\ nm})_{\ pH\ 4.5}$. The

monomeric anthocyanin pigment concentration was calculated as cyanidin-3-glucoside, being the concentration determined by the equation: Monomeric anthocyanin pigment (mg Cy 3-glu/l) = $A \times MW \times DF \times 1000/(\varepsilon \times 1)$, where A = absorbance difference, MW = molecular weight (449.2), DF = dilution factor, and ε = molar absorptivity (26,900). All measurements were performed in duplicate.

Flavonoids

The total flavonoid content was determined by the method described by Viuda-Martos et al. [12], with slight modifications. One ml of juice solutions at different concentrations were mixed with 0.3 ml of $NaNO_2$ (5%, m/v). After 5 min, 0.3 ml of $AlCl_3$ (10%, m/v) were mixed. After 6 min, 2 ml of NaOH (1 M) were added. The absorbance was read at 510 nm and flavonoids were quantified using a standard curve of quercetin (10–160 µg/ml). The results were expressed in mg quercetin equivalents per 100 ml juice (mg QE/100 ml juice).

Hydrolyzable tannins

The content of hydrolyzable tannins was determined by the method described by Elfalleh et al. [47]. To different concentrations of juice (1 ml), 5 ml of 2.5% KIO_3 was added and stirred for 10 s. The absorbance was measured at 550 nm. The blank was made with methanol/water (4:1, v/v). Different concentrations of tannic acid solutions (0.025–1.6 g/l) were used for calibration. Results were expressed in mg of tannic acid equivalent per 100 ml juice (mg TAE/100 ml juice).

Vitamin C

Vitamin C (ascorbic acid) content was determined by redox titration using iodine. In an Erlenmeyer flask, 2 ml of pomegranate juice were mixed with 18 ml of water and five drops of starch solution. The mixture was titrated with a standardized iodine solution. Results of vitamin C content were expressed as mg ascorbic acid per 100 ml juice (mg AA/100 ml juice).

Antioxidant activity

Total reducing capacity

The total reducing capacity of each sample was determined by the Folin–Ciocalteu method, described by Falcão et al. [48]. To 100 µl of juice solutions, 7.90 ml of deionized water and 500 µl of Folin–Ciocalteu reagent were added. The blank and standards were prepared similarly, replacing the sample by methanol and standard, respectively. After 3–8 min, 1.5 ml of saturated sodium carbonate solution was added. After 2 h the absorbance values were read at 765 nm. A calibration curve was obtained with gallic acid (0.25–5 mg/l), and the results expressed on mg gallic acid equivalent per 100 ml juice (mg GAE/100 ml juice).

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical-scavenging activity

DPPH radical-scavenging activity was determined by the procedure described by Delgado et al. [49], with some modifications. 0.0024 g of DPPH was dissolved in 100 ml of methanol to obtain a solution 6.09×10^{-5} mol/l. For each cultivar, different juice solutions were prepared in methanol:water (4:1, v/v) and 300 µl of these solutions were added to 2.7 ml of DPPH methanolic solution. After 1 h in the dark at room temperature, the absorbance was determined at 517 nm. Antioxidant activity was expressed by the percentage of scavenging effect according to the formula in Eq. 1:

$$\text{DPPH radical} - \text{scavenging effect}(\%) = \frac{A_{\text{DPPH}} - A_{\text{Sample}}}{A_{\text{DPPH}}} \times 100 \qquad (1)$$

A_{DPPH} was the absorbance of the DPPH solution and A_{Sample} the absorbance in the presence of the sample. The blank was made with methanol/water. The EC_{50} values corresponded to the juice concentration with a DPPH radical-scavenging effect of 50%.

Reducing power

The reducing power values of the juices were determined by the procedure described by Delgado et al. [49]. To 1.0 ml of the juice solutions at different concentrations were added 2.5 ml of phosphate buffer 0.2 M (pH 6.6) and 2.5 ml of $K_3[Fe(CN)_6]$ 1% (w/v). After shaking, the mixture was incubated at 50 °C for 20 min. 2.5 ml of 10% trichloroacetic acid (w/v) was added with further stirring. A volume of 2.5 ml of the mixture was transferred to another test tube, to which were added 2.5 ml of distilled water and 0.5 ml of $FeCl_3$ 0.1% (w/v). The absorbance values were read at 700 nm. From the graph $Abs_{700\ nm}$ versus solution concentration, the EC_{50} values were determined corresponding to the concentration with an absorbance of 0.5.

HPLC-ESI-MS (qualitative) and HPLC-ESI-MS/MS (quantitative) techniques and conditions

The juices were analyzed according to the method described by Gil et al. [6] with slight modifications. Phenolic compounds were characterized using a HPLC 1200 series from Agilent (Waldbronn, Germany), equipped with an autosampler, a pump, a Zorbax Eclipse XDB C18 column (4.6 × 150 mm, 5 μm) and a diode array detector (Agilent G1315C). This HPLC system was connected in series with an Agilent 6410B triple quadrupole mass spectrometer (MS) (Waldbronn, Germany) fitted with an ESI source.

The HPLC mobile phase consisted of 2.5% (v/v) acetic acid in water (eluent A) and 2.5% (v/v) acetic acid in methanol (eluent B). The flow rate was 1 ml/min, and the gradient profile was the following: 5–15% B (15 min), 15–30% B (35 min), 30–40% B (40 min), 40–60% B (50 min), 60–90% B (55 min), and 100% B isocratic (75 min). Total run time was 75 min. The injection volume for all samples was 20 μL. The diode array detector was set at 280, 360, and 520 nm.

The LC eluate was introduced directly into the ESI interface without splitting at a flow rate of 1 ml/min and the phenolic compounds were analyzed in negative ionization mode and anthocyanins in positive ionization mode. The temperature of the nebulizing gas was 350 °C at a pressure of 35 psi. The flow rate of the gas was 10 l/min and the capillary voltage of 4000 V. Analyses were carried out using full scan from m/z 100 to 1600. Compounds identification was performed by their molecular weights, taking into account the data reported by Mena et al. [50] and Calani et al. [51]. Aditionally, gallic acid, ellagic acid, cyanidin-3,5-di-*O*-glucoside, cyanidin, 3-*O*-glucoside and pelargonidin-3-*O*-glucoside chloride were quantified using standard solutions by HPLC-ESI-MS/MS.

Statistical analysis

SPSS Statistic software, version 18.0 (SPSS Inc., Chicago, USA), was used for the statistical treatment of the data. Normality and homogeneity of variance were tested by Shapiro–Wilk and Levene's tests, respectively. Analysis of variance (one-way ANOVA) or ANOVA Welch was carried out to evaluate if there were significant differences ($p < 0.05$) between samples. Additionally, significant post hoc analyses were performed (Tukey HSD test if variances in the different groups were identical or Games–Howell test if they were not). The correlations between variables were determined by Pearson correlation coefficient.

A principal component analysis (PCA) was also performed to total reducing capacity, hydrolyzable tannins, flavonoids, vitamin C, anthocyanin, and EC_{50} values of DPPH and reducing power assays of the nine pomegranate cultivars. The PCA score plot was used to differentiate them.

Results and discussion

Physical characteristics

The weights of the nine pomegranate cultivars grown in Spain, as well as of their constituents (skin, pellicle, arils and seeds), are presented in Table 1. Mollar de Elche cultivar had the heaviest fruits (478.64 g) unlike White that presented the lowest (175.95 g). Regarding Mollar de Elche cultivar, our results were higher than reported by Martínez et al. [36] of 251.05 g for ME15 cultivar to 261.72 g for ME14, as well as, Legua et al. [35] who obtained 280.58 g for ME5 cultivar to 351.48 g for ME16, indicating that our Mollar de Elche cultivar presented bigger fruits than those studied by the authors mentioned. In other studies of different pomegranate cultivars the fruit weight was similar to ours, ranging from 189.4 to 595.9 g in Croatia [52], and between 173.5 and 622.3 g in Italy [33, 53], while Tehranifar et al. [26] found lowest values, between 197 and 315 g.

When taking into account the classification of the Codex Alimentarius Commission [41], it was detected that the nine cultivars had different size codes; however, most of them were classified in B (401–500 g) and C (301–400 g) size codes, except Katirbasi (D) and White (E) cultivars that presented the smallest fruits. White cultivar showed the lowest values for the four component weights (skin—78.27 g; pellicles—3.31 g; arils—89.05 g and seeds—16.83 g). The skin percentage ranged between 36.18% for Parfianka and 57.74% for Cis 127. Arils (edible part) were the other major component of pomegranates, representing 49.40% (Cis 127) to 59.68% (Parfianka) of the fruit. Finally, the seed percentage varied from 6.01 to 10.65% of fruit weight for Mollar de Elche and Wonderful 1, respectively, as well as from 11.51 to 21.47% of arils weights for the same cultivars. In this order, Mollar de Elche had the highest juice yield due to its high arils weight and low percentage of seeds, indicating to be the most appropriate for juice industry.

TSS, pH, TA, and maturity index

Table 2 shows that significant differences ($p < 0.05$) among the nine cultivars were observed for TSS contents, varying between 14.87 and 18.04 ºBrix for Parfianka and Wonderful 1 cultivars, respectively. When comparing this range with other authors, who also studied cultivars grown in Spain, including Mollar de Elche (ME), Valenciana (V), and Wonderful (W) beyond others, our range was quite similar. Melgarejo et al. [44] reported TSS contents between 14.31 (CRO2) and 15.81 ºBrix (ME2); Melgarejo et al. [54] of 11.94 (CRO2) to 14.84

Table 1 Physical properties of nine pomegranate cultivars grown in Spain

Cultivar	Fruit weight (g)	Size Code	Skin weight (g)	Skin (%)	Pellicles weight (g)	Pellicles (%)	Arils weight (g)	Arils (%)	Seeds (g)	Seeds (%)	Seeds weight in the arils (%)
Mollar de Elche	478.64 ± 13.54^{a}	B	197.59 ± 11.79^{a}	41.33 ± 3.28^{b,c}	9.72 ± 1.72^{a}	2.04 ± 0.40^{a,b,c,d}	247.96 ± 29.28^{a}	51.79 ± 5.71	28.78 ± 6.73^{a,b}	6.01 ± 1.38	11.51 ± 1.45^{b}
Valenciana	438.05 ± 49.49^{a,b}	B	178.36 ± 18.47^{a,b}	40.75 ± 0.44^{b,c}	9.66 ± 0.55^{a}	2.22 ± 0.17^{a,b,c}	235.57 ± 26.26^{a}	53.79 ± 0.99	32.95 ± 5.80^{a,b}	7.51 ± 0.84	13.98 ± 1.84^{a,b}
White	175.95 ± 28.81^{c}	E	78.27 ± 16.32^{c}	44.54 ± 7.27^{a,b,c}	3.31 ± 0.60^{c}	1.88 ± 0.21^{a,b,c,d}	89.05 ± 19.51^{b}	50.52 ± 6.09	16.83 ± 4.41^{b}	9.52 ± 1.54	18.79 ± 0.90^{a,b}
CG8	335.72 ± 8.50^{b}	C	161.79 ± 25.58^{a,b}	48.11 ± 6.63^{a,b}	7.73 ± 0.72^{a,b,c}	2.30 ± 0.16^{a,b}	198.54 ± 81.67^{a,b}	59.34 ± 25.09	25.96 ± 4.73^{a,b}	7.71 ± 1.20	14.64 ± 6.54^{a,b}
Cis 127	324.62 ± 50.43^{a,b,c}	C	186.06 ± 22.01^{a,b}	57.74 ± 5.80^{a}	8.96 ± 3.22^{a,b}	2.72 ± 0.64^{a}	161.15 ± 33.37^{a,b}	49.40 ± 4.24	24.48 ± 6.17^{a,b}	7.55 ± 1.54	15.22 ± 2.15^{a,b}
Katirbasi	275.10 ± 32.68^{b,c}	D	118.79 ± 10.48^{b,c}	43.30 ± 2.01^{a,b,c}	7.00 ± 1.41^{a,b,c}	2.54 ± 0.33^{a}	141.57 ± 22.55^{a,b}	51.31 ± 2.87	19.72 ± 4.24^{b}	7.12 ± 0.83	13.84 ± 0.88^{a,b}
Parfianka	412.46 ± 84.63^{a,b,c}	B	148.25 ± 22.54^{a,b,c}	36.18 ± 1.77^{c}	5.87 ± 2.44^{a,b,c}	1.39 ± 0.28^{b,c,d}	247.21 ± 59.20^{a,b}	59.68 ± 1.95	31.17 ± 11.58^{a,b}	7.45 ± 1.72	12.46 ± 2.66^{b}
Wonderful 1	368.55 ± 25.00^{b}	C	168.89 ± 29.21^{a,b}	45.61 ± 4.94^{a,b,c}	4.56 ± 0.37^{b,c}	1.25 ± 0.19^{d}	181.83 ± 2.88^{a,b}	49.49 ± 3.49	39.02 ± 3.07^{a}	10.65 ± 1.51	21.47 ± 1.92^{a}
Wonderful 2	415.17 ± 39.36^{a,b}	B	180.32 ± 48.62^{a,b}	42.95 ± 8.16^{b,c}	5.45 ± 1.12^{a,b,c}	1.31 ± 0.23^{c,d}	218.71 ± 33.58^{a,b}	52.73 ± 6.97	37.28 ± 4.05^{a}	9.07 ± 1.69	17.50 ± 4.46^{a,b}

Values in the same column with different letters are statistically different ($p < 0.05$)

°Brix (BA1); Martínez et al. [36] of 12.36 (ME14) and 16.32 °Brix (PTO7); Mena et al. [3] of 13.73 (V.111) to 17.60 (WSN); and Legua et al. [35] of 14.79 (ME17) to 15.81 (MO6). Our results showed that there is one cultivar significantly sweeter than Mollar de Elche (the best known by consumers), namely Wonderful 1; however, as previously mentioned, this cultivar had one of the lowest arils percentage (49.49%) and the highest seed percentage (10.65%), making it less appreciated and chosen by the consumers and juice industry.

Regarding pH values (Table 2), significant differences between cultivars were found, ranging from 2.56 (Wonderful 2) to 4.31 (Valenciana). US Food and Drug Administration [55] reported a pH range of 2.93–3.20 for the edible portions (arils) of pomegranate in the natural state. In our study, Cis 127 and Wonderful 1 were the only cultivars that showed values within that range, presenting the other cultivars higher pH values, with the exception of Parfianka and Wonderful 2. These had pH values lower than the range referred, indicating to be the most acid. Nevertheless, after comparing our results with other authors who had studied different cultivars, similar pH ranges were found. Tehranifar et al. [26] determined pH values between 3.16 and 4.09; Gadže et al. [30] of 2.9 and 4.0; Ozgen et al. [29] of 2.98 and 3.68; Ferrara et al. [33] of 2.93 and 3.59; Legua et al. [35] of 3.94 and 4.07; Raduníc et al. [52] of 2.81–3.90; and Melgarejo-Sánchez et al. [56] of 3.49 and 5.14. Several factors such as fruit variety, maturity status, and postharvest handling will contribute to differences in pH values [57]. Regarding Mollar de Elche cultivar, Melgarejo et al. [44, 54] obtained similar pH values to our (3.97) for several clones from 3.96 (ME12) to 4.27 (ME1), and 4.06 (ME2) to 4.11 (ME14), respectively. Identical pH values were also obtained by Martínez et al. [36] (4.28 for ME14 and ME15); Mena et al. [3] (3.84 for M.29–4.00 for M.Leon.1); and Legua et al. [35] (3.99 for ME16 and 4.07 for ME5). Concerning Valenciana cultivar, our result (4.31) was higher than Mena et al. [3], 3.60 for V.111–3.67 for V.46i, indicating that our cultivar had lower acidity than those. On the other hand, when comparing our results of Wonderful 1 and 2 cultivars (2.97 and 2.56, respectively) with Mena et al. [3] (2.52 for W.7–3.71 for W.2), similar results were obtained. Nevertheless, some of these physicochemical parameters had different values than ours because arils' juices were prepared in different ways, namely: using a commercial/domestic blender [29, 30, 35, 36, 44, 57], making pressure on the arils against a nylon mesh [3, 56] or through layers of cheesecloth [52].The organic acids present in the arils of pomegranate include, mainly, citric, malic, oxalic, acetic, fumaric, and tartaric acids [10]. According to Mars et al. [58] cited by Pavez [54], pomegranate cultivars can be classified

Table 2 TSS, pH, TA, and maturity indexes of juices of nine pomegranate cultivars grown in Spain

Cultivar	TSS (°Brix)	pH	TA (% citric acid)*	TSS/TA (°Brix/ % citric acid)	Maturity index
Mollar de Elche	15.84 ± 0.06[b,c,d]	3.97 ± 0.05[b]	0.32 ± 0.01[d,e] (Sweet)	49.18	Sweet
Valenciana	16.37 ± 0.01[b,c]	4.31 ± 0.03[a]	0.28 ± 0.01[e] (Sweet)	57.73	Sweet
White	15.70 ± 0.29[b,c,d]	3.45 ± 0.01[d]	0.60 ± 0.07[c] (Sweet)	26.06	Sour–sweet to Sweet
CG8	15.87 ± 0.87[b,c,d]	3.57 ± 0.01[c]	0.74 ± 0.02[c] (Sweet)	21.41	Sour–sweet
Cis 127	16.87 ± 0.50[a,b]	3.03 ± 0.04[e]	0.52 ± 0.05[c,d] (Sweet)	32.28	Sweet
Katirbasi	16.04 ± 0.29[b,c,d]	3.42 ± 0.02[d]	0.60 ± 0.07[c] (Sweet)	26.79	Sour–sweet to Sweet
Parfianka	14.87 ± 0.50[d]	2.74 ± 0.02[f]	2.11 ± 0.06[b] (Sour)	7.04	Sour
Wonderful 1	18.04 ± 0.50[a]	2.97 ± 0.04[e]	1.92 ± 0.08[b] (Sour–Sweet)	9.40	Sour (a little higher than the limit)
Wonderful 2	15.20 ± 0.29[c,d]	2.56 ± 0.02[g]	2.68 ± 0.18[a] (Sour)	5.68	Sour

TSS Total soluble solids, *TA* total titratable acidity, *TSS/TA* maturity index

Values in the same column with different letters are statistically different ($p < 0.05$)

* The values in parenthesis corresponded to juice acidity, taking into account the classification proposed by Mars et al. (1997)

by juice acidity (expressed as citric acid percentage) in sweet, sour–sweet and sour. Sweet cultivars have acidity lower than 0.9% and are mainly destined for fresh consumption. Sour–sweet cultivars have acidity between 1 and 2% and are used for the production of soft drinks. However, the ratio of sugars/acids is very important for human consumption and different ratios may be appreciated by people in different countries. Sour cultivars have acidity higher than 2% and may be used by the food industry for acid extraction (De Palma and Novello [59], cited by Pavez [60]). In this order, the majority of our cultivars were sweet and suitable for fresh consumption, with the exception of Parfianka (2.11%) and Wonderful 2 (2.68%) that were sour and Wonderful 1 (1.92%) that was sour–sweet. The TA value of our Mollar de Elche (0.32%) cultivar was slightly higher than those reported for other Mollar de Elche clones. For example, Melgarejo et al. [44, 54] obtained values between 0.20% (ME11) and 0.25% (ME14), and 0.24% (ME1) and 0.25% (ME2 and ME14), respectively, as well as Martínez et al. [36] of 0.26% (ME15) to 0.27% (ME14) and Legua et al. [35] of 0.23% (ME17) to 0.26% (ME5).

Concerning the maturity index (TSS/TA) and according to the classification suggested by Melgarejo et al. [44] for Spanish cultivars, our Mollar de Elche, Valenciana and Cis 127 were sweet, whereas Parfianka, Wonderful 1 and Wonderful 2 were sour. This was in line with the previous results on juice acidity. The other cultivars had intermediate maturity indexes. In generally, our result for Mollar de Elche (49.18) was lower than Melgarejo et al. [44, 54] who obtained values of 56.97–75.07 and 61.90–64.23, respectively; Legua et al. [35] of 59.14–64.40; and Mena et al. [3] of 64.15–89.28. Regarding the maturity indexes of our Valenciana (57.73), Wonderful 1 (9.40), and Wonderful 2 (5.68) cultivars, these were similar to Mena et al. [3], who determined maturity indexes between 52.15 and 61.67 for Valenciana, and 5.19 and 29.08 for Wonderful cultivars. For other cultivars the TSS/TA ratio varied from 11.5 to 33 for Croatian [52], 5.4–37.7 and 4.8–37.7 for Italian [33, 53, 61] and 37.4–77.6 for Moroccan cultivars [62].

Taking into account the total titratable acidity and maturity index, Chace et al. [63] referred that pomegranate is appropriate for fresh market when its acidity content is lower than 1.8% and its maturity index is between 7 and 12. Moreover, when maturity index ranges between 11 and 16, pomegranates are considered to be quite tasty. Thus, taking into account our results all cultivars studied were appropriate for fresh market and considered tasty, with exception of Parfianka, Wonderful 1 and Wonderful 2 than had higher acidity (>1.9% citric acid).

Total monomeric anthocyanin

The total monomeric anthocyanin contents of the nine pomegranate cultivars analyzed in the present study varied significantly among them, ranging from 43.4 (Cis 127) to 293.5 mg Cy 3-glu/l (Wonderful 2), closely followed by CG8 (284.6 mg Cy 3-glu/l). This range was smaller than that reported by Sepúlveda et al. [64], 168–1328 mg Cy 3-glu/l, for genotypes of different regions of Chile. When comparing our results with Mena et al. [3], a similar trend was obtained, namely: Wonderful > Mollar de Elche > Valenciana. In generally, our results were higher than Elfalleh et al. [64] who reported values between 28.15 (Gabsi3) and 48.27 (Chetoui) mg Cy 3-glu/l for Tunisian cultivars. On contrary, the values obtained for Wonderful cultivars (254.8 and 293.5 mg Cy 3-glu/l) were within the range reported by Gil et al. [6] of 161.9–387.4 mg Cy 3-glu/l for different juices of the same cultivar.

Anthocyanins are considered responsible for the red color of pomegranate and its arils, which is an attribute of quality [7]. The red color depends on the concentration and type of anthocyanins. In this order, in the present study Cis 127 cultivar showed the lowest level of anthocyanins and the juice with the lowest reddish color, as can be seen in Fig. 1b.

pH is an important factor for color expression of anthocyanins, being these compounds more stable in acidic than alkaline or neutral medium. In acidic medium, there is a shift in anthocyanins chromophores equilibrium to the flavylium cation, the most stable anthocyanin form [60, 66]. The juices of Wonderful 1, Wonderful 2 and Katirbasi cultivars were the most reddish (Fig. 1) due to their high anthocyanin pigment contents and low pH (2.56–3.42). Even though CG8 presented a high anthocyanin pigment concentration, its higher pH (3.57) may explain its lower reddish color.

Flavonoids

The flavonoid contents of the nine cultivars studied in the present work ranged from 20.8 to 189.4 mg QE/100 ml juice. Katirbasi and CG8 cultivars were those that had the highest flavonoid contents, whereas Parfianka, Wonderful 2, and Cis 127 the lowest values. Our range was slight higher than Orak et al. [67] who obtained values between 38.78 and 45.50 mg QE/100 ml for Turkish pomegranates, taking into account the extraction yields and flavonoid contents of aqueous extracts.

Hydrolyzable tannins

Significant differences on hydrolyzable tannin contents were detected between pomegranate cultivars (Table 3), ranging from 26.0 to 325.3 mg TAE/100 ml juice. Our range was slight higher than Elfalleh et al. [47, 65] of 197–338 mg TAE/100 ml juice for Tunisian cultivars. Our Katirbasi value (325.3 mg TAE/100 ml juice) was within the range reported by Orak et al. [67] of 309–378 mg TAE/100 ml juice for Turkish pomegranates of the same cultivar.

Katirbasi and Parfianka had the highest values of hydrolyzable tannins, whereas Mollar de Elche and Wonderful 1 the lowest contents. Tannin content is an important factor for fruit acceptability by consumers, as it is associated with astringency [60]. In this order, Katirbasi would be the most astringent and Mollar de Elche the less. This in line with Melgarejo et al. [44] who reported that Mollar de Elche family is characterized by its sweetness, low sourness, and soft and easy to eat pith.

Vitamin C

Vitamin C contents in the juice of the nine cultivars studied in the present work varied between 25.6 and 110.2 mg AA/100 ml juice for the Wonderful 2 and Katirbasi cultivars, respectively. Our values were generally higher than Ferrara et al. [53] (8.90–23.63 mg AA/100 ml juice); Mena et al. [3] (8.0–20.0 mg AA/100 ml juice); Paul and Ghosh [68] (19.8 mg AA/100 ml juice); Zarei et al. [27] (8.68–15.07 mg AA/100 g); and Tehranifar et al. [26] (9.91–20.92 mg AA/100 g), indicating that the cultivars grown in Spain presented higher vitamin C contents than those analyzed by the referred authors.

Antioxidant activity

Total reducing capacity

The Total reducing capacities of the juices of the nine cultivars are represented in Fig. 2a, varying from 94.7

Table 3 Anthocyanin, flavonoids, hydrolyzable tannins and vitamin C contents, as well as, antioxidant activity of juices of nine pomegranate cultivars grown in Spain

Cultivar	Monomeric anthocyanin pigment (mg Cy 3-glu/l)	Flavonoids (mg QE/100 ml juice)	Hydrolyzable tannins (mg TAE/100 ml juice)	Vitamin C (mg AA/100 ml juice)	EC_{50} DPPH (µl juice/ml)	EC_{50} Reducing power (µl juice/ml)
Mollar de Elche	116.2 ± 5.7^{c}	$53.0 \pm 1.6^{c,d}$	26.0 ± 1.8^{c}	$79.3 \pm 3.7^{b,c}$	9.78 ± 0.13^{b}	92.5 ± 0.9^{a}
Valenciana	52.8 ± 3.8^{d}	$52.1 \pm 7.9^{c,d}$	$133.8 \pm 20.4^{b,c}$	$83.4 \pm 3.7^{b,c}$	$9.78 \pm 0.26^{a,b}$	21.8 ± 1.0^{c}
White	245.5 ± 2.4^{b}	63.6 ± 8.8^{c}	$102.6 \pm 8.3^{b,c}$	88.8 ± 5.2^{b}	$6.37 \pm 0.05^{b,c}$	20.9 ± 1.6^{c}
CG8	284.6 ± 1.9^{a}	123.3 ± 2.5^{b}	121.8 ± 4.2^{b}	76.3 ± 5.5^{c}	7.16 ± 0.01^{b}	12.6 ± 0.2^{c}
Cis 127	43.4 ± 8.5^{d}	48.6 ± 0.3^{d}	114.6 ± 22.0^{b}	50.7 ± 1.0^{d}	7.65 ± 0.02^{b}	16.8 ± 0.1^{c}
Katirbasi	256.5 ± 0.1^{b}	189.4 ± 6.0^{a}	325.3 ± 15.3^{a}	110.2 ± 3.7^{a}	$4.97 \pm 0.11^{b,c}$	$22.8 \pm 2.9^{b,c}$
Parfianka	109.2 ± 3.3^{c}	20.8 ± 1.8^{e}	257.0 ± 19.3^{a}	17.9 ± 0.1^{e}	16.62 ± 0.11^{a}	$52.9 \pm 0.7^{a,b}$
Wonderful 1	254.8 ± 2.4^{b}	65.1 ± 1.4^{c}	$78.7 \pm 25.2^{b,c}$	39.3 ± 3.1^{d}	7.59 ± 0.03^{b}	$23.6 \pm 0.3^{b,c}$
Wonderful 2	293.5 ± 0.6^{a}	47.7 ± 1.2^{d}	$97.9 \pm 14.4^{b,c}$	25.6 ± 1.0^{e}	1.97 ± 0.27^{c}	$38.7 \pm 0.1^{a,b,c}$

Cy 3-glu cyanidin 3-glucoside, *QE* quercetin equivalent, *TAE* tannic acid equivalent, *AA* ascorbic acid

Values in the same column with different letters are statistically different ($p < 0.05$)

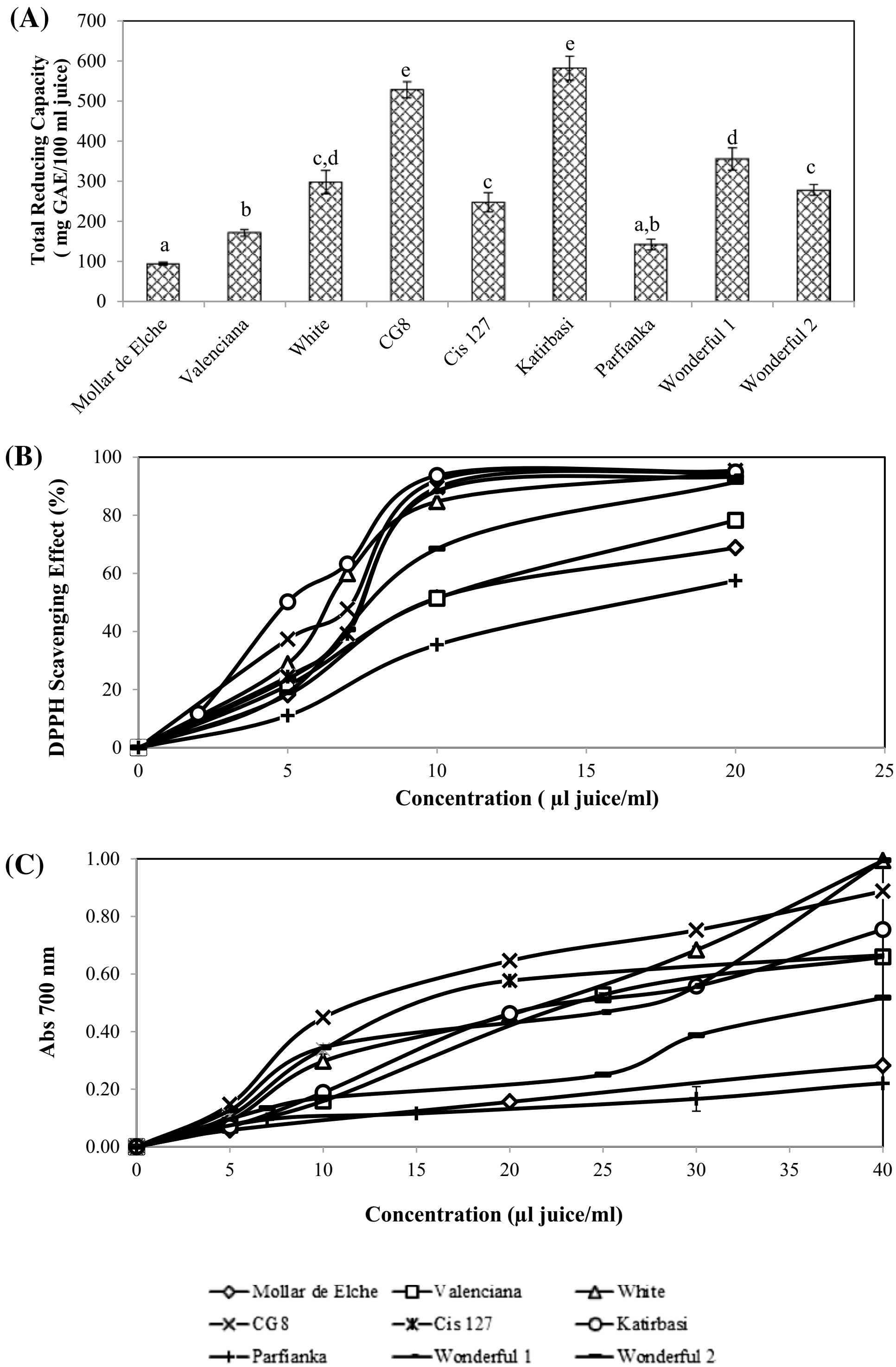

Fig. 2 Antioxidant activity of juice of nine pomegranate cultivars grown in Spain: Total Reducing Capacity (**a**), DPPH radical-scavenging activity (%) versus concentration of juice (**b**) and Reducing Power (Abs 700 nm) versus concentration of juice (**c**)

(Mollar de Elche) to 581.0 (Katirbasi) mg GAE/100 ml juice, due to the presence of antioxidants. According to Elfalleh et al. [62], pomegranate is a natural source of these compounds, namely tannins, polyphenols, flavonoids, and vitamin C. This is in line with the present study because Katirbasi cultivar showed the highest values of flavonoids (189.4 mg QE/100 ml juice); vitamin C (110.2 mg AA/100 ml juice) and hydrolyzable tannins (325.3 mg TAE/100 ml juice). On the contrary, Mollar de Elche was one of the cultivars with low values of flavonoids (53.0 mg QE/100 ml juice) and hydrolyzable tannins (26.0 mg TAE/100 ml juice), explaining its low phenolic content, estimated by total reducing capacity. Our range of total reducing capacity was higher than

Ferrara et al. [33, 53] with values from 30.3 to 282.9 mg GAE/100 ml of juice for Italian pomegranates; Radunić et al. [31, 52] of 198.56–294.87 mg GAE/100 ml for cultivars grown in Croatia; Ozgen et al. [29] of 124.5–207.6 mg GAE/100 ml for Turkish cultivars; and Sepúlveda et al. [64] of 67.6–128.0 mg GAE/100 ml for Chilean pomegranate genotypes. On the contrary, Tezcan et al. [69] reported higher values than ours, namely 14.4–1008.6 mg GAE/100 ml. When comparing our results with Mena et al. [3], who also studied Spanish cultivars, our value for Mollar de Elche was lower than theirs (94.7 vs. 150.0–200.0 mg GAE/100 ml), whereas for Valenciana and Wonderful cultivars our values were similar to their ranges (171.4 vs. 200.0–250.0, and 277.8–355.4 vs. 200.0–400.0 mg GAE/100 ml, respectively). Even though Mollar de Elche, Valenciana and White are the most known cultivars by consumers, others such as CG8 and Katirbasi, presented interesting total reducing capacities, and so antioxidant potential.

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical-scavenging activity

The antioxidant activity determined by DPPH method for the nine cultivars studied in present work (Fig. 2b and Table 3) showed significant differences between them. The DPPH free radical-scavenging activity increased with juice concentration (Fig. 2b). The EC_{50} values ranged between 1.97 and 16.62 µl juice/ml for Wonderful 2 and Parfianka cultivars, respectively (Table 3). In this order, Wonderful 2 followed by Katirbasi had the highest antioxidant potential, since they showed the lowest EC_{50} values. Until now, few studies have determined DPPH free radical-scavenging activity of pomegranate juice; however, Elfalleh et al. [47] for Tunisian cultivars detected EC_{50} values ranging from 15.98 to 23.98 µl juice/ml, similar to our Parfianka, indicating lower antioxidant capacity of those cultivars.

Reducing power

Regarding reducing power, significant differences were detected among cultivars, increasing Reducing Power with juice concentration (Fig. 2c). As shown in Table 3, it can be seen that the EC_{50} values ranged between 12.6 and 92.5 µl juice/ml for CG8 and Mollar de Elche cultivars, respectively. In this order, CG8 closely followed by Cis 127, presented the highest Reducing Power, as they had the lowest EC_{50} values.

Identification and quantification of phytochemicals compounds in pomegranate juices

A total of 53 compounds of polyphenols were identified, including, 20 hydrolyzable tannins, 15 phenolic acid derivates, 12 non-colored flavonoids, four lignans, and two organics acids (Table 4). Nevertheless, only 17 compounds were detected in all of the nine cultivars. Cis 127, White, Wonderful 2, and CG8 were the cultivars with the highest number of compounds identified, whereas Valenciana and Mollar de Elche presented the lowest number of compounds. This data showed that the nine cultivars studied presented different polyphenols profiles.

As already reported in the literature, hydrolyzable tannins are the most abundant antioxidant polyphenolic compounds in pomegranate juices [50, 51] and include pedunculagin (11, 12, 15), punicalin (10), punicalagin (17), HHDP glucoside (7) and ellagic acid (3, 4, 5, 6, 13, 14). The highest number of hydrolyzable tannins was detected in CG8, Cis 127, and Wonderful 1 (17 compounds). On the contrary, Mollar de Elche and Valenciana had the lowest number of identified compounds, namely 8 and 4, respectively. Katirbasi was the cultivar that presented the highest hydrolyzable tannins content (325.3 mg TAE/100 ml juice), being detected 16 individual compounds, as well as, the highest content of ellagic acid (4.83 mg/l) (Table 5). Our range for ellagic acid (0.69–4.83 mg/l) was higher than that reported by Li et al. [70] for Chinese cultivars (0.25–1.02 mg/l), suggesting some variability in the content of this compound among pomegranate cultivars. Furthermore, Nuncio-Jáuregui et al. [11] found higher values of derivatives of ellagic acid in sour–sweet cultivars, being Katirbasi one of these. Wu et al. [20] showed that punicalagin and ellagic acid inhibit the activity of the fatty acid synthase, having potential in the prevention and treatment of obesity. So, some pomegranate cultivars may be recommended in weight-loss diets.

Phenolic acids have two parent structures: hydroxycinnamic acid and hydroxybenzoic acid. Hydroxycinnamic acid derivatives include ferulic (31, 34), caffeic (33), and *p*-coumaric (32, 35), while hydroxybenzoic acid derivatives consist of gallic (22), vanillic (21, 27, 28), syringic (29, 30), and protocatechuic (24, 26) acids. White and Cis 127 were the cultivars with the highest number of phenolic acids derivates identified compounds, namely 14 compounds, while Mollar de Elche, CG8, and Wonderful 1 presented 13 compounds. In the present work the gallic acid was tentatively quantified (Table 5), being Mollar de Elche and Kartibasi cultivars those that presented the highest contents, 2.67 and 2.68 mg/l, respectively.

Table 4 Identification of phytochemical compounds by HPLC–MS in juices of different pomegranate cultivars

Id.	Compounds	[M−H]⁻ (*m/z*)	Cultivars (Juice)								
			Mollar de Elche	Valenciana	White	CG8	Cis 127	Katirbasi	Parfianka	Wonderful 1	Wonderful 2
	Hydrolyzable tannins										
	Gallotannins										
1	Galloyl glucoside	331	+	+	+	+	+	+	+	+	+
2	Digalloyl glucoside	483	−	−	+	+	+	+	+	+	+
	Ellagitannins										
3	Ellagic acid	301	+	+	+	+	+	+	+	+	+
4	Ellagic acid pentoside	433	−	−	+	+	+	+	+	+	+
5	Ellagic acid rhamnoside	447	+	+	+	+	+	+	+	+	+
6	Ellagic acid glucoside	463	+	−	+	+	+	+	+	+	+
7	HHDP glucoside	481	+	+	+	+	+	+	+	+	+
8	Galloyl HHDP glucoside	633	−	−	+	+	+	+	+	+	−
9	Galloyl HHDP gluconate (lagerstannin C)	649	+	−	+	+	+	+	+	+	−
10	α-punicalin	781	−	−	−	+	−	−	−	−	+
11	bis-HHDP glucoside(pedunculagin I)	783	+	−	+	+	+	+	+	+	+
12	Digalloyl HHDP glucoside (pedunculagin II)	785	−	−	+	+	+	+	+	+	−
13	Ellagic acid derivative	799	−	−	−	−	+	−	−	−	−
14	Ellagic acid derivative	805	−	−	−	−	+	−	−	−	−
15	Pedunculagin III (galloylpunicalin)	933	−	−	−	+	−	+	−	+	−
16	Trisgalloyl HHDP glucose isomer /Galloyl HHDP DHHDP glucoside (granatin B)	951	−	−	+	+	+	+	−	+	+
17	Punicalagin isomer/α-punicalagin/β-punicalagin	1083	−	−	+	+	+	+	+	+	−
18	Digalloyl gallagyl hexoside	1085	−	−	+	+	+	+	+	+	−
19	Punicalagin-like	1101	−	−	−	−	−	−	−	+	−
20	Digalloyl triHHDP-diglucose (sanguiin H10) isomer	1567	+	−	+	+	+	+	+	+	+
	Phenolic acid derivates										
	Hydroxybenzoic acid derivatives										
21	Vanillic acid	167	+	+	+	+	+	+	+	+	+
22	Gallic acid	169	+	−	+	+	+	+	−	+	−
23	Syringaldehyde	181	+	+	+	−	+	+	+	−	−
24	Protocatechuic acid pentoside	285	+	−	+	−	−	−	−	+	+
25	Hydroxybenzoic acid hexoside	299	+	+	+	+	+	−	+	+	+
26	Protocatechuic acid hexoside	315	+	+	+	+	+	+	+	+	+
27	Vanillic acid hexoside	329	+	+	+	+	+	+	+	+	+
28	Vanillic acid derivative	363	+	+	+	+	+	+	+	+	+
29	Syringic acid derivative	391	−	+	+	+	+	+	+	+	+
30	Syringic acid derivative	555	+	+	+	+	+	+	+	+	+

Table 4 continued

Id.	Compounds	$[M-H]^-$ (*m/z*)	Cultivars (Juice)								
			Mollar de Elche	Valenciana	White	CG8	Cis 127	Katirbasi	Parfianka	Wonderful 1	Wonderful 2
	Hydroxycinnamic acids										
31	Ferulic acid	193	−	−	−	+	+	+	+	+	+
32	Coumaric acid hexoside	325	+	+	+	+	+	−	+	−	−
33	Caffeic acid hexoside	341	+	+	+	+	+	+	+	+	+
34	Ferulic acid hexoside	355	+	+	+	+	+	+	+	+	+
35	Coumaric acid derivative	429	+	+	+	+	+	+	+	+	+
	Non-Colored Flavonoids										
	Flavan-3-ols										
36	(+)-Catechin/(-)-Epicatechin	289	−	−	−	−	+	−	−	−	−
37	(+)-Gallocatechin	305	−	+	+	+	+	+	−	+	+
	Flavonols										
38	Eriodictyol hexoside	449	+	+	+	+	+	+	+	+	+
39	Taxifolin hexoside	465	+	+	+	+	+	+	+	+	+
40	Myricetin hexoside	479	−	−	+	+	+	−	+	+	+
41	Syringetin hexoside	507	+	+	+	+	+	+	+	+	+
42	Kaempferol rutinoside	593	+	−	+	+	+	+	+	+	+
	Flavanones										
43	Pinocembrin	255	+	+	+	+	+	+	+	+	+
44	Naringenin-like	271	+	+	+	+	+	+	+	+	+
45	Eriodictyol hexoside	449	+	+	+	+	+	+	+	+	+
	Flavones										
46	Trihydroxyflavone	269	−	−	+	−	+	+	+	−	−
	Dihydrochalcones										
47	Phloretin	273	−	−	−	+	−	−	−	−	+
	Lignans										
48	Pinoresinol	357	+	−	+	+	+	−	+	−	−
49	Syringaresinol	417	−	−	−	+	−	−	−	−	−
50	Cyclolariciresinol hexoside	521	+	−	+	+	+	−	+	−	−
51	Secoisolariciresinol hexoside	523	+	−	+	−	+	+	−	−	+
	Organic acids										
52	L-Malic acid	133	+	−	−	−	+	−	−	−	−
53	Citric acid	191	−	+	−	−	+	+	−	−	+

Key: +, detected; −, not detected

HHDP hexahydroxydiphenoyl, *DHHDP* dihexahydroxydiphenoyl

Table 5 Individual compounds analyzed by HPLC–MS in pomegranate juices (mg/l)

Cultivars	Ellagic acid	Gallic acid	Cyanidin-3,5-di-*O*-glucoside chloride	Cyanidin 3-*O*-glucoside chloride	Pelargonidin 3-*O*-glucoside chloride
Mollar de Elche	1.90	2.67	35.6	20.6	12.9
Valenciana	2.77	<DL	8.0	0.4	0.2
White	1.75	0.56	57.0	24.1	3.7
CG8	2.14	0.41	108.3	12.4	1.4
Cis 127	0.99	0.73	5.9	0.5	<DL
Katirbasi	4.83	2.68	63.0	–	3.0
Parfianka	0.69	<DL	34.6	19.1	2.5
Wonderful 1	1.72	0.19	54.1	27.6	2.5
Wonderful 2	2.54	<DL	72.9	26.2	2.0

DL detection limit

Comparing our results with Li et al. [70], who had studied 10 Chinese cultivars, higher ranges were found, namely: 0.70–15.93 mg/l.

Thirteen different flavonoids belonging to five subclasses of non-colored flavonoids (flavan-3-ols, flavonols, flavanones, flavones, and dihydrochalcones) were identified, all previously reported in pomegranate juice [50, 51]. The highest number of non-colored flavonoids compounds (11 compounds) was detected in the juice of Cis 127.

Among the lignans identified, pinoresinol (48), cyclolariciresinol hexoside (50), and secoisolariciresinol hexoside (51) were detected in five cultivars, while syringaresinol (49) was only detected in CG8. Moreover, in Valenciana and Wonderful 1 cultivars, no lignans were detected.

Regarding organic acids, citric acid (53) and L-malic acid (52) have been pointed out as the main organic acids in pomegranate juices [46]. In the present study, Mollar de Elche and Cis 127 were the only cultivars with L-malic acid, whereas, citric acid was presented in Valenciana, Cis 127, Katirbasi and Wonderful 2.

Furthermore, the pomegranate juice color is due to the presence of anthocyanins. So, in the present work the cyanidin-3,5-di-*O*-glucoside, cyanidin-3-*O*-glucoside and pelargonidin-3-*O*-glucoside chloride were quantified (Table 5). Regarding their individual concentrations, the highest value of cyanidin-3,5-di-*O*-glucoside chloride was obtained for CG8 cultivar (108.3 mg/l), while the lowest concentrations were observed for Cis 127 (5.9 mg/l) and Valenciana (8.0 mg/l). Concerning cyanidin-3-*O*-glucoside chloride, compound that increases the fibrinolytic potency of vascular endothelial cells and may prevent thrombus formation, the Wonderful 1 cultivar was the one with the highest content (27.6 mg/l), closely followed by Wonderful 2 (26.2 mg/l). Mollar de Elche presented the highest content of pelargonidin-3-*O*-glucoside chloride (12.9 mg/l) that is an anthocyanin able to protect successfully membrane lipids against oxidation induced by both chemical such as AAPH (2,2′-azobis(2-methylpropionamidine) dihydrochloride) radicals and physical (UVC radiation) agents [71]. For both compounds, Valenciana and Cis 127 were the cultivars that again presented the lowest concentrations, in line with the results obtained for the monomeric anthocyanin pigment contents (Table 3). Regarding the anthocyanins concentration, they follow the next order: cyanidin-3,5-di-*O*-glucoside, cyanidin-3-*O*-glucoside, and pelargonidin-3-*O*-glucoside chloride. Similar order was reported for cultivars grown in Georgia [46]. However, this order was different than that reported for Raduníc et al. [52], who observed higher values of cyanidin-3-*O*-glucoside

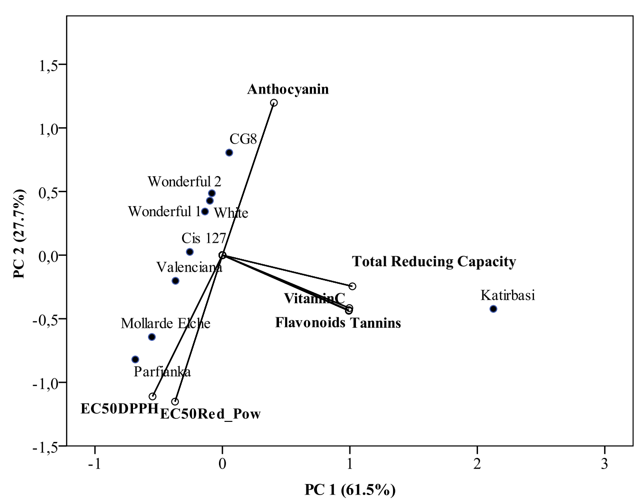

Fig. 3 Principal component analysis plot of nine pomegranate cultivars grown in Spain

than for cyanidin-3,5-di-*O*-glucoside in Croatian cultivars.

Principal component analysis

Principal component analysis (PCA) was applied to find any clusters within the analyzed pomegranate cultivars. The scores of the first two principal components for the nine pomegranate cultivars are presented in Fig. 3. The first two principal components took into account 89.2% (PC1 = 61.5% and PC2 = 27.7%, respectively) of the total variation. PC1 was highly contributed by total reducing capacity, flavonoids, hydrolyzable tannins, and vitamin C. PC2 was mainly correlated positively to monomeric anthocyanin pigment and negatively to EC_{50} values of DPPH and Reducing Power assays. Katirbasi could be separated from the other cultivars, with high scores in PC1 due to its high values of total reducing capacity, hydrolyzable tannins, flavonoids, and vitamin C. Regarding other cultivars, CG8 and Wonderful 2 presented the highest monomeric anthocyanin contents, whereas Mollar de Elche and Parfianka showed the highest EC_{50} values of DPPH and reducing power, indicating less antioxidant activity.

Conclusions

The presented study demonstrated that different pomegranate cultivars presented different physicochemical and antioxidant properties, which are factors of great importance to characterize pomegranate cultivars with respect to their future use. Mollar de Elche presented one of the highest TSS/TA ratio in juice and the lowest seeds % in arils and hydrolyzable tannins contents (less astringent), being appropriate for fresh consumption and juice industry. On the other hand, Katirbasi cultivar presented distinctive characteristics from the other cultivars because its juice showed the highest values of flavonoids, hydrolyzable tannins, and vitamin C, explaining its high antioxidant potential, measured by total reducing capacity, suggesting health benefits for the consumers. Even though Katirbasi presented the smallest fruits, this cultivar should be valorised by its juice chemical properties. A total of 53 polyphenols were identified, including 20 hydrolyzable tannins, 15 phenolic acid derivates, 12 non-colored flavonoids, four lignans, and two organic acids. Among the nine pomegranate cultivars' juices different compounds were detected and quantified such as ellagic acid, cyanidin-3-glucoside and pelargonidin-3-*O*-glucoside chloride, compounds with potential in the prevention and treatment of diseases. This study might provide valuable information on pomegranate cultivars in order to better characterize them and for developing new beverages or products where cultivars less appreciated by consumers may be added.

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Compliance with ethical standards

Conflict of interest None.

Compliance with ethics requirements This article does not contain any studies with human or animal subjects.

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