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Qualitative and varietal characterization of pomegranate peel: High-value co-product or waste of production?

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ABSTRACT

Fruit peel (i.e. pericarp + mesocarp) is the most abundant by-product of the agri-food pomegranate processing chain. In recent decades, pomegranate fruit peel is attracting a lot of attention due to the presence of bioactive molecules suitable to be used in the production of food supplements. This study aimed at investigating and characterizing the nutritional and nutraceutical quality of the peels of seven important pomegranate cultivars selected considering the fruit ripening period (early, medium and late), the type of cultivar (sweet, semi-acid and acid cultivar), and the seed hardness. Early (Purple Queen, Acco, and 29–101) and medium (MR100) ripening cultivars showed higher percentages of crude fiber (>2.2%) than the late ripening cultivars (Wonderful, Kingdom and ME17). The highest content of organic acids, punicalagin and its derivates were observed in the peel of acid and semi-acid cultivars (Wonderful, Kingdom and Acco). Acco fruit peel exhibited the highest antioxidant and radical scavenging activity (1.30 and 0.88 mmol Trolox g^{-1} on a dry weight basis d.w., respectively) as well as total soluble polyphenol (249 mg GAE g^{-1} d.w.) content. No consistent relationship considering sugar concentration and mineral composition was observed. The wide quantitative and qualitative diversity of bioactive compounds and nutrients observed in the analysed fruit peels showed that the overall biochemical profile is genotype dependent. So far, the findings of this research further highlighted that pomegranate fruit peel contains a wide variety of beneficial compounds, thus showing a high potential in terms of nutrients and nutraceutics, so confirming the importance of pomegranate fruit peels for pharmaceutical and medical purposes.

Abbreviations

TSP	total soluble polyphenol
RSA	radical scavenging activity
AA	antioxidant activity
NMR	nuclear magnetic resonance
GAE	gallic acid equivalent
D.W.	dry weight.

1. Introduction

The estimated worldwide production of fruits is about 800 millions of metric tonnes (Food and Agriculture Organization (FAO) 2021) from which processing industries generate more than 500 million tonnes of waste (Banerjee et al., 2017). The amount and availability of this feedstock for recovery of bioactive compounds such as pectin, lipids, polyphenols, dietary fiber, etc., has encouraged researchers to perform detailed studies on the value that could be added to fruit production chains from fruit processing waste. Peels, pomace, kernels and seed fractions from processed fruits could potentially be a good source of energy, fibers and bioactive compounds. Fruit by-products represent relevant sources of functional compounds, which could be efficiently used for various industrial applications, such as the production of fertilizers, animal feed, cosmetics and supplements. This sounds strategically important since it allows the creation of new products with high quality features without using the material intended for human consumption. On the other hand, fruit residues have been inadequately disposed leading to pollution problems as well as high costs (Shalini and Gupta, 2010), whilst nowadays special attention is paid to recover residues, separating them into specific components that can have different

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applications depending on the market, thus meeting the modern concepts of waste reuse and circular economy.

Pomegranate (*Punica granatum* L.) is a fruit species appreciated since ancient times for its hardiness, being capable of fruiting in adverse soil conditions (i.e. salt and heat stress). Nowadays, pomegranate has gained major attention due to the multifunctional nutritional quality and health benefits of their fruits. Pomegranate fruit, botanically denominated balausta, shows a quite complex structure, since it is formed by fleshy seeds (with the testa containing the juice and the tegmen holding the embryo and its structures) grouped in compartments (carpels) and separated by carpelar membranes, the latter forming the mesocarp together with the placentar membrane, and the pericarp (rind) that wraps the whole fruit (Melgarejo et al., 2020). Generally, pomegranate pulpy seeds are mainly used for fresh consumption, or industrially processed to obtain fruit juice, according to Directive 2012/12 EU. Conversely, the pomegranate pomace (i.e. rind and internal membranes), is discarded, thus representing a waste.

In the last decade, pomegranate peel has been thoroughly investigated given the high content and number of different phytochemicals (Singh et al., 2018; Balli et al., 2020a,b). Notably, the most cited groups are represented by phenolic acids and hydrolysable tannins, such as ellagic acid and punicalagin, since they are responsible for mediating the antioxidant capacity and radical scavenging activity response against several diseases and inflammations (Akhtar et al., 2015; Sorrenti et al., 2019). Furthermore, other less investigated compounds in the pomegranate peel are individual sugars and organic acids. As for the edible part of fruit, organic acids contribute to increase the peel antioxidant activity (Pande and Akoh, 2009), while the characterization of sugars offers important information on fiber and polysaccharide fractions providing probiotic activity (Hasnaoiu et al., 2014). Minerals can also contribute to increase the nutritional values of pomegranate fruit, and some macro-elements such as K and Ca are mostly accumulated in the fruit external tissues (i.e. rind and mesocarp) rather than seeds (Fawole and Opara, 2012). A sound and specific knowledge on the elemental composition of pomegranate fruit parts is relevant in dietary information and, in particular, in the quest to enrich food composition.

The presence and amount of the above-mentioned compounds in pomegranate fruits are determined by a combination of environmental, agronomic and genetic factors, the latter one being scarcely investigated when referring to the bioactive compounds of the pomace, in spite of the substantial changes of the biochemical profile among different varieties of pomegranate fruits (Balli et al., 2020a).

In this context, the aim of this research was to investigate and compare the nutritional and nutraceutical composition of the peel fruit (rind plus internal membranes) of traditional varieties and recently released cultivars.

Table 1
Main characteristics of the seven pomegranate cultivars studied in this work. Ripening time related to Ojòs (Murcia, Spain).

	Seed hardness	Type of cultivar
Early ripening cultivar (*from August to September*)		
Acco	Soft	Semi-acid
Purple Queen	Soft	Sweet
29–101	Soft	Sweet
Medium ripening cultivar (*from September to October*)		
MR100	Soft	Sweet
Late ripening cultivar (*from October to November*)		
Wonderful	Hard	Acid
ME17	Soft	Sweet
Kingdom	Hard	Semi-acid

2. Materials and methods

2.1. Plant material and sample processing

Seven pomegranate varieties have been chosen for the trial (**Table 1**). Three traditional varieties (29–101, ME17 and Wonderful) and four novel cultivars (MR100, Acco, Purple Queen and Kingdom) were selected on the basis of their commercial and quality characteristics according to previous researches carried out by Tozzi et al. (2020), on the nutraceutical and nutritional profile of the edible part of the same fruit varieties.

Pomegranate trees were cultivated under homogeneous conditions in an experimental farm located in the southeast of Spain (Ojós, Murcia), in suitable areas for pomegranate cultivation (Tozzi et al., 2020). Pomegranate trees were about 8 and 10 years-old, presented appropriate sanitary conditions and were cultivated under conventional management practices. Pomegranate fruits were harvested in 2018 at commercial ripening from three trees per cultivar and transported immediately to the laboratory. For each cultivar, 15 fruits in good conditions, without any defects in peel (sunburns, cracks, cuts, or bruises) were chosen and randomly separated in three replicates (5 fruits each) for analysis. The main pomological traits for each cultivar are presented in **Table 2**.

After washing the fruits with tap and Milli-Q water, the non-edible part (i.e. pericarp + mesocarp) was gently separated from the seeds, weighted, and then freeze-dried (Christ Alpha 2–4, LSCplus) until constant weight. Successively, the samples were finely grinded and passed through a 0.5 mm sieve and then stored under constant temperature (−5 °C) until analysis. For all genotypes, the analysis was carried out maximum one week after samples storage.

2.2. Analysis of the crude fiber

Crude fiber contents were determined through acid and alkaline digestion by a digester (Ankon fiber analyzer model A220), according to the official methodology established by the Spanish Ministry of Agriculture, Fisheries and Food (M.A.P.A., 1993). Briefly, the acid digestion was prepared with sulfuric acid (H_2SO_4, 1.25% *w/v*) for removing sugar and starch, while alkaline digestion was carried out with sodium hydroxide (NaOH, 1.25% *w/v*) for removing proteins. Between the two extractions, samples were washed with abundant water to eliminate reagents residues and to neutralize the pH. As the last step, a cold extraction with acetone was made due to the oil content in pomegranate samples.

2.3. Sugars and organic acids determination

Sugars and organic acids were extracted and quantified according to Tozzi et al. (2020) with some modifications. An aliquot (about 50 mg) of freeze-dried pomegranate peel was extracted in a methanol/water

Table 2
Mean ($n = 15$) and standard deviations (in bracket) of the weight values (g) of total fruit, peel (pericarp and mesocarp), and seeds (testa and tegmen), peel percentage in the whole fruit, and skin thickness (mm) of the analysed pomegranate cultivars.

Cultivar	Fruit weight	Peel weight	Seed weight	Peel percentage	Skin thickness
Wonderful	501 (31)	208 (13)	293 (21)	42 (2)	3.8 (0.6)
ME17	403 (47)	178 (21)	225 (34)	44 (4)	4.5 (0.6)
Acco	184 (25)	79 (11)	105 (17)	42 (4)	2.2 (0.8)
Kingdom	568 (53)	265 (43)	303 (34)	47 (5)	4.8 (0.9)
29–101	382 (46)	175 (27)	207 (32)	46 (4)	4.3 (0.7)
MR100	406 (52)	150 (28)	256 (29)	37 (3)	3.9 (0.6)
Purple Queen	319 (33)	134 (11)	185 (24)	42 (2)	3.9 (0.3)

(50/50 *v/v*) solution. The extract was vortexed for 1 min, sonicated for 3 min and finally centrifuged at 5000 x g for 20 min at 4 °C. The supernatant was collected, dried in a spider vacuum at 27 °C, and resuspended in 800 μL of potassium dihydrogen phosphate (100 mM KH_2PO_4 pH= 6.0) buffer in deuterium oxide (D_2O), containing 0.58 mM trimethyl silyl propionic acid sodium salt (TSP). The mixture was centrifugated at 4000 × g for 5 min at 4 °C and the supernatant (600 μL) was introduced into a 5 mm nuclear magnetic resonance (NMR) tube for analysis. All spectra were recorded on a Bruker AV-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 = 4.0 μs (90°), and relaxation delay = 2.0 s. Free induction decay was Fourier-transformed with line broadening = 1 Hz, Gaussian broadening = 0, and peak-picking sensitivity = 1.0. The 1H-NMR spectra were analysed with Chenomx Profiler (v. 8.0., Edmonton, Canada) to obtain the concentrations of primary metabolites in pomegranate peel. 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.

2.4. Extraction and determination of TSP, RSA, AA and individual phenolic compounds

The sample extraction for the determination of TSP, RSA, AA, and individual phenolic compounds was performed through a water decoction according to Balli et al. (2020a). More in detail, a freeze-dried pomegranate peel aliquot (5 g dry weight, d.w.) was boiled with 200 mL of Milli-Q water for 1 h. The decoction was centrifuged (4500 x g, 8 min, 4 °C) in order to remove the supernatant and then taken to a final volume of 200 mL with Milli-Q water. Before the analysis, each aqueous extract was centrifuged at 4000 x g for 4 min and passed through a 0.45 μm PTFE filter (Waters, Milford, USA).

All spectrophotometric measurements were carried out using a UV–VIS Spectrophotometer (Termospectromic Helios Gamma UVG 1002 E, Cambridge, UK), according to Renai et al. (2021) with some modifications. TSP were analysed according to the Folin–Ciocalteu method: 100 μL of the extract was mixed with 200 μL of Folin–Ciocalteu reagent and 2 mL of Milli-Q water. After 3 min, 1 mL of an aqueous solution saturated with sodium carbonate was added. Then, the solution was dark incubated for 1 h and afterwards the absorbance was measured at 765 nm. Calibration lines were previously prepared with gallic acid as reference standard. Results were expressed as mg of gallic acid equivalent (GAE) g^{-1} d.w.

RSA was determined through the methods based on 2,2-diphenyl-1-picrylhydrazyl (DPPH). Briefly, 10 μL of the extract was mixed with 40 μL of methanol (MeoH/$H_2$0 80:20 v/v plus 1% of HCl) and added to 950 μL of DPPH solution. The mixture was shaken and placed in a dark room for 15 min. The decrease in absorbance was measured at 515 nm. In addition, AA was measured via ferric reducing antioxidant power (FRAP) assays. This method is based on measuring the absorbance increase at 593 nm due to the reduction of the ferric ion (Fe^{3+}) to the ferrous ion (Fe^{2+}) promoted by the antioxidant compounds. The results were expressed as millimoles of Trolox equivalents g^{-1} d.w.

Chromatographic analysis was performed as described by Tozzi et al. (2020) using an Agilent 1200 series HPLC-ESI-DAD-MS^n Ion Trap (Agilent 1200 series 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: (A) water-formic acid (95:5, *v/v*) and (B) 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 for 2 min. The identification of the compounds was performed by the fragmentation patterns obtained from mass spectra. The quantification of the phenolic compounds was performed by comparing chromatography with pure standards of ellagic (Sigma Chemical Co. St. Louis, MO, USA) and punicalagin (Chengdu Biopurify Phytochemicals Ltd., Sichuan, China) and their maximum absorbance spectrum at an emitted wavelength at 290 nm and 320 nm through a diode array detector (DAD) integrated in the HPLC and connected in line to the mass spectrometer.

2.5. Determination of macro and micro minerals

The elemental analysis (K, Ca, P, Mg, Na, Fe, Zn, Cu, Mn and Ni) was performed on the freeze-dried peel samples. Freeze-dried peel aliquots (500 mg) were finely grinded and mineralized with 10 mL of nitric acid by microwave (model Ethos 1, Milestone, Bergamo, Italy). The following microwave digestion program was adopted: from ambient temperature to 200 °C in 15 min and then isotherm at 200 °C for 15 min. After mineralization, each sample was diluted with Milli-Q water to obtain a final volume of 25 mL and then, the extracts were analysed by inductively coupled plasma (ICP) (iCAP 7400 DUO ICP-OES; Thermo Fisher Scientific, Waltham, MA, USA).

2.6. Statistical analysis

The Shapiro–Wilk test was used to evaluate the normality of the data. Data were processed with a one-way analysis of variance (ANOVA) followed by Fisher's Least Significant Difference (LSD) procedure at 95.0% confidence level were performed (Minitab ® 17.1.0, Minitab Inc., State College, PA, USA).

3. Results

3.1. Crude fiber

The cultivars showed significant differences in terms of crude fiber percentage in the peel (Fig. 1). The highest percentage was found in MR100 (2.8%), followed by Acco, Purple Queen and 29–101, which showed similar values, equal to about 2.2%. ME17 and Kingdom displayed lower values with an average of 1.6%, while Wonderful resulted the cultivar with the lowest percentage (1.1%).

3.2. Sugars and organic acids profile

Table 3 illustrates individual (fructose, glucose, sucrose, xylose, ribose and myo-inositol) and total sugar concentrations found in pomegranate peel. The highest total sugar concentration was found in 29–101 (524 mg g^{-1}), followed by ME17 (472 mg g^{-1}), MR100 (411 mg g^{-1}), Purple Queen (395 mg g^{-1}), Kingdom (392 mg g^{-1}), and Wonderful (389 mg g^{-1}), whereas Acco exhibited the lowest value (338 mg g^{-1}). Since fructose and glucose were the most abundant individual sugars, their concentrations followed the same statistical trend of the

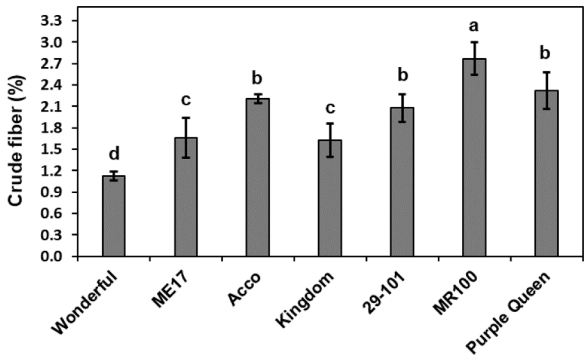

Fig. 1. Mean values ($n = 3$) of crude fiber (%) found in pomegranate peels. Bars represent standard deviations. Different letters mean statistically significant differences ($P < 0.05$).

Table 3
Mean values ($n = 3$) and standard deviations (in bracket) of individual, total sugars, individual and total organic acids (mg g^{-1} dw.) found in pomegranate peels. Within a same row, different letters mean statistically significant differences ($P < 0.05$).

Parameters	Wonderful	ME17	Acco	Kingdom	29–101	MR-100	Purple Queen
Sugars							
Fructose	179 (5) d	223 (4) b	155 (2) e	180 (2) d	263 (5) a	191 (3) c	180 (2) d
Glucose	185 (2) d	229 (4) b	160 (5) e	188 (2) d	244 (3) a	203 (8) c	190 (3) d
Sucrose	1.17 (0.03) d	1.56 (0.03) c	2 (0.1) a	1.1 (0.1) d	1.8 (0.1) b	1.2 (0.1) d	1.5 (0.1) c
Xylose	13 (1) a	8 (1) c	8 (0.3) c	12 (1) a	8 (1) c	8 (1) c	10 (0.5) b
Ribose	9 (1) b	8 (1) c	11(1) a	9 (1) b	4.6 (0.4) e	6 (0.3) d	12 (1) a
Myo-inositol	1.6 (0.1) b	2.2 (0.1) a	2.1 (0.1) a	1.7 (0.1) b	2.4 (0.2) a	1.7 (0.3) b	2.1 (0.2) a
Total	389 (2) d	472 (8) b	338 (4) e	392 (3) d	524 (2) a	411 (11) c	395 (6) d
Organic acids							
Citric	103 (4) a	16 (2) c	18 (1) c	106 (2) a	52 (5) b	15 (1) c	17 (1) c
Malic	2.8 (0.3) d	8 (1) ab	5 (1) c	2.8 (0.2) d	7 (1) b	8.3 (0.3) ab	9 (1) a
Fumaric	0.31 (0.03) d	0.21 (0.02) e	0.38 (0.04) c	0.34 (0.02) bcd	0.32 (0.02) cd	0.37 (0.03) bc	0.56 (0.04) a
Succinic	0.08 (0.01) b	0.04 (0.003) d	0.13 (0.02) a	0.08 (0.01) b	0.039 (0.002) d	0.040 (0.004) d	0.07 (0.01) c
Total	106 (4) a	24 (2) c	24 (1) c	109 (2) a	59 (5) b	24 (1) c	27 (2) c

total sugar concentration. Taking into account sucrose, Wonderful, Kingdom, and MR-100 peel varieties showed values fairly constant (1.1–1.2 mg g^{-1}), whereas an increasing trend was found in the order Purple Queen ≈ ME17 < 29–101 < Acco, the last one exhibiting a sucrose concentration of 2 mg g^{-1}. Pomegranate peels also showed the presence of other sugars such as xylose, ribose and myo-inositol. For these sugars, statistically significant differences have been observed among the samples with wide variations in their concentrations, i.e. 8–13 mg g^{-1}, 4.6–12 mg g^{-1} and 1.6–2.4 mg g^{-1}, for xylose, ribose and myo-inositol, respectively.

Individual (citric, malic, fumaric and succinic) and total organic acid concentrations found in the peel of each cultivar are illustrated in Table 3. The highest total organic concentration was found in Wonderful and Kingdom peel (106 and 109 mg g^{-1}, respectively), followed by 29–101 (59 mg g^{-1}), while the remaining cultivars showed similar and almost equal values (24–27 mg g^{-1}). Citric acid was by far the predominant individual organic acid in pomegranate peel, thus following the same statistical trend of total organic acids. As regards malic acid, Purple Queen, MR100, and ME17showed the highest concentration (8–9 mg g^{-1}), followed by 29–101 (7 mg g^{-1}), Acco (5 mg g^{-1}) and Kingdom/Wonderful (2.8 mg g^{-1}). Taking into account fumaric acid, Purple Queen and ME17 presented the highest and the lowest concentrations (0.56 and 0.21 mg g^{-1}, respectively), while the other cultivars had quite similar values (0.31–0.38 mg g^{-1}). Finally, succinic acid was found at the highest concentrations in the Acco cultivar (0.13 mg g^{-1}), followed by Wonderful, Purple Queen and Kingdom with comparable concentrations (0.07–0.08 mg g^{-1}), whilst the other cultivars exhibited the same concentrations (0.04 mg g^{-1}).

3.3. TSP, RSA, AA and individual phenolic compounds

The peel decoctions of the seven cultivars showed a consistent trend of TSP, DPPH-RSA and FRAP-AA values (Table 4). Statistically significant differences were observed, being the peel of the Acco cultivar the richest in TSP (249 mg GAE g^{-1}) and exhibiting the highest RSA and AA of 0.88 and 1.30 mmol Trolox g^{-1}, respectively. In the other cultivars, TSP ranged from 115 to 196 mg GAE g^{-1} in Wonderful and MR100, RSA from 0.39 to 0.72 mg Trolox g^{-1} in ME17 and Purple Queen, and AA from 0.59 to 1.07 mg Trolox g^{-1} in Wonderful and Purple Queen. The most predominant ellagitannins were represented by α- and β-punicalagin, followed by pedunculagin and β-punicalin. These compounds were found significantly higher in Acco, Kingdom, and Wonderful peels, displaying mean values of 23.7, 32.2, 11.3, 6.9 mg g^{-1}, while the other cultivars showed lower values of about 17.8, 26.4, 9.5 and 4.2 mg g^{-1}, respectively. Acco peel exhibited the highest concentration of ellagic acid (5.3 mg g^{-1}), coumaric acid derivative (4.9 mg g^{-1}), galloyl-HDDP-gluconic acid (1.7 mg g^{-1}), galloyl-HDDP-hexoside (2.8 mg g^{-1}). Ellagic acid hexoside and HHDP-hexoside were significantly higher in Wonderful (6.3 and 3.2 mg g^{-1}, respectively), and lower in ME17 and 29–101 (2.9 and 1.7 mg g^{-1}, respectively).

3.4. Macro and micro-mineral concentration

Mineral concentration of pomegranate peels is reported in Table 5. For K, Ca and P, despite the significant differences, the cultivars did not exhibit wide variations with ranges from 9 g kg^{-1} (ME17 and Kingdom) to 12 g kg^{-1} (Wonderful, 29–101 and Purple Queen) for K, from 1.03 g

Table 4
Mean values ($n = 3$) and standard deviations (in bracket) of total soluble polyphenols (TSP, mg GAE g^{-1} dw.), antiradical and antioxidant activities, as measured by DPPH and FRAP methods (mmol Trolox g^{-1} dw.) and individual phenolic compounds (mg g^{-1} dw.) found in pomegranate peels. Within a same row, different letters mean statistically significant differences ($P < 0.05$).

Parameters	Wonderful	ME17	Acco	Kingdom	29–101	MR-100	Purple Queen
Total soluble polyphenols							
TSP	115 (3) f	180 (4) d	249 (4) a	146 (3) e	191 (3) b	196 (2) bc	188 (3) c
Antiradical/antioxidant activities							
DPPH	0.40 (0.07) c	0.39 (0.05) c	0.88 (0.13) a	0.47 (0.05) c	0.41 (0.06) c	0.51 (0.12) c	0.72 (0.03) b
FRAP	0.59 (0.02) e	0.75 (0.04) de	1.30 (0.24) a	0.82 (0.04) cd	0.83 (0.05) cd	0.97 (0.05) bc	1.07 (0.10) b
Phenolic compounds							
Ellagic acid	4.2 (0.2) c	3.4 (0.2) d	5.3 (0.2) a	2.8 (0.1) e	2.1 (0.1) f	3.9 (0.2) c	4.8 (0.3) b
Ellagic acid hexoside	6.3 (0.4) a	2.9 (0.2) d	4.6 (0.2) b	4.8 (0.2) b	3.2 (0.2) cd	3.0 (0.1) cd	3.3 (0.1) c
Coumaric acid derivative	4.5 (0.2) b	1.2 (0.1) f	4.9 (0.2) a	3.7 (0.1) c	2.5 (0.2) d	1.6 (0.1) e	2.6 (0.1) d
Galloyl-HDDP-gluconic acid	1.4 (0.2) b	1.2 (0.1) bc	1.7 (0.2) a	1.2 (0.1) bc	1.13 (0.08) c	1.10 (0.05) c	1.3 (0.1) bc
Galloyl-HDDP-hexoside	1.2 (0.1) d	1.0 (0.1) e	2.8 (0.1) a	1.9 (0.1) c	1.2 (0.1) d	1.10 (0.03) de	2.2 (0.1) b
HHDP-hexoside	3.2 (0.2) a	2.1 (0.2) de	2.9 (0.2) ab	2.3 (0.5) cd	1.7 (0.1) e	2.6 (0.2) bc	2.9 (0.3) ab
Pedunculagin	11.8 (0.3) a	10.2 (0.3) b	11.8 (0.1) a	10.3 (0.3) b	8.2 (0.5) d	9.1 (0.2) c	10.7 (0.6) b
α-punicalagin	22.5 (0.7) b	15.8 (0.7) e	24.7 (1.5) a	24.0 (0.9) a	17.4 (0.4) d	18.5 (0.7) cd	19.3 (0.8) c
β-punicalagin	31.3 (1.1) a	25.3 (1.6) b	32.9 (1.6) a	32.3 (1.2) a	26.7 (1.6) b	26.1 (1.9) b	27.6 (1.2) b
β-punicalin	6.7 (0.5) a	3.1 (0.2) d	7.1 (0.3) a	7.0 (0.7) a	5.3 (0.2) b	3.9 (0.1) cd	4.6 (0.3) bc

Table 5
Mean values ($n = 3$) and standard deviations (in bracket) of K, Ca, P (expressed as g kg^{-1} dw), Mg, Na, Fe, Zn, Cu, Mn and Ni (expressed as mg kg^{-1} dw.) found in pomegranate peels. Within the same column, different letters mean statistically significant differences ($P < 0.05$).

Cultivar	K	Ca	P	Mg	Na	Fe	Zn	Cu	Mn	Ni
Wonderful	12 (0.1) a	1.6 (0.03) b	0.80 (0.01) b	325 (5) a	97 (4) c	18 (1) c	5 (1) ab	2.16 (0.02) cd	3.5 (0.2) b	0.07 (0.01) d
ME17	9 (0.1) c	0.7 (0.01) f	0.51 (0.01) e	204 (3) e	62 (4) e	19 (3) c	4.2 (0.3) bc	2.0 (0.1) cde	1.7 (0.1) d	0.15 (0.02) c
Acco	10 (0.3) b	1.86 (0.03) a	0.53 (0.01) e	223 (6) d	137 (8) b	26 (4) b	4.4 (1) abc	1.95 (0.3) e	4.3 (0.2) a	0.28 (0.04) b
Kingdom	9 (0.2) c	1.34 (0.03) c	0.70 (0.02) c	258 (8) b	86 (4) d	26 (3) b	5.1 (0.4) a	2.2 (0.3) bc	2.5 (0.3) c	0.14 (0.01) c
29–101	12 (0.4) a	1.03 (0.03) e	0.86 (0.02) a	232 (8) cd	70 (3) e	47 (5) a	5.1 (0.2) a	2.6 (0.2) a	3.6 (0.1) b	0.38 (0.06) a
MR100	11 (0.1) b	1.13 (0.02) d	0.71 (0.01) c	236 (4) c	235 (5) a	31 (1) b	3.6 (0.2) c	2.47 (0.03) ab	2.31 (0.02) c	0.04 (0.01) d
Purple Queen	12 (0.1) a	1.17 (0.01) d	0.6 (0.01) d	175 (2) f	133 (4) b	27 (1) b	4.0 (0.8) c	1.9 (0.1) e	4.12 (0.03) a	0.05 (0.01) d

kg^{-1} (29–101) to 1.86 g kg^{-1} (Acco) for Ca, and from 0.51 g kg^{-1} (ME17) to 0.86 g kg^{-1} (29–101) for P. On the contrary, Mg and Na peel concentrations undergo great differences among cultivars, with values between 175 (Purple Queen) and 325 (Wonderful) mg kg^{-1} and between 62 (Acco) and 235 (MR100) mg kg^{-1}, respectively for Mg and Na. Iron concentrations varied from 18 (Wonderful) to 47 (29–101) mg kg^{-1}, Zinc from 3.6 (MR100) to 5.1 (Kingdom and 29–101), Cu from 1.9 (Purple Queen and Acco) to 2.6 (29–101) mg kg^{-1}, Mn from 1.7 (ME17) to 4.12 (Purple Queen) mg kg^{-1}, and Ni from 0.04 (MR100) to 0.38 (29–101) mg kg^{-1}.

4. Discussion

To date, it is unequivocal that pomegranate must be recognized as one of the most outstanding 'super fruits' thanks to the relevant phytochemical composition of the whole fruit (Melgarejo-Sánchez et al., 2021). The fact that the nutritional and nutraceutical properties of this fruit are not exhausted with the consumption of the edible part, adds value to the use of this fruit in food supplements and pharmaceutical products. However, while much attention has been given to the main edible part of the fruit (the fleshy part of the seeds), limited knowledge is currently available about the peel, the most abundant by-product of the food supply chain. In this context, the varietal characterization may provide useful information for addressing innovative production and market strategies, able to convert a by-product into a co-product.

Starting from the first parameter analysed in this study, the crude fiber, it can be noted that the peel of the selected varieties showed quite similar percentages (1.1–2.8%) (**Fig. 1**) to those present in the seeds of the same varieties (1.3–2.5%) (Tozzi et al., 2020). Interestingly, early and medium varieties exhibited higher crude fiber content (i.e. above 2%) compared to late varieties probably due to the shorter development period to reach fruit maturity, since, during fruit maturation, the fiber will be transformed into other compounds such as sugars, organic acids, among others (Anderson and Chen, 1979). The amount of crude fiber observed in the studied fruits confirms the potential of using pomegranate peel as preservative food additives in different food processes due to their water retention capacity (Sánchez-Zapata et al., 2009). Despite the fact that some studies already showed the use of pomegranate peel as a food preservative and/or animal feed (Hasnaoui et al., 2014; Sharma and Yadav, 2020), the finding here reported on the variability between cultivars represents a tool in decision-making for the improvement of the efficiency of the fiber extracting process based on varietal selection.

Taking into account sugar peel composition, fructose and glucose were the two most abundant sugars, contributing to similar amount (47 and 48%, respectively) to the total sugar (**Table 3**). Xilose and ribose equally accounted for the 2%, while sucrose and myo-inositol were the minor individual sugars representing 0.4 and 0.5% of the total sugar, respectively. Different individual sugars and total sugar compositions were individuated by other studies in fruits from other genotypes. More in details, in Tunisian varieties, Hasnaoui et al. (2014) reported xilose and arabinose as the main components accounting for the 60% of the total content, while glucose represented only the 10%. Conversely, Gavlighi et al. (2018) observed in an unknown Iranian variety that glucose was the most abundant sugar (45–68% of the total), while other sugars (i.e. galactose, mannose, arabinose and rhamnose) represented a lower amount (<19%). Only a study detected the presence of fructose in 29 Israeli accessions fruit peel (Dafny-Yalin et al., 2010). The presence of sucrose within the peel of all the selected cultivars was not completely expected, since its content tends to decrease with the hydrolysis of sucrose into fructose and glucose as the ripening process proceeds. Sometimes, the conversion of sucrose can be so pronounced to make it unquantifiable in both juice (Melgarejo et al., 2019) and peel (Dafny-Yalin et al., 2010). In this study, the major sucrose concentration was found in Acco peel, agreeing with its significantly lowest fructose and glucose values in the peel (**Table 3**). Based on pomegranate juice, the richest varieties in sugar concentration are those characterized by longer fruit development periods, as observed in medium late or late ripening varieties such as Kingdom and Wonderful, respectively (Tozzi et al., 2020; Borochov-Neori et al., 2009). Surprisingly, the low concentrations of glucose, fructose, sucrose and total sugars detected in Wonderful and Kingdom peel suggested an opposite trend, which could indicate a loss of sugars in the rind in favor of a greater translocation and accumulation towards the edible part as a consequence of the ripening process. These findings indicated that deeper investigations are advisable to explore the physiological mechanisms driving sugar synthesis and accumulation within pomegranate peel tissues. Pomegranate peels were also characterized by the presence of other minor sugars generally not quantified in pomegranate juice. Xylose is a fraction of the lignocellulosic biomass generally present in the peel of some fruit species, and it gained attention since it can be used for biorefinery purposes (i.e. bioethanol production) (Kamoldeen et al., 2017) or for either pharmaceutical and dietary purposes (i.e. xylitol production, a popular sweeteners suitable for diabetic patients) (Rahman et al., 2007). In this study, xylose accounted for an average of 2% of the total sugars, in accordance with values reported in other pomegranate varieties (Abid et al., 2017) or in plant species producing fruits with high peel-based by-products such as citrus, banana, apple and pear (Choi et al., 2015). Interestingly, Wonderful and Kingdom exhibited the highest concentrations of xylose (13 and 12 mg g^{-1}, respectively) and this finding could be attributed to their hardness of the seeds. At this regard, it is interesting to notice that recently specific comparative genetic analyses revealed many genetic differences between soft- and hard-seeded pomegranate varieties, indicating that genomic variations and selective genes may have contributed to the genetic divergence between soft- and hard-seeded pomegranate varieties (Lou et al., 2018). Myo-inositol is a six-carbon sugar alcohol, very diffuse in plant since it covers multiple metabolic and biological functions. In berry fruits (i.e. kiwifruit and blueberry), myo-inositol was associated with the maintenance of turgor during cell expansion stage (Cui et al., 2013), thus its occurrence in pomegranate peel could be ascribable to its role as an osmoprotectant and/or substrate for cell wall precursor (Mamat et al., 2020) or other relevant metabolites such as ascorbic acid (Songh et al., 2016) as indicated for other berry fruits.

Regarding organic acids, citric, malic, fumaric and succinic acids were individuated and quantified in the peel of all cultivars (**Table 3**), while tartaric acid was not quantified, even though appears frequently in the edible part of the fruit, especially in early genotypes (Tozzi et al., 2020). Citric and malic, accounting the 79 and 20% of the total acid,

definitely predominated over fumaric and succinic, representing only the 1 and 0.2%, respectively. Similar percentages were observed in peel by Pande and Akoh (2009). It can be observed a broad variability for citric acid among the cultivars, with significantly similar and higher concentrations for Wonderful and Kingdom. The amount of citric acid within the edible part is usually subjected to great fluctuation depending on the genotype, which determines the type of cultivars in terms of sweetness or acidity. Thus, this data indicated that in sour cultivars, the greater acidity of the juice, the greater citric content in the peel. Moreover, Wonderful and Kingdom, acid and semi-acid cultivars respectively, were also characterized by the lowest malic concentration (i.e. 2.8 mg g^{-1}) compared to sweet varieties (i.e. ME17, Purple Queen, 29–101, MR100), confirming again the same behavior usually observed in the edible part of pomegranate fruit (Alcaraz-Mármol et al., 2017; Tozzi et al., 2020). Regarding succinic acid, Acco cultivar showed the highest concentration (0.13 mg g^{-1}) with a value about 2.2 times greater than that observed for the other peels. The other cultivars followed the trend: Kingdom and Wonderful > Purple Queen > ME17, MR100 and ME17. Succinic is a minor acid, commonly present in trace amounts in pomegranate juice (Mekni et al., 2019) and for this reason scarce information is present in literature. Only two studies revealed the occurrence of succinic acid in pomegranate peel, reporting values slightly higher than those found in this study (Pande and Akoh, 2009; Dafny-Yalin et al., 2010). Nevertheless, these results suggest that there might be a preference for acid and semi-acid (Acco, Kingdom and Wonerful) cultivars to synthetize greater succinic acid than sweet cultivars (Purple Queen, ME17, MR100 and ME17) within the peel tissues. Fumaric acid was also detected in fruit peel, and, despite the significant differences, the cultivars displayed moderate variations, without showing a specific trend. Overall, the comparison of these results with literature is difficult since very few studies have assessed both sugar and organic acid profile in pomegranate peel. Thus, to the best of our knowledge, this is the first study investigating and characterizing the organic acids in the peel of these seven important and widely cultivated cultivars. Moreover, it is important to observe that the concentrations of sugars and organic acids found in peel of the selected cultivar peel is 27–34 times higher than the amount often found in the seeds (Melgarejo et al., 2000; Mekni et al., 2019), denoting pomegranate peel as a relevant by-product, rich in bio-compounds with high nutritional value, potentially reusable in other productive sectors, such as animal feed, supplements and cosmetic products.

Taking into account phenolic compounds, pomegranate peels were characterized by the presence of two phenolic acids (i.e. ellagic acid and coumaric acid derivative) and 8 ellagitannins (i.e. ellagic acid hexoside, galloyl-HDDP-gluconic acid, galloyl-HDDP-hexoside, HHDP-hexoside, pedunculagin, α- and β-punicalagin and β-punicalin) (**Table 4**). The range of concentration values found in our study is in line with those reported by Balli et al. (2020a) which used the same extraction method based on aqueous peel decoction. Among the phenolic compound individuated, α- and β-punicalagins were the most abundant, accounting approximately for 65–68% of the total ellagitannins. Punicalagin and its derivatives are the bioactive compounds that contribute most to the antioxidant capacity of the pomegranate peel (Singh et al., 2018). Peels from acid and semi-acid cultivars such as Wonderful, Kingdom and Acco were found with the highest concentrations of punicalagins compared to the other sweet cultivars. Nevertheless, only Acco peel possessed the greatest antioxidant and anti-radical performances and was denoted by the highest polyphenols content, while the peels from the other two acid and semi-acid cultivars displayed higher values, and the extent of this trend is particularly pronounced in Wonderful peel showing the lowest capacity (**Table 4**). These data suggest that the type of variety in relation to its acidic content influenced the amount of phenolic compounds, in particular ellagitannins, whereas the overall antioxidant and antiradical activity appeared to be affected by other parameters intrinsic to the genotype, such the peel color. Accordingly, the highest phenolic compound and antiradical and antioxidant activity found in Acco peel, can be explained by the color of the mesocarp. Acco peel is a cultivar denoted by a pronounced saturated red pigmentation, as previously reported by Tozzi et al. (2020), which observed high a* and Chrome index values. Red-fruited cultivars, such as Acco, have been associated with increased synthesis and accumulation of anthocyanins (Gözlekçi et al., 2011; Abid et al., 2017) an important group of flavanoids. Moreover, Acco peel decoction was found with the highest content of flavanoids among fifteen pomegranate cultivars (Balli et al., 2020a). Therefore, the high free radical scavenging potential of Acco peel may be associated to the presence of other bioactive compounds such as anthocyanins and other flavonoids typical of pomegranate peel (i.e. quercetin, kaempferol) (Zhao et al., 2014).

Considering macro and micro elements, the following trend was observed for all the studied samples: K > Ca > P > Mg > Na > Fe > Cu and Mn > Ni, (**Table 5**) confirming the same patterns also reported in Iranian (Mirdehghan and Rahemiand, 2007) and Turkish (Gözlekçi et al., 2011) pomegranate peel cultivars. This finding clearly suggests that, notwithstanding the different genotypes, geographical locations and agro-climatic environments, pomegranate peel followed a typical pattern of element absorption and accumulation in response to the physiological status. Nevertheless, mineral peel compositions seemed not to be influenced by the type of the cultivars (i.e. early or late ripening), since a clear trend was not individuated. As expected, K resulted the most abundant element in pomegranate fruit peel, as it is also the predominant element of the fruit edible portion (Tozzi et al., 2020). Potassium concentrations of the fruit peels ranged from 9 to 12 g kg^{-1}, in accordance with previous studies (Mirdehghan and Rahemi, 2007; Gözlekçi et al., 2011). Potassium plays an important role in fruit enlargement and cell turgidity, positively influencing fruit yield and quality (Davarpanah et al., 2017). Moreover, K has a favourable effect on fruit cracking, since this element is involved in the mechanism of opening and closing of stomata, providing greater flexibility and elasticity to the peel skin of the cellular wall. Pomegranate peels were rich in Ca, a very important element, especially for preventing fruit cracking disorder (Davarpanah et al., 2018). Peel Ca concentrations ranging from 0.7 to 1.86 g kg^{-1} (**Table 4**) were higher compared to those generally reported for seeds (0.21–0.56 g kg^{-1}; Tozzi et al., 2020), likely due to the low mobility of calcium in the phloem (Davarpanah et al., 2018). Taking into account micro-mineral, Fe, Zn, Cu, Mn and Ni concentrations were comparable to values previously reported in peel samples from other pomegranate varieties (Mirdehghan and Rahemiand, 2007).

5. Conclusions

The results of this study highlighted the compositional diversity of the peels among the different cultivars studied. This variability can be extrapolated when comparing the results of the peel with the edible pomegranate part (seeds and/or juice). Interestingly, organic acids and phenolic compounds showed synthesis and accumulation patterns similar to those of the edible part as in the case of acid and semi acid-cultivars (Wonderful, Acco and Kingdom). The crude fiber content appears to be influenced by the short ripening period of Acco, MR100, Purple Queen and 29–101 fruits. On the contrary, sugars and minerals appear to be unrelated and independent on the categories here considered.

In a practical way, the information obtained in this study can be used as a potential indicator that helps in decision-making for the definition of one pomegranate cultivar versus another based on its use/application and/or final consumption. This prior directionality for the use of the whole fruit (edible and inedible part) could help to complement the industry with agriculture sector in an integrated and sustainable way with the reduction and conversion of agro-food waste into raw materials with interest and economic value in a perspective of circularity of the agricultural, food and pharmaceutical supply chain.

Author contributions

All the authors contributed to the drafting of the manuscript. FT, DN, PL MDB and PM performed the analysis. FT, DN, PL, MDB, EG and 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. **Dámaris Núñez-Gómez:** Writing – original draft, Data curation, Formal analysis, Validation, Writing – review & editing. **Pilar Legua:** Formal analysis, Validation, Supervision. **Massimo Del Bubba:** Formal analysis, Validation, Supervision. **Edgardo Giordani:** Methodology, Supervision. **Pablo Melgarejo:** Conceptualization, Resources, Supervision.

Declaration of Competing Interest

None.

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