Adv. Hort. Sci., 2020 34(3): 277-286

DOI: 10.13128/ahsc-8961

AHS
Advances in Horticultural Science

Phenolic fingerprint in wild growing pomegranate fruits from Azerbaijan

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Key words: anthocyanins, phenolics, tannins, wild-growing pomegranate.

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Citation:
ZEYNALOVA A., NOVRUZOV E., BARTOLINI P., BRUNETTI C., MASERTI B., 2020 - *Phenolic fingerprint in wild growing pomegranate fruits from Azerbaijan.* - Adv. Hort. Sci., 34(3): 277-286

Data Availability Statement:
All relevant data are within the paper and its Supporting Information files.

Competing Interests:
The authors declare no competing interests.

Received for publication 1 December 2019
Accepted for publication 5 June 2020

Abstract: The demand for pomegranate (*Punica granatum* L.) juices is increasing worldwide due to its documented health-promoting effects which likely derive from phenolic compounds. This study reports the phenolic composition of the juices obtained from eight wild-growing pomegranate accessions collected in eight areas of Azerbaijan, characterized by different climate and soil composition. The anthocyanins found in all the accessions were cyaniding derivatives and pelargonidin derivatives, while only two accessions contained also delphinidin-3,5-O-diglucoside. The main hydrolysable tannins contained in the juices were punicalagin and ellagic acid derivatives. These bio-active metabolites found in the juices varied qualitatively and quantitatively among the eight accessions, thus constituting specific traits for selecting promising accessions that can be used as a nutritious food source. The different phenolic profiles might be determined both by genotype and the growing environmental conditions, or by their interaction. Our results suggest that some of the studied wild-growing pomegranate accessions might have a commercial value because of their richness in bioactive metabolites and might constitute a suitable source of genes for breeding programs.

1. Introduction

Punica granatum L. (pomegranate) originated in the region extending from Iran to northern India has been used since ancient times as fruit and for medical purpose. Recently an increasing of pomegranate cultivation and consumption has been reported because of its beneficial effects on human health (Kalaycıoğlu and Erim, 2017). In particular, the juice of pomegranate shows potent antioxidant (Lee *et al*., 2012; Aloqbi *et al.*, 2016), anti-atherosclerotic (Aviram *et al*., 2000) and anticancer effects (Derakhshan *et al*., 2018), due to its high concentration of punicalagin derivatives, ellagic acid derivatives (both in its free and conjugated forms), gallotannins and anthocyanins. The high concentration of polyphenols in pomegranate extracts has been also related to a strong anti-inflammatory, hepatoprotective and antigenotoxic properties has been recently investigated (Sartippour *et al.,* 2008; Santhini *et al.,* 2011). Moreover, pomegranate extract has been reported to be beneficial to enhance fruit post-harvest stability due to its antimicrobial and antifungal effects, mainly correlate to the content of punicalagin derivatives (Rongai *et al*., 2018, 2019).

Azerbaijan is one of the richest floristic regions and a major focus in the South-West Asian and it is the origin center of many cultivated plants including pomegranate (Zeynalova and Novruzov, 2017). Pomegranate carries a deep cultural and historical heritage in Azerbaijan and occupies a special place in cuisine and traditional medicine (Karasharli, 1979). In several regions of Azerbaijan, wild pomegranate accessions grow spontaneously in self-maintaining populations in natural or semi-natural ecosystems independently of direct human action. In particular, wild pomegranate accessions can be found at the foothills and in lower mountain zones of the Greater and Lesser Caucasus, in the Kur-Araz and Samur-Divichinsk lowlands and in the mountain belt of the Talysh Plateau, on dry slopes up to 700 m above sea level (Asadov and Asadov, 2001).

Wild plant accessions of cultivated fruits gained attention to scientific community, since they have developed a high adaptability to climatic pressures and resistance to diseases and pests, accumulating high amount of secondary metabolites respect to cultivated varieties (Howard *et al*., 2003; Kassim *et al*., 2009). The genetic background of each accessions plays a key role in the responses to environmental conditions, resulting in accessions more or less sensitive to different environmental pressures (Schwartz *et al*., 2009), however, in most cases, climate effects on arils' composition is substantial since phenol metabolism, both synthesis and oxidation, is induced by stressful conditions, especially thermal stress (Di Stefano *et al*., 2019). At this regards, it has been shown that seasonal warming increases the content of diglucosylated anthocyanins (Borochov-Neori *et al*., 2011).

In the framework of a systematic study on the functional properties of wild pomegranate plants in Azerbaijan, this work reports the phenolic fingerprinting of the juices obtained from wild growing pomegranate accessions collected in eight different areas of Azerbaijan (Zeynalova *et al.*, 2019), as a starting point to select wild-growing suitable genotypes that can be used in breeding programs for the production of fruits and juices with a high content of bioactive metabolites.

2. Materials and Methods

Plant material collection

Fresh ripe pomegranate (*Punica granatum* L.) fruits were collected from seven districts of Azerbaijan. Geographical coordinates and altitude corresponding to each surveyed area are shown in (Fig. 1 and Table S1). The soil of the different areas was analyzed. Pomegranate fruits were collected at the same maturity stage from of 25th September and October 2nd 2018 during a field campaign extensively described in a companion paper (Zeynalova *et al*., 2019). Each accession sample (Pg) was the pool of three fruits from three different trees collected in each sampling station. Details on the physio-chemical and organoleptic characteristics of the fruits are reported in Zeynalova *et al*. (2019).

Juice preparation

After collection, fruit juices were prepared as described by Zeynalova *et al.* (2019). Briefly each fruit was weighed individually, then the arils, peel and calyx membranes were manually divided and juice was extracted by mechanical press, directly from arils to prevent the contamination of phenolic components from the skin or peels membrane into the juice, and then stored at -80°C until HPLC analysis.

Chemicals

All reagents and solvents were of HPLC grade (Sigma Aldrich, Italy). The following standards were used for identification and quantification purposes with HPLC-DAD: cyaniding 3-glucoside, delphinidin 3-

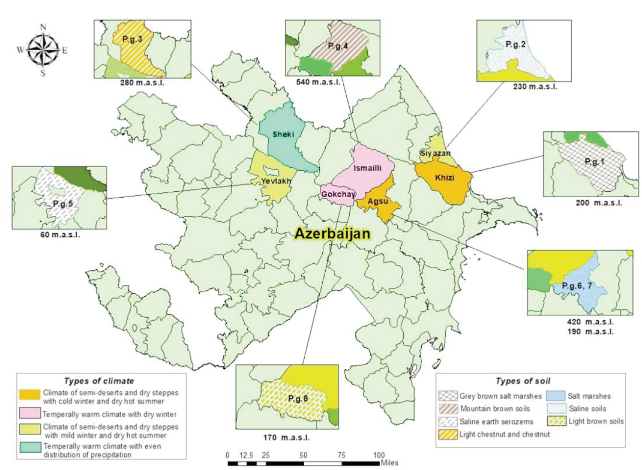

Fig. 1 - Selected sampling stations in Azerbaijan districts. The type climate and of soil of each district is reported. The pomegranate accession harvested in each area are also indicated on the map.

glucoside, pelargonidin 3-glucoside, cyanidin 3-rutinoside (Extrasynthese, Lyon, France); gallic acid (Fluka, Buchs, Switzerland); ellagic acid (Extrasynthese, Lyon, France); punicalagin A+B mixture (Sigma Aldrich, Italy).

Quantification of phenolic compounds by HPLC analysis

The juices obtained from the fruits of the eight pomegranate accessions were directly injected in the HPLC system. HPLC system consisted of Perkin Elmer Flexar HPLC equipped with a quaternary 200Q/410 pump and a LC 200 photodiode array detector. Anthocyanins and non-anthocyanin phenolics were separated and quantified using two different gradient elution conditions, as previously described in Fischer *et al*. (2011). The separation was carried out injecting 10 µL of the juices with analytical Zorbax SB-C18 (5 µm, 250 × 4.6 mm) column (Agilent Technologies, Italy), operating at 30°C and at a flow rate of 0,6 L min^{-1}. Individual phenols were identified comparing UV-spectral characteristics with those of authentic standards and on the basis of the identification reported in Fischer *et al.* (2011). The diode array detector was set at an acquisition range between 200 and 600 nm. Quantification was performed at 520 nm for anthocyanins and at 280 nm for tannins, utilizing calibration curves of corresponding standards.

Statistical analysis

Data corresponds to the juice extracted from a pool of three fruits for each pomegranate accession. Three technical replicates of each sample were analyzed. A one-way ANOVA with the "accession" as factor followed by Tukey post hoc test ($p<0.05$) was used to compare the compound in the different accessions. Heat map and PCA biplot analysis have been performed with ClustVis software (Metsalu and Vilo, 2015).

3. Results

Plant material

The climate was different in the seven districts, ranging from temperate warm climate (Gokchay, Ismailly) to semidesert dry steppes with hot summer (Agsu, Khizi). The sampling area were located at different altitude, ranging from 60 m (Yevlak) to 540 m (Ismailli). In the Agsu district two accessions were collected, one accession in the Agsu mountaın pass, whereas the other accession at 190 m a.s.l. (Fig. 1). Four areas presented salinized soil (Slyazan, Yevlakh and the two sampling points in the district of Agsu). Two areas have a typical mountain humus rich soil (Sheki, Ismailli).

Morphological traits

The shape and dimension of the fruits were very similar, except for Pg7 which showed a higher fruit length. The colors of the extracted juice ranged from a strong red (Pg 2) to light pink (Pg8) (Fig. 2 A, B).

Anthocyanins content in pomegranate juice

Six different anthocyanins were present in the pomegranate juice, namely delphinidin-3,5-O-diglucoside (Del-3,5-diglc), cyanidin-3,5-O-diglucoside (Cya-3,5-digl), cyanidin-3-O-glucoside(Cya-3-glc), cyanidin-3-O-pentoside (Cya-3-pent), pelargonidin-3,5-O-diglucoside (Pel-3,5-digl), pelargonidin-3-O-glucoside (Pel-3-glc) (Table 1). The anthocyanin profiles were significantly different qualitatively and qualitatively among the accessions. The accessions Pg3-8 contained only cyanidin derivatives, namely Cya-3,5-digl, Cya-3-glc and Cya-3-pent.The highest content of these compounds was detected in Pg 5,6,7 (Table 1). The accessions Pg 1 and Pg 2 showed a different anthocyanin composition, characterized by the presence ofDel-3,5-diglc, Cya-3,5-digl, Pel-3,5-digl and Pel-3-glc.The highest concentration Cya-3,5-digl of was determined in Pg1 (152.6±2.21mg L^{-1}) and Pg2 (102.3±1.39mg L^{-1}), whereas the lower level was measured in Pg 4 (5.450±0.63 mg L^{-1}) (Table 1). The compounds Cya-3-pent and Pel-3-glc are present in low concentration in the juices of all the accessions.

Content of hydrolyzable tannins and their derivates in pomegranate juice

Among the hydrolysable tannins, punicalagin α and β isomers, punicalagin derivatives,ellagic acid, ellagic acid glucoside, galloyl-glucose and hexahydroxydiphenoyl (HHDP)-hex-derivative were found in the studied accessions.All the pomegranate accession contained the punicalagin isomer α and β. Punicalagin isomer α concentration ranged from ~8mg L^{-1}inPg2 and Pg8 to 37.52 mg L^{-1}inPg1. The highest content of punicalagin isomer βwas found in Pg1 (61.65 mg L^{-1}), whereas the lowest was in Pg 2 and Pg 8 (~14mg L^{-1}) (Fig. 3).

In the juices obtained from the different pomegranate accessions were found also other punicalagin derivatives (Table 2). These compounds were identified because of their UV/VIS absorption spectrum which showed the maxima at 378 and 258

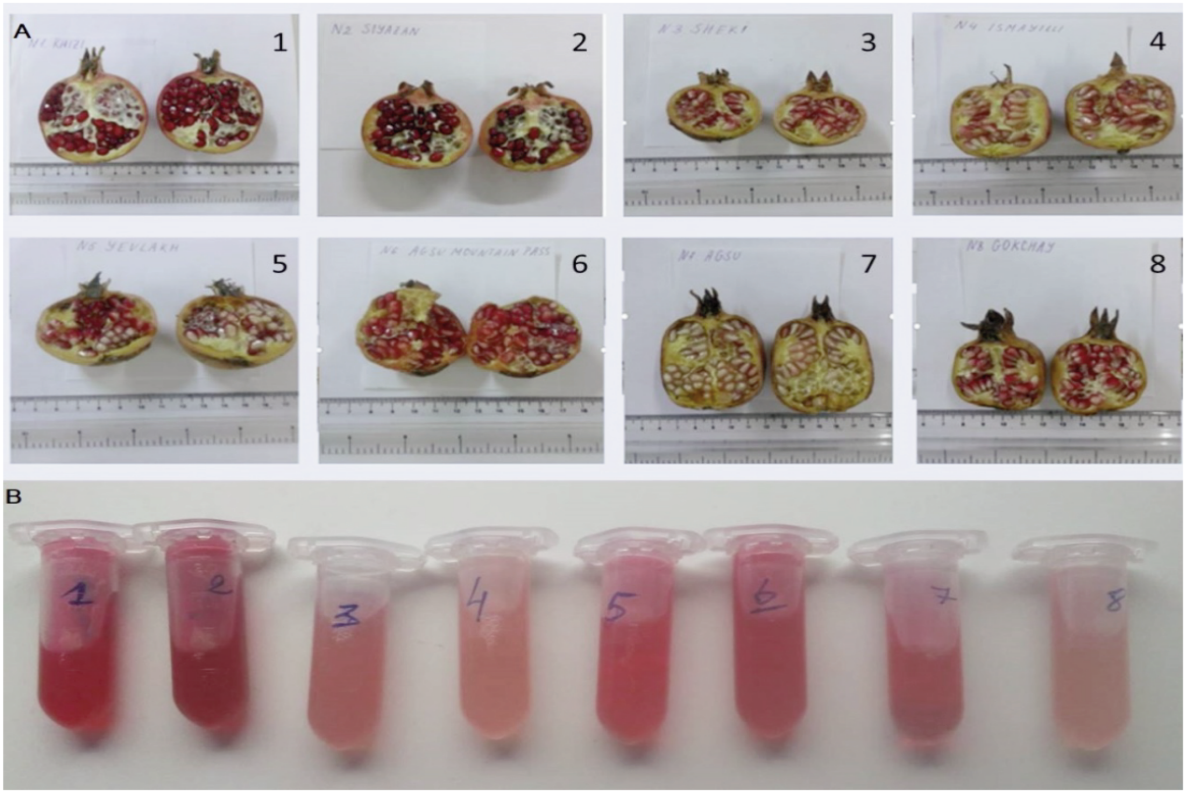

Fig. 2 - A typical fruit harvested in each sampling area (A) and an aliquot of the corresponding extracted juice (B).

Table 1 - Content of anthocyanin derivatives in the juices obtained from pomegranate fruits harvested in each sampling area

Pomegranate accessions	Del-3,5-diglc (mgL^{-1})	Cya-3,5-digl (mgL^{-1})	Pel-3,5-digl (mgL^{-1})	Cya-3-glc (mgL^{-1})	Pel-3-glc (mgL^{-1})	Cya-3-pent (mgL^{-1})
Pg1	75.58 ± 0.46 b	152.6 ± 2.21 a	0.102 ± 0.001 b	-	1.67 ± 0.165 a	-
Pg2	114.7 ± 1.85 a	102.3 ± 1.39 b	80.54 ± 1.20 a	-	0.65 ± 0.03 b	-
Pg3	-	7.910 ± 0.30 e	-	10.20 ± 0.04 d	-	0.551 ± 0.06 e
Pg4	-	5.450 ± 0.63 f	-	5.051 ± 0.08 f	-	0.061 ± 0.003 f
Pg5	-	26.94 ± 2.49 d	-	30.34± 2.85 c	-	2.451 ± 0.26 c
Pg6	-	49.75 ± 17.8 c	-	41.32 ± 5.04 a	-	6.581 ± 0.28 a
Pg7	-	41.12 ± 1.46 c	-	33.53 ± 1.09 b	-	4.663 ± 0.15 b
Pg8	-	7.680 ± 0.61 e		9.52 ± 0.48 e		0.532 ± 0.002 e

Values are reported as mean ± SD (n=3).
Numbers followed by different letters in the same column are statistically different according to Tukey's test ($p \leq 0.05$).
Del-3,5-diglc=delphinidin-3,5-O-diglucoside, Cya-3,5-digl=cyanidin-3,5-O-diglucoside, Cya-3-glc=cyanidin-3-O-glucoside, Cya-3-pent=cyanidin-3-O-pentoside, Pel-3,5-digl=pelargonidin-3,5-O-diglucoside, Pel-3-glc=pelargonidin-3-O-glucoside.

nm, as also observed for punicalagin isomer α and β and previously reported in other studies on pomegrante composition (Gil *et al.*, 2000). Punicalagin derivatives 1 and 2 were more abundant in Pg 1, whereas punicalagin derivatives 2 was higher in Pg 6 and Pg 8. Punicalagin derivative 4 was maximum in Pg3 and Pg4, reaching concentration more than 19 mg L^{-1}), whereas the minimum content was found in Pg5 (4.54±0.49mg L^{-1}) (Table 2). Overall, considering the sum of all punicalagin derivatives, the highest content was found in Pg 1.

A significant variation in the content of ellagic acid derivatives was found among the accessions. In particular, the concentration of ellagic acid ranged between 2.01 to 18.81mg $L^{-1}$and increasing from Pg2< Pg1< Pg6 = Pg5< Pg8< Pg7< Pg3=Pg 4. A similar trend was also observed for the elleagic glucoside, whichranged from 2.92 to 27.36 mg L^{-1}, showing the highest values in Pg3 and Pg4 and the lowest in Pg2 (Fig. 4).

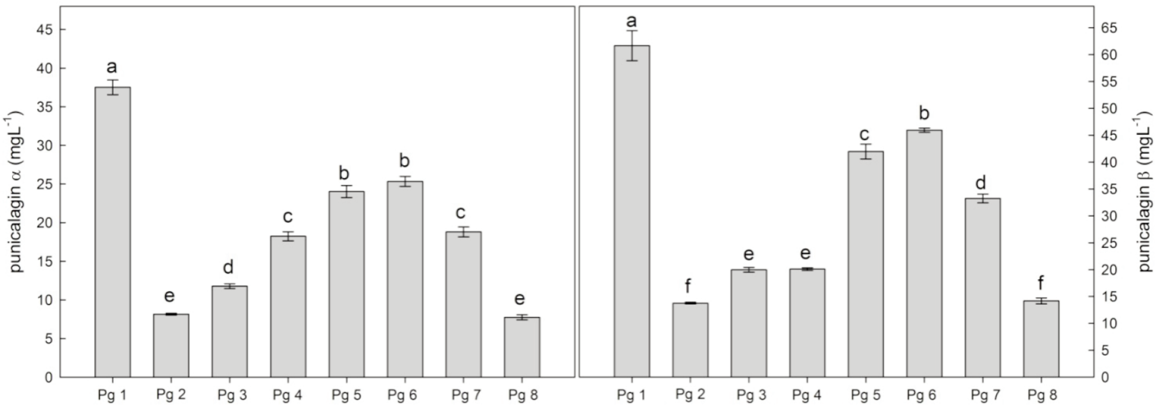

Fig. 3 - Concentration of punicalagin α and β in the juices obtained from pomegranate fruits harvested in each sampling area. The bars indicate the standard deviation (n=3). Different lowercase letters indicate significant differences according to Tukey's test ($p \leq 0.05$).

Table 2 - Content of four punicalagin derivatives in the juices obtained from pomegranate fruits harvested in each sampling area

Pomegranate accessions	Punicalagin derivative_ 1 (mgL^{-1})	Punicalagin derivative_2 (mgL^{-1})	Punicalagin derivative_3 (mgL^{-1})	Punicalagin derivative_4 (mgL^{-1})	Sum of all punicalagin derivatives (mgL^{-1})
Pg1	14.1 ± 0.73 a	21.7 ± 0.18 a	7.94 ± 0.19 b	8.44 ± 0.17 c	151.3 ± 4.66 a
Pg2	9.09 ± 0.03 c	8.17 ± 0.10 c	2.20 ± 0.01 e	6.15 ± 0.13 d	47.54 ± 0.38 f
Pg3	4.12 ± 0.06 d	10.3 ± 0.33 b	8.36 ± 0.18 b	19.7 ± 0.43 a	74.28 ± 1.41 e
Pg4	4.15 ± 0.05 d	10.4 ± 0.10 b	7.49 ± 0.18 b	19.7 ± 0.02 a	80.13 ± 1.13 d
Pg5	1.95 ± 0.09 f	8.72 ± 0.38 c	4.54 ± 0.49 d	4.54 ± 0.49 f	88.93 ± 3.29 b
Pg6	11.6 ± 0.48 b	-	9.66 ± 0.44 a	-	92.51 ± 0.88 b
Pg7	3.07 ± 0.02 e	11.0 ± 0.32 b	6.15 ± 0.18 c	11.3 ± 0.10 b	83.60 ± 1.29 c
Pg8	4.10 ± 0.15 d	6.53 ± 0.25 d	9.27 ± 0.40 a	5.71 ± 0.23 e	47.54 ± 1.90 f

The sum of all punicalagin derivatives include also punicalagin α and β.
Values are the mean ± SD (n =3). Values followed by the different letter in the same column are statistically different according to Tukey's test ($p \leq 0.05$).

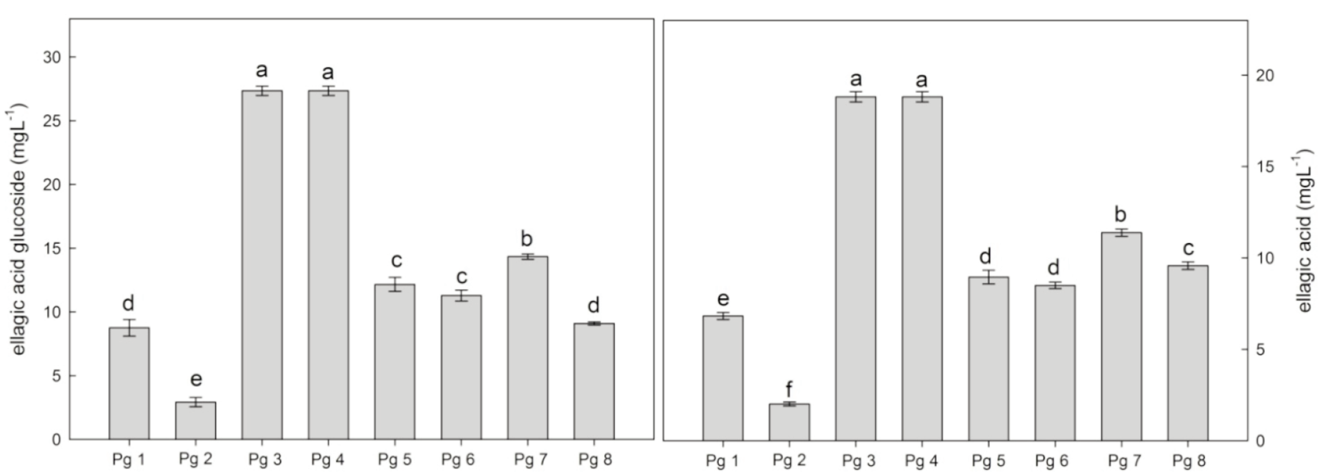

Fig. 4 - Concentration of ellagic acid glucoside and ellagic acid in the juices obtained from pomegranate fruits of the different accessions. The bars indicate the standard deviation (n=3). Different lowercase letters indicate significant differences according to Tukey's test ($p \leq 0.05$).

Other compounds were putatively identified, on the basis of the retention time and their UV/VIS absorption spectrum (Fischer *et al*., 2011), as galloyl-glucose and hexahydroxydiphenoyl-(HHDP)-hex-derivatives (following named HHDP-hex-derivatives). Indeed, the peak of galloyl-glucose showed a typical UV spectra similar to that of gallic acid (λmax = 269 nm), whereas the HHDP-hex-derivatives have similar retention time and λmax to pedunculag in derivatives (λmax = 258 and 375nm) reported in Fischer *et al*. 2011. Galloyl-glucose concentration increased from the minimum in Pg2 (~6 mg L^{-1})to the maximum in Pg7 (~18 mg L^{-1}) (Fig. 5). The concentration of HHDP-hex-derivatives was highest in Pg 1, Pg 3 and Pg 4. In particular, the HHDP-hex-derivative 1 was found in similar content in Pg 3 and Pg 4 (~50mg L^{-1})while HHDP-hex-derivative 2 ranged from 8.44 mg L^{-1}in Pg6 and Pg8 to 20.07 mg L^{-1}in Pg1 (Fig. 5).

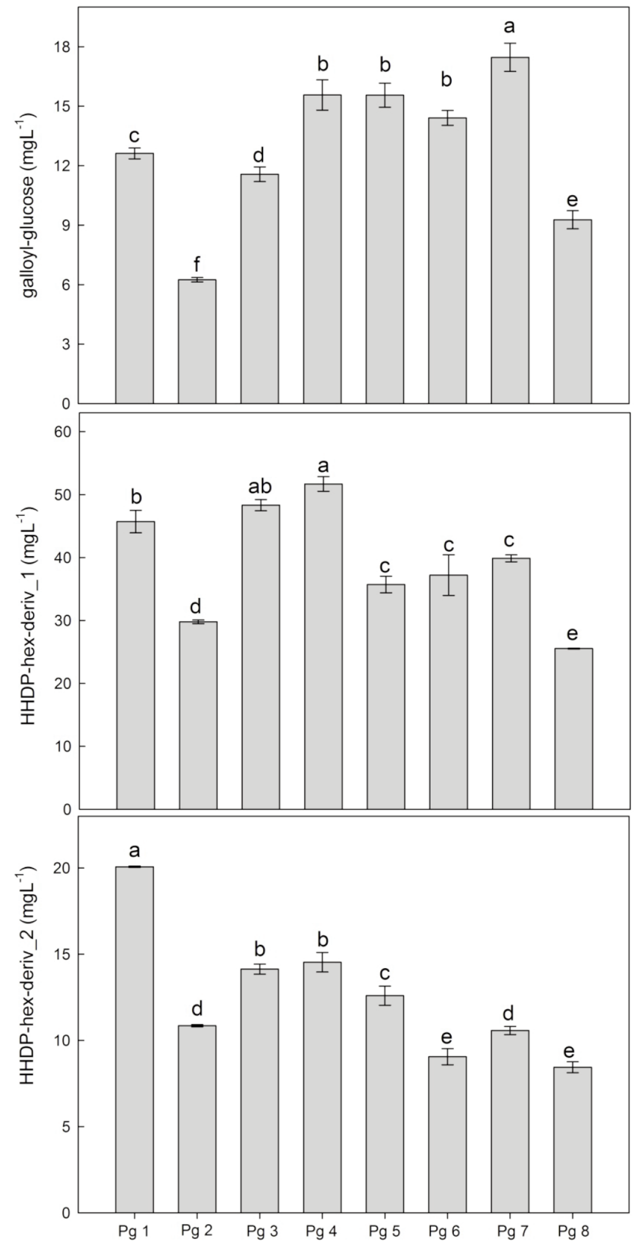

Fig. 5 - Concentration of galloyl-glucose and two HHDP-hex-derivatives (HHDP-hex-deriv_1 and HHDP-hex-deriv_2) in the juices obtained from pomegranate fruits of different accessions. The bars indicate the standard deviation (n=3). Different lowercase letters indicate significant differences according to Tukey's test ($p \leq 0.05$).

4. Discussion and Conclusions

The current study reported interesting differences in thejuice phenolic fingerprinting of eight wild-growing pomegranate accessions harvested in eight areas of Azerbaijan characterized by different climate conditions, soil composition and altitude.

Specific anthocyanin composition of the eight pomegranate accessions

Anthocyanins are natural plant pigments that have beneficial effects for the plantsas well as for humans and animals. The presence of six anthocyanins, 3-*O*-glucosides and 3,5-*O*-diglucoside of delphinidin, cyanidin and pelargonidin, is in agreement with the anthocyanin composition determined in registered Turkish varieties (Türkyilmaz, 2013).The compound Cya-3,5-digl was present in all samples (Ben-Simhon *et al.,* 2015), however the higher value found in Pg1 and Pg2, and moderately in Pg6 and Pg7,accounted for the prominінent red color showed by the four accessions respect to the other ones (Fig. 2B and Table 1). These outcomes are in agreement with those reported by Hasnaoui et al. (2011), who found that Cy-3,5-digl was the main anthocyanin in Tunisian pomegranate juice as well as in the Turskish "Izmir 1513" variety (170 mg L^{-1}),which were characterized by a strong red color of the juice (Türkyilmaz, 2013). In addition, in the accessions Pg 1 and Pg 2, the low concentration of Pel-3-glc, a pigment responsible for the orange pigmentation,is in agreement with those reported by Ben-Simhon *et al.* (2015), who found similar values for Pel-3-glc content in the pomegranate cultivar "Wonderful" (Table 1).

The quantitative and qualitative anthocyanin variations among the accessions might be related to the environmental conditions during the ripening period, as well as the to genotype adaptation mechanisms to the growing area (Fig. 1). Borochov-Neori *et al*. (2011, 2014) reported an increment in diglucosylated anthocyanins after seasonal warming, and our data may concurr to suggest a correlation between high level of the anthocynain diglucosides and hot summer. Indeed, Pg 1 and Pg 2, which can be found in dry regions characterized by hot summers, contain the highest levels of delphinidin-3,5-O-diglucoside and cyanidin-3,5-O-glucoside, presumably due to a higher stability of 3,5-diglucosides compared to 3-glucosides (García-Viguera and Bridle, 1999; Hernandez *et al*., 1999). However, the high levels of anthocyanins might be also the consequence of adaptation to saline soil or marshes as high amount of anthocyanin leads to increased salt tolerance in Arabidopsis (Oh *et al.,* 2011). The hypothesis that Pg1, Pg2, Pg6, Pg7 can be more tolerant to saline soil should be further explored in view of their possible use as sources of resistance genes in breeding programs. Additionally, the high and peculiar concentration of anthocyanins in Pg1 and Pg2 makes the two accessions of particular interest for utilization in human health, given the activity of these compounds as cellular antioxidant and against inflammation status (Blesso, 2019).

Different content of hydrolyzable tanninsin the eight pomegranate accessions

In addition to anthocyanins, the studied pomegranate juiceswere also rich of hydrolyzable tannins which are synthesized from galloyl glucose, with pentagalloyl glucose serving as the precursor for both higher-molecular-weight gallotannins and ellagitannins. Ellagitannins, which contain two or more neighboring galloyl groups that are oxidatively coupled to form rigid groups such as hexahydroxydiphenoyl (HHDP) units, are particularly abundant in pomegranate fruits and can be extracted in significant levels into the juice (Fig. 3, 4, 5) (Seeram *et al*., 2005). The high levels of ellagitannins, in particular punicalagin derivatives, found in our juices are in line with the those reported in literature (Fig. 3) (Mena *et al.,* 2012; Akhavan *et al*., 2015; Kalaycıoğlu and Erim, 2017; Balli *et al*., 2020). Even if these compounds can determine an unpleasant taste in juices, due to their interactions with the salivary proteins, they have been identified as the active antiatherosclerotic compounds in pomegranate juices responsible for the ability of this juice to protect human low-density lipoprotein cholesterol from oxidation *in vivo* (Aviram *et al*., 2000). In addition, ellagitannins show also strong radical scavenging and antioxidant ability as well as interesting anti-mutagenic and anticancer properties (Vattem and Shetty, 2005). Other than the well-known punicalagin application in human health (Scalbert and Williamson, 2000; Zarfeshany *et al*., 2014), it is noteworthy the noticeable antifungal activities of punicalagins due to the ability, similar to that of amphotericin B to form pore-like aggregates in the cellular membranes of fungi (Rongai *et al*., 2018). These recent findings suggest further potential utilization of punicalagins extracted from pomegranate fruits as environmentally-friendly natural formulations to control fungus and bacteria in crop cultivation.

Besides their multiple applications for human health and agriculture, ellagitannins have also important functions for plant physiology, indeed they can offer protection against biotic stresses (Furlan *et al*., 2011). Lastly, the concentration of ellagic acid derivatives seems to increase under salinity stress (Borochov-Neori *et al*., 2014), probably because of the ability of these compounds to alleviate osmotic stress as previously reported in other species (El-Souda *et al*., 2013).

Selection of pomegranate accessions through the phenolic fingerprint

The phenolic fingerprint of the juices obtained from the accessions collected in eight different areas of Azerbaijan allowed to select promising wild pomegranate accession particularly rich both in anthocyanins and ellagitannins. Among the investigated accessions, Pg 1 resulted the richest both in anthocyanins and hydrolysable tannins, particularly in punicalagin derivatives (Table 1-2 and Fig. S1). In this accession, collected in the region of Khizi, characterized by hot summer and cold winter but partially mitigated by its proximity to the Caspian Sea, the interaction between environmental conditions and the genotype induces the accumulation of both more stable anthocyanins, such as del-3,5-diglc and cya-3,5-digl, and different punicalagin derivatives. Similar anthocyanin composition was also found in Pg 2, which however showed an unbalalanced composotion because of the very low amount of tannins (Table 1-2 and Fig. S1). Indeed, the accumulation of both classes of polyphenols can be stimulated under warming conditions, but also somehow drastically reduced under prolonged and severe drought conditions (Mena *et al*., 2013). A PCA biplot was applied to highlight the correlations between the contents of phenolic compounds of various accession fruits (Fig. S2). The two PCs explained almost the 70% of the variance of the data.

The closer the accessions lie on the plot, the more similar they are in the composition of phenolic compounds. By contrast, distant accessions have significantly different compositional characteristics. Indeed, PC1 discriminates very well Pg 1 and Pg 2 from the other accessions, since the samples on the left of the biplot are more abundant in anthocyanins. PC2 allows to discriminate accessions on the basis of their tannin content, and it clearly shows the presence of three different groups: the richest accession (Pg1), accessions with similar tannin content (Pg 3,4,5,6,7) and the lowest accessions (Pg 2 and Pg 8). The accessions Pg 3,4,5,6,7 have a more balanced content of anthocyanins and tannins (Fig. S1). However, also among these accessions, environmental influences can be osbserved considering the accessions Pg 5, 6, 7, which have been collected in areas with hot summer conditions and salinity soils and result to have higher anthocyanin and punicalagin contents compared to Pg 3 and Pg 4 (Table 1, 2 and S1). By contrast, these latest accessions, collected in regions with a temperate climate and dark loamy soils, are characterized by a higher content of ellagic acid and its glycosylated derivative (Fig. 4 and Table S1). The results of this study, considering the recently work on the pomegranate genome (Qin *et al*., 2017), provides a valuable resource for selecting pomegranate accessions with interesting metabolic traits that could be used in breeding efforts to increase the production of this bioactive compounds.

The results reported in this study contribute to provide significant information about phenolic fingerprinting of juices extracted from different wild growing pomegranate accessions in the territory of Azerbaijan Republic. Data showed that those accessions are rich in bioactive metabolites, which are useful for human health and might be correlated to adaptation to specific environmental conditions. The results of our study suggest that Azerbaijan wild-growing accessions are promising sources of bioactive metabolites and they might be exploited in pomegranate breeding programs as donors of valuable traits for creating pomegranate varieties with high nutritional and medical properties and/or to ameliorate the resistance of commercial varieties to harsh conditions.

Acknowledgements

Authors kindly acknowledge Prof Valida Ali-zada, Director of Institute of Botany of the Azerbaijan National Academy of Sciences (ANAS) and the former Director of CNR- Institute for Sustainable Plant Protection for signing the collaboration agreement between IB (ANAS) and CNR-IPSP.

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