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ARTICLE

# Color, Sugars and Organic Acids Composition in Aril Juices and Peel Homogenates Prepared from Different Pomegranate Accessions

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The current study describes differences in pomegranate fruit size and aril weight of 29 accessions grown in Israel. The contents of sugars and organic acids in their aril juices and peel homogenates, as well as color parameters, were determined. While the levels of total soluble solids (TSS) and soluble sugars in the aril juices differ only slightly, those of titratable acidity (TA) and citric acid changed significantly, suggesting that they are the main contributors to juice taste. In general, significant positive correlations were found between TA values and the red color parameters, and these two parameters, as well as TSS, appeared to be higher in the juices of accessions harvested late in the season. Peel homogenates exhibited lower levels of TSS, TA, soluble sugars and organic acids than aril juices. Some red color parameters, TA and citric acid were found to correlate significantly between the aril juices and peel homogenates.

**KEYWORDS: Color; fruit quality; organic acids; pomegranate; *Punica granatum* L.; sugars**

## INTRODUCTION

The traditional importance of the pomegranate fruit (*Punica granatum* L., Punicaceae) as a medicinal plant is now supported by data obtained from modern science showing that the fruit contains anticarcinogenic (*1*), antimicrobial (*2*) and antiviral compounds (*3*). Recent biological studies have proven that certain compounds contained in pomegranate juice, which has been shown to reduce blood pressure, are antiatherosclerotic and significantly reduce LDL oxidation (*4*). Due to the extensive knowledge about the pomegranate's health attributes and increasing public awareness about functional food, the demand for pomegranate fruit and its byproduct has increased tremendously in the Western world. There is a growing demand for good quality fruits both for fresh use and for processing into juice, syrup, squash, wine and anardana (*5*). As a result of this trend, the extent of pomegranate growth was increased significantly in many regions throughout the world, and industries that produced pomegranate products were developed (*6*).

As for many fruit species, pomegranate varieties differ in their taste, ranging from sweet to sour (*6*). This is related directly to the quality and quantity of the organic acids and sugars found in the fruit, and indeed a great diversity in these components and their contents has been detected in different pomegranate juices collected from several regions around the world (*7*−*10*). The desired pomegranate taste varies, however, in different countries and regions. In North Africa, for example, almost all commercialized pomegranate belong to the sweet varieties (*11*), while in Russia and other northern countries more sour accessions are commercialized (*12*).

We previously reported the variability in antioxidant activity, total polyphenol content, total anthocyanin content, and the level of four hydrolyzable tannins in aril juice and peel homogenates of 29 accessions grown in Israel (*13*). In this study, we further characterized these 29 accessions, concentrating on the compounds that are contributors to the taste and color of the juice. The objectives are (i) to determine total soluble solids (TSS) and titratable acidity (TA), two parameters whose ratio defines the taste of pomegranate juice, and to study them in two fruit parts arils juice and peel homogenates; (ii) to study the level of each organic acid and sugar in these 29 pomegranate accessions in both aril juices and peel homogenates, and to determine the relationship between these two parts of the fruit; (iii) to study the relationship between fruit skin and aril color to TA and TSS contents; and (iv) to define fruit size and aril weight in each of these accessions in order to gain more knowledge about the juicy potential of the fruits.

## MATERIALS AND METHODS

**Plant Materials and Fruit Processing.** The 29 pomegranate accessions grown at the Newe Ya'ar Research Center, ARO [registered in Israel Gene Bank for Agriculture Crops (IBG, Web site: http://igb.agri.gov.il)] that were previously used to study the antioxidant activity of different fruit parts (*13*) were used in this study. These accessions differed in peel and aril

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**Table 1.** Harvest Days of Pomegranate Accessions Grown at Newe Ya'ar Examined in 2006

| accessions | harvest day |
|---|---|
| PG 106-7 | August 26 |
| PG 205-216 | |
| PG 128-29 | September 7 |
| PG 203-214 | |
| PG 201-212 | |
| PG 200-211 | |
| PG 202-213 | |
| EVE | |
| PG 103-4 | September 14 |
| PG 109-10 | |
| PG 112-13 | |
| PG 114-15 | |
| PG 119-20 | |
| PG 120-21 | |
| PG 121-22 | |
| PG 123-24 | |
| PG 105-6 | September 21 |
| PG 130-31 | |
| PG 106-7 | September 28 |
| PG 118-19 | |
| A17 | October 5 |
| PG 116-17 | |
| PG 206-217 | |
| PG 101-2 | |
| PG 100-1 | October 10 |
| PG 104-5 | |
| PG 102-3 | October 15 |
| PG 108-9 | |
| PG 127-28 | October 28 |

color, size and taste (*13*). The different accessions were harvested from the end of August up to the end of October in the year 2006 (**Table 1**). The trees were at least five years old and planted at a 3 × 5 m distance in 3−5 replicate trees per accession. Ten fruits from each accession (3 to 4 fruits per tree) were harvested when fully matured according to commercial practice. The fruits were transported via a ventilated car to the laboratory, where they were characterized by physical (fruit weight, peel and aril weights) and chemical parameters, as described below.

The arils and peels from each fruit were separated and weighed. The arils were squeezed using a nylon sieve to produce the aril juice. The peels (150 g) were homogenized (for 2 min) with 300 mL of cold distilled water. The homogenates and aril juices were then centrifuged (4,000 rpm for 15 min) and the sups collected. Five pools were prepared from 10 fruits of each accession, each pool containing the homogenates or juice prepared from two fruits. The aril juice and homogenates were then frozen at −20 °C for further analysis.

**Physical Analysis.** Every fruit from each accession, as well as their arils and peels, was weighed on a balance having an accuracy of 0.001 g.

**Determination of Total Soluble Solids (TSS) and Titratable Acidity (TA).** Measurements were made on fresh aril juices and homogenates. TSS level was measured using a digital refractometer (ATAGO RR-1 serial no. 602055, Tokyo, Japan, calibrated using distilled water). The instrument was set to measure % TSS using the temperature compensated mode. A Metrohm titration unit (Brinkmann, Metrohm ch-9101 Herisau, Switzerland) equipped with a 719S Titrino titration assembly was used for total TA determination. Two milliliters of juice was diluted with 10 mL of distilled water and titrated with 0.1 N NaOH to pH 8.2. TA was calculated as g citric acid/100 g fresh weight or as percentage of citric acid. Measurements were replicated twice for each sample.

**Determination of Total Anthocyanins Content.** Anthocyanin pigments undergo reversible structural transformations with a change in pH manifested by strikingly different absorbance spectra (*14*, *15*). The colored oxonium form predominates at pH 1.0 (25 mM potassium chloride buffer) and the colorless form at pH 4.5 (0.4 M sodium acetate buffer). The samples were diluted by a potassium chloride buffer until the absorbance of the sample at a 510 nm wavelength was within the linear range of the spectrophotometer. This dilution factor was used later to dilute the sample with the sodium acetate buffer. The wavelength reading was performed after 15 min of incubation, four times per sample diluted in the two different buffers and at two wavelengths of 510 and 700 nm. The absorbance was then calculated according to the following equation: $A = (A_{510} - A_{700})_{pH1.0} - (A_{510} - A_{700})_{pH4.5}$. Results were expressed as mg of cyanidin-3-glucoside per 1 L of juice using a molar absorptive coefficient ($\varepsilon$) of 26,900 and a molecular weight of 449.2.

**Determination of Aril Juice and Peel Color.** Pomegranate skin color was measured using a colorimeter (Chroma Meter CR-301, Minolta, Ramsey, NJ) (*16*). Color was assessed according to the Commission International del'Eclairage (CIE) and expressed as $L^*$, $a^*$, $b^*$, $C$, and $H°$ color values. $L^*$ defines lightness, $a^*$ and $b^*$ define red−greenness and blue−yellowness, respectively, and $C^*$ defines saturation. Hue angle ($H°$) was calculated as hue angle arctangent [$b^*/a^*$], where 0° = red-purple; 90° = yellow; 180° = bluish-green and 270° = blue. The mean values for 10 points on the peel surface were calculated for each fruit. Juice color was measured using a Minolta CR-301 colorimeter. An aliquot of each juice was analyzed using a plate (5 cm diameter) and a white background. Blank measurements were made using the plate filled with distilled water (*17*).

**Contents of Organic Acids and Sugars in Aril Juices Using HPLC.** The aril juice was diluted 1:10 with distilled water and then filtered through a 0.45 $\mu$m Millipore membrane filter. The diluted juices were injected into a Hewlett-Packard HPLC series 1090 equipped with an Aminex 87H (Bio-Rad) column (30 cm × 7.8 mm). The mobile phase consisted of 0.1% phosphoric acid running isocratically at a flow rate of 0.6 mL $min^{-1}$. The organic acids were detected by their absorbance at 210 nm using a UV detector. Ascorbic acid was detected at 254 nm, while the detection of sugars was obtained by a refractive index detector. A standard curve of pure organic acids and sugars purchased from Sigma (Poole, Dorest, U.K.) was used for quantification. Results were expressed as mg of ascorbic acid 100 $g^{-1}$ (*18*), and those for sugars as parts per million (ppm) sugars of the juices (*19*).

**Contents of Organic Acids and Sugars in Peel Homogenates Using GC−MS.** Metabolitic analysis by GC−MS was carried out using a method modified from that described previously (*20*). Peel homogenates (200 $\mu$L) were mixed with 1,300 $\mu$L of methanol, and 120 $\mu$L of internal standard (0.2 mg $mL^{-1}$ ribitol in water) was subsequently added as a quantification standard. The mixture was extracted for 15 min at 70 °C. After centrifugation at 4,000 rpm, supernatants were added to 2,250 $\mu$L of chloroform:water (1:2 v/v). After vortexing and centrifuging at 4,000 rpm, 600 $\mu$L of the methanol−water phase was dried by freeze-drying using lyophilizer. The dried residues were dissolved and derivatized for 120 min at 37 °C with 40 $\mu$L of 20 mg $mL^{-1}$ methoxyamine hydrochloride in pyridine and 30 min treatment with 70 $\mu$L of *N*-methyl-*N*-(trimethylsilyl)-trifluoroacetamide at 37 °C, respectively.

Five microliters of a relative retention time (RRT) standard mixture (0.2% v/v *n*-dodecane, *n*-pentadecane, *n*-nonadecane, *n*-docosane, *n*-octacosane, *n*-dotracontane, and *n*-hexatriacontane dissolved in pyridine) was added to 60 $\mu$L of trimethylsilylation mixture.

Sample volumes of 1 $\mu$L were then injected onto the GC column in the splitless injection mode using a split ratio of 1:50 (each sample prepared in two repeats). The GC−MS system consisted of a 7683 series autosampler, a 6890N network gas chromatography (GC) system, and a 5973 network quadrupole mass spectrometer (Agilent Technology). The mass spectrometer was calibrated according to the manufacturer's recommendations using tris(perfluorobutyl)amine (CF43). The GC separation was performed on a 30 m Rxi-5sil MS with integrated guard column with 0.25 mm i.d. and 0.25 m film thickness (Restek International). Injection temperature was 300 °C, the interface set to 250 °C, and the ion source adjusted to 280 °C. The carrier gas used was helium set at a constant flow rate of 1 mL $min^{-1}$. The temperature program was 5 min isothermal heating at 70 °C, followed by a 5 °C $min^{-1}$ oven temperature increase to 320 °C, and a final 10 min heating at 320 °C. The system was then temperature equilibrated for 1 min at 70 °C prior to injection of the next sample. Mass spectra were recorded with a mass-to-charge ratio of 35 to 200 (start at 0 min), 35 to 450 (start at 10 min) and 50 to 700 (start at 20 min) (7.96, 3.5, and 2.2 scans per second, respectively) scanning range. More than 120 peaks were counted in each sample whereas the chromatograms and mass spectra were evaluated using the MSD ChemStation program (Agilent Technology). Retention time and mass spectral library for automatic peak quantification of metabolite derivatives were measured

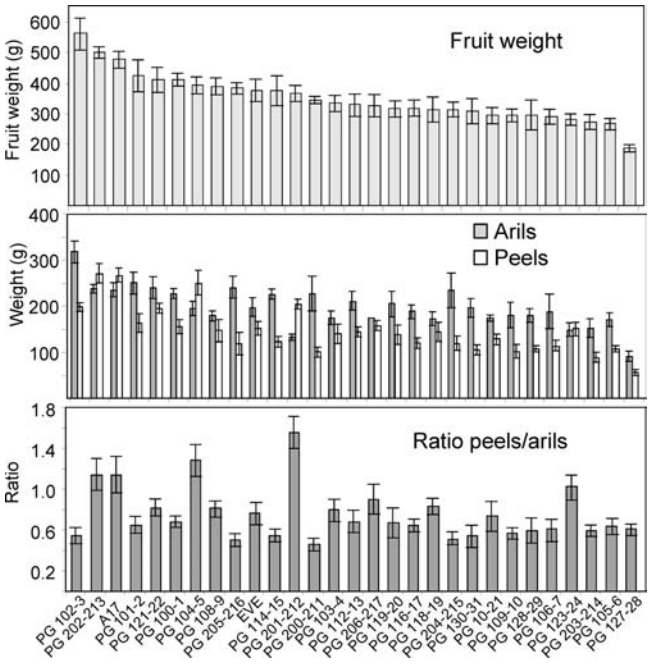


**Figure 1.** Fruit weight, aril (gray bars) and peel (white bars) weights, and the ratio between arils and peels in 29 pomegranate accessions. The data represent the mean ± SD of ten replicates from each accession.

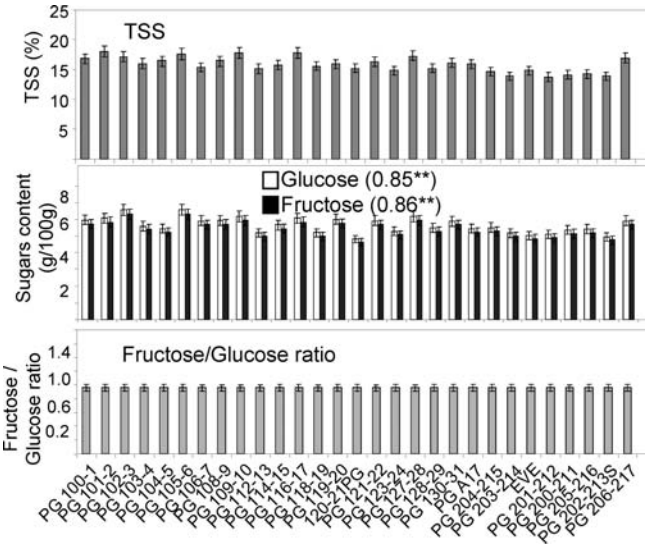


**Figure 2.** TSS, glucose (□) and fructose (■) contents measured by HPLC, and the fructose-to-glucose ratio in aril juices of 29 pomegranate accessions. The data represent the mean ± SD of five replicates from each accession. The $R^2$ value was calculated against the TSS; the significance ($p < 0.01$) is identified by two asterisks.

use the MSD ChemStation method. The absolute concentrations of sugars and organic acids were determined by comparing them to standard calibration curve response ratios of various concentrations of standard substance solutions, including the internal standard ribitol, which was derivatized concomitantly with tissue samples.

**Statistical Analysis.** The data obtained from this study were analyzed statistically using SPSS software adapted to Windows, ver. 14. In this software, the Spearman test was used for the correlation studies and their significance.

## RESULTS AND DISCUSSION

**Weight, Size and Juice Content.** Fruit juice industries are seeking accessions that have the appropriate taste and color but also high juice yield. In the current study, we examined the 29 accessions that had been used previously to measure antioxidant activity level and total polyphenols content (*13*). The weight of the whole fruits was determined (**Figure 1**). Various fruit weights were found in the accessions examined, ranging from 186 ± 36 g in accession PG 127-28 to 551 ± 48 g in accession PG 102-3 (about 3-fold) (**Figure 1**). Next, the weights of the peels and arils from each accession were examined. The ratio between the weights of these parts was then calculated. A low ratio will indicate greater juice content in each fruit. The ratio between peel and aril weights varied between 0.45 in accession PG 200-211 to 1.55 in accession PG 201-12. When calculated to percentage of arils from whole fruits, the values varied from 36% to 75%. These values are broader relative to values previously reported for Turkish accessions, showing that juice content of the fruit accounted for about 45−65% of the whole fruit (*7*).

The results suggest that genetic background of these accessions contributed greatly to juice content. However, other factors are also known to be involved in determining aril content in the fruit, since it was recently reported that fruits from the same genetic background grown in Mediterranean climates have more juice than those grown in desert climates (*21*). It was suggested that climate, mainly radiation, temperature and humidity that vary significantly between these two habitats, affects aril numbers and juice content (*21*). In addition, it was reported that fruit size and weight increase during fruit maturation and ripening (*19*, *22*, *23*), indicating that the harvest day can also affect the fruit size.

**TSS and Sugar Levels in the Aril Juice Did Not Differ Significantly in the Different Pomegranate Accessions.** The arils are the edible part of the pomegranate fruit and are usually consumed directly. They are also used for the preparation of fresh juice or canned beverages, as well as alcoholic beverages, jellies, jams and for flavoring and coloring drinks (*4*). The 29 accessions chosen for this study differ significantly in taste, ranging from those having a strong sweet taste (e.g., PG 203-214, PG 204-215, PG 106-7) to those having a strong sour taste (e.g., PG 109-10, PG 104-5, PG 118-19). To get an indication of the variation in aril taste from the different accessions, the parameters commonly used to evaluate fruit taste, i.e., TSS and TA, were examined. TSS content was first determined. Determination of TSS is important not only to establish the organoleptic quality of the juice, but also because TSS content is the major parameter determining the accessions that can be used for wine preparation (*24*). The results show that the TSS values range from 13.7 ± 1.8 g/100 g in PG 201-212 to 17.8 ± 2.3 g/100 g in PG 101-2 and PG 109-10 (**Figure 2**). This range is in accordance with those reported from other collections grown in different regions around the world. In Spain, the TSS level ranged from 11.4 to 13.5 g/100 g (*8*), while in those collected in Turkey, the TSS ranged from 13.9 to 16.1 g/100 g (*7*). In Macedonia, it ranged from 8.4 to13.2 g/100 g (*25*), and in Russia from 15.2 to 20.5 g/100 g. The values obtained from the Russian collection are higher than those from other reported regions (*7*). The reason for the higher TSS level in the Russian accessions is not known, but we previously reported that growth conditions could significantly affect the TSS level. Eleven accessions having the same genetic background that were grown in a Mediterranean climate in Israel exhibited significantly higher TSS contents than those grown in the desert (*21*). This suggests that colder climates promote the accumulation of compounds contributing to the TSS level, as previously shown for peanuts (*26*) and potatoes (*27*), whereas higher temperatures exceeding 40 °C decrease sugar and TSS contents, as observed for grape berries (*28*). The harvest period can also affect TSS content since the TSS values increase during maturation and ripening of pomegranate fruits (*19*, *21*).

This suggests that the high TSS values obtained in the Russian accessions could be attributed to the late harvest in this region.

A strong correlation was found previously in pomegranate juices for TSS to sugar content (*19*, *21*), and therefore the levels of the sugars were next determined using HPLC. Determining the composition of the sugars is also important because, in terms of sweetness, if sucrose rated 1, then fructose rated 1.75 and glucose 0.75 (*29*, *30*). Using HPLC, glucose and fructose were shown to be the major sugars in pomegranate aril juice. Their contents varied from 4.8 ± 2.9 to 6.6 ± 3.4 g/100 g. Maltose was also determined in accession PG 102-3 at a negligible content of 0.02 g/100 g. Maltose was also reported in one Spanish accession at content of 0.17 g/100 g (*8*, *10*).

Next the fructose-to-glucose ratio was calculated (**Figure 2**). Determining this ratio is important since a high ratio may cause diarrhea or abdominal pain due to excess fructose ferments in the large intestine. High levels of fructose also lead to a higher LDL level plus greater insulin resistance, which is consistent with metabolic syndrome, while a high level of glucose did not show this effect (*30*). Juices such as orange juice that contain equal amounts of glucose and fructose are the most recommended. The results have shown that pomegranate aril juices in general have equal amounts of glucose and fructose as recommended and as we previously found (*19*, *21*) (**Figure 2**). Similar ratios were obtained in fruits found in Turkey (*8*). However, fructose-to-glucose ratio differed in the accessions of different collections. It is reported that the Spanish accessions have almost always higher levels of fructose than glucose (*8*), but in another study it was reported that glucose was found to be the more predominant sugar than fructose (*31*). In the Russian accessions, more glucose than fructose was found (*8*), which was similar to the finding in Saudi Arabia (*41*). This ratio is most probably not dependent on environmental conditions, since it was found that fruits obtained from desert and Mediterranean habitats have the same ratio in these sugars (*21*).

The soluble sugars are most likely the major contributors to TSS content, since as previously reported and also found in this study, TSS level correlated strongly to soluble sugars level (**Figure 2**) (*19*, *21*).

**TA Content Significantly Affects Aril Juice Taste.** The results described above show that TSS values do not differ significantly between accessions having a sweet taste and those having a sour taste. Furthermore, accessions having a sour taste, such as PG 109-10, PG 104-5 and PG 118-19, have higher TSS values compared to those having a sweet taste, such as PG 203-214, PG 204-215 and PG 203-216 (**Figure 2**). The finding that significant and positive correlation is found between TA and TSS ($R^2 = 0.57, p < 0.01$) suggests that in general sour accessions contain more sugars than sweet accessions. Therefore, TSS contents cannot explain the major differences between aril juice tastes. To gain greater knowledge about the factors determining taste, we next studied an additional major contributor to taste, TA. Acidity can play an important role in the perception of fruit quality. It not only affects the fruit's sour taste but also its sweetness by masking the taste of the sugars. TA values were determined, and the accessions were graded from the accession having the highest level to that having the lowest level (**Figure 3**). The results show major differences in accessions, varying from 0.22 ± 0.04 g/100 g in accession PG 200-214 to 1.97 ± 0.26 g/100 g in accession PG 109-10 (about 9-fold higher). These values are in the range found in the Spanish accessions, which varied between 0.22 g/100 g and 2.9 g/100 g (*8*). The data on TA values found in different collections grown in different locations around the world suggest that TA content and, thus, pomegranate taste depend on climate and growth conditions. Sour accessions were

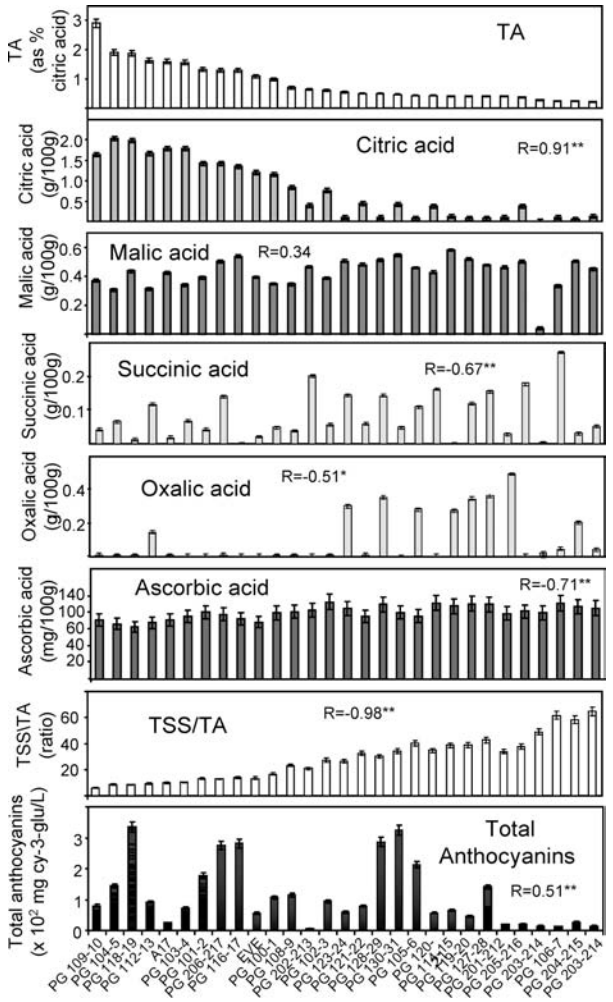


**Figure 3.** Titratable acidity (TA), organic acids levels (measured by HPLC), the TSS and TA ratio, and total anthocyanins level in mg cyanidin 3-glucoside equivalents per L juice in aril juices prepared from 29 pomegranate accessions. The data represent the mean ± SD of five replicates from each accession. The $R^2$ value was calculated against the TA. Significance of the $R^2$ value ($p < 0.01$) is identified by two asterisks.

characterized more in northern and cold regions, while sweet accessions having low TA values appeared more in regions having hot/dry conditions. For example, in northern regions such in Turkey, Russia, Georgia and Macedonia, TA ranged from 1.73% to 4.6% (*7*), 0.52% to 2.3% (*12*), 0.6% to 2.2% (*33*), and 0.37% to 2.80% (*25*), respectively. However, in hot climates such as in India, Egypt and Saudi Arabia, TA values dropped to 0.12% to 0.13% (*32*, *34*), 0.03% to 0.1%, and 0.02% to 0.14%, respectively (*11*). This assumption is supported by a previous study showing that climatic conditions significantly affect total TA level of several pomegranate accessions, which decreased in a hot and dry climate compared to a Mediterranean climate (*21*, *22*). This is also in agreement with the results obtained for tomatoes, whereby TA levels decreased by 25% when temperature increased from 21 to 26 °C (*35*). However, the higher TA values found in the northern countries could also be attributed to the different demand of customers in these regions, which led the breeders to breed for a sourer taste in the aril juices than those found in India, Egypt and Saudi Arabia.

Next, organic acids content, which contributed to the TA of these juices, was examined. Organic acid profiles can determine juice flavor, freshness or spoilage and are important for their

contribution to sensory attributes, as well as for their potential health benefits (*36*). Organic acids also influence the growth rate of microorganisms in fruit and their products, and therefore affect juice quality and shelf life (*37*). Five organic acids were detected by HPLC in aril juice, namely, citric, malic, ascorbic, oxalic and succininc acids (**Figure 3**). Acetic, tartaric, fumaric and lactic acids, which were detected in the Spanish and Turkish accessions (*7*, *8*), and shikimic, maleic, and fumaric acids detected in the Iranian accessions (*36*), were not detected in our system. However, succinic acid, which was found in the juice of accessions grown in Israel, was not detected in the Spanish accessions (*8*).

Although several organic acids were found in pomegranate aril juice, the major acid accounting for TA is citric acid, which is the major organic acid in many accessions, and its level shows a strong, positive and significant correlation to TA [($R^2 = 0.91$, $p < 0.01$); **Figure 3**]. Citric acid was the predominant organic acid in 17 out of 25 accessions from Iran (*36*), as well in all six accessions from Georgia (*33*). However, in some of the Spanish accessions, malic acid was found to be the most predominant, followed by citric acid (*31*). These two organic acids were synthesized in different parts of the fruit cells: malic acid was synthesized by phosphoenolpyruvate carboxylase and NAD-dependent malate dehydrogenase in the cytosol, while mitochondrial citrate synthase was implicated in citric acid accumulation (*38*). Therefore, competition for the same precursors is not expected. The levels of oxalic and succinic acids were found to differ significantly in the accessions; some accessions have high content, and some show negligible levels. Notably, these two organic acids showed negative correlations to TA (**Figure 3**), and are more dominant in accessions having a sweet taste found on the right side in **Figure 3**. In addition to these organic acids, it was reported that a high fumaric level was detected in Iranian accessions (*36*), and tartaric and malic were determined to be the second most abundant organic acid in the Georgia collection (*33*), demonstrating the diversity of organic acids in pomegranate fruits.

Ascorbic acid (vitamin C) is an abundant acid in many fruits and has numerous biological functions, which include playing a role in many aspects of redox control and antioxidant activity that prevent, for example, the browning of tissues. Here, we show that ascorbic acid has a negative correlation to TA (**Figure 3**). This was in accordance to our previous data showing that ascorbic acid content increased during pomegranate fruit development, whereas TA level decreased significantly (*19*). Its level was not correlated to antioxidant activity ($R^2 = -0.09$), showing that it is not major contributor to this activity of the aril juices.

Taken together, the current results show that citric acid is the main contributor to TA content and to the sour taste in pomegranate juice. Notably, accessions having a sweet taste have higher contents of succinic, oxalic and ascorbic acids. These results agree with those obtained in the Spanish accessions demonstrating that sweet accessions have high levels of oxalic acid, while sour accessions have higher levels of citric acid (*8*).

Organic acids, such as ascorbic acid and citric acid, can contribute to the antioxidant activity of the juice (*39*). In order to get an indication whether they contribute to the antioxidant activity of aril juices and peel homogenates, correlations were made between their levels and antioxidant activity, as previously described (*13*). The results do not show a significant correlation, implying that in aril juices anthocyanins and other phenolic compounds are the main contributors to this activity, as previously suggested (*13*).

As mentioned above, overall consumer appreciation is related more to TSS/TA ratio than to soluble sugars content, or to levels of the different organic acids alone (*40*). The values, for example, of the 'Wonderful' accession that is considered to have a sour-sweet taste, varied from 11 to 16 (*22*). Therefore, we next calculated the TSS/TA ratio. This ratio was found to differ significantly between the various accessions, ranging from 6.1 in PG 109-10 to 64.6 in PG 200-2111 (about 10-fold higher). Screening of the Spanish accessions has shown that in sour-sweet accessions, the TSS/TA ratio ranges from 17 to 28, while accessions considered to have a sour taste have values ranging from 32 to 96 (*8*). However, the most abundant accessions in Spain found on the market are the sweet accessions having a low ratio (*23*).

**Red Pomegranate Accessions Usually Have in General a More Sour Taste than Pink-White Accessions.** Tasting the arils of the 29 accessions left the impression that those accessions having a sour taste had a more red-magenta color than those having a sweet taste. To test this further, color parameters were examined in the aril juices as well as the level of total anthocyanins content, which are the main contributors to aril color (**Table 2**; **Figure 3**) (*23*). It was found that total anthocyanins content had a positive and significant correlation to TA ($R^2 = 0.51$, $p < 0.01$, **Figure 3**). The accessions exhibiting a very low level of anthocyanins (pink and white aril juice color) in general have a low TA level (**Figure 3**). The acidity of the fruit is known to affect anthocyanin color (*37*). Many anthocyanins are red under acidic conditions and turn blue under less acidic conditions; some of them even turn colorless as acid levels drop (*38*). These two parameters, acidity and anthocyanins content, appear to be influenced by climatic and geographical conditions (*41*). High temperatures reduced the levels of both, which can explain why pomegranate accessions grown in desert climates show low TA as well as low level of anthocyanins compared to fruit grown in Mediterranean climates (*21*). A positive correlation between TA and anthocyanins content was also reported for cherry (*42*), black berries (*43*, *44*), blood orange (*41*) and grape berries (*28*).

Although the level of sugars in the different accessions did not vary to a great extent, a significant correlation was found to anthocyanins and TSS values, as well as to glucose/fructose content (**Table 3**). This positive correlation was observed in many plant species, showing that sugars induced accumulation of anthocyanins (*45*). For example, sugars induce biosynthesis of anthocyanins and pigment accumulation in developing corollas of *Petunia* hybrida (*46*), in *Vitis vinifera* cells (*47*), and in radish (*Raphanus sativus*) hypocotyls (*48*), and many anthocyanin biosynthetic genes are induced by sugars (*49*−*51*).

Anthocyanins contribute to fruit color, however, some of these compounds do not have a strong red-purple color. To determine the relationship between anthocyanins levels and aril juice color, correlation matrix was preformed (**Table 3**). The results obtained from the anthocyanins measurements were in agreement with those obtained from the color measurements, showing a significant negative correlation of level of anthocyanins to the color parameter $L^*$, which defines lightness, and the $H°$ parameter, which defines hue angle (**Table 3**). The $a^*$ and $C$ parameters, which define green−red transition and saturation, respectively, show a positive correlation to anthocyanins, whereas no correlation was found to the parameter $b^*$, which defines blue−yellowness.

TA and citric acid show a significant correlation to all color parameters, with the exception of $H°$ (**Table 3**). A high correlation between $a^*$ values define the transition from a green to red color and to TA levels, which are also reported during the development and ripening of pomegranate fruit (*19*), suggesting the major role of TA in determining aril juice color.

**Accessions Harvested Early in the Season Have Lower TSS, TA and Color Parameters Compared to Those Harvested Late in the Season.** At Newe Ya'ar, the fruits of the various accessions were

**Table 2.** Color Determination in Pomegranate Aril Juices of 29 Accessions

| name | $L^*$ | $a^*$ | $b^*$ | $C$ | $H$ |
|---|---|---|---|---|---|
| PG 100-1 | 20.70 ± 1.04 | 3.50 ± 0.42 | 2.10 ± 0.32 | 4.10 ± 0.57 | 31.6 ± 4.42 |
| PG 101-2 | 20.10 ± 1.01 | 3.30 ± 0.40 | 1.80 ± 0.27 | 3.70 ± 0.52 | 28.4 ± 3.98 |
| PG 102-3 | 21.50 ± 1.08 | 3.80 ± 0.46 | 2.50 ± 0.38 | 4.60 ± 0.64 | 32.9 ± 4.61 |
| PG 103-4 | 21.80 ± 1.09 | 4.60 ± 0.55 | 2.30 ± 0.35 | 5.10 ± 0.71 | 26.5 ± 3.71 |
| PG 104-5 | 19.70 ± 0.99 | 5.40 ± 0.65 | 2.50 ± 0.38 | 5.00 ± 0.70 | 26.2 ± 3.67 |
| PG 105-6 | 20.00 ± 1.40 | 4.10 ± 0.12 | 1.20 ± 0.21 | 4.30 ± 0.00 | 16.8 ± 3.20 |
| PG 106-7 | 24.10 ± 1.21 | 0.50 ± 0.06 | 2.30 ± 0.35 | 2.30 ± 0.32 | 78.6 ± 11.00 |
| PG 108-9 | 20.40 ± 1.50 | 4.30 ± 0.90 | 2.20 ± 0.50 | 4.80 ± 0.90 | 29.3 ± 5.80 |
| PG 109-10 | 20.90 ± 1.05 | 5.30 ± 0.64 | 2.20 ± 0.33 | 5.70 ± 0.80 | 22.2 ± 3.11 |
| PG 112-13 | 22.00 ± 1.10 | 4.30 ± 0.52 | 1.80 ± 0.27 | 4.60 ± 0.64 | 22.2 ± 3.11 |
| PG 114-15 | 23.30 ± 1.17 | 4.40 ± 0.53 | 1.00 ± 0.15 | 4.50 ± 0.63 | 12.8 ± 1.79 |
| PG 116-17 | 19.20 ± 0.96 | 3.10 ± 0.37 | 1.80 ± 0.27 | 3.60 ± 0.50 | 30.1 ± 4.21 |
| PG 118-19 | 21.60 ± 0.70 | 3.20 ± 0.20 | 1.50 ± 0.10 | 3.50 ± 0.20 | 25.0 ± 0.40 |
| PG 119-20 | 23.80 ± 0.90 | 3.00 ± 0.60 | 1.10 ± 0.17 | 3.10 ± 0.50 | 20.1 ± 3.40 |
| PG 120-21 | 24.30 ± 1.22 | 3.00 ± 0.36 | 0.80 ± 0.12 | 3.10 ± 0.43 | 14.7 ± 2.06 |
| PG 121-22 | 20.60 ± 1.03 | 4.60 ± 0.55 | 1.40 ± 0.21 | 4.80 ± 0.67 | 16.6 ± 2.32 |
| PG 123-24 | 22.30 ± 1.12 | 3.10 ± 0.37 | 1.30 ± 0.20 | 3.40 ± 0.48 | 22.7 ± 3.18 |
| PG 127-28 | 19.80 ± 0.99 | 4.40 ± 0.53 | 1.80 ± 0.27 | 4.80 ± 0.67 | 22.4 ± 3.14 |
| PG 128-29 | 19.60 ± 0.98 | 3.50 ± 0.42 | 1.30 ± 0.20 | 3.70 ± 0.52 | 20.1 ± 2.81 |
| PG 130-31 | 19.90 ± 1.00 | 1.80 ± 0.22 | 0.40 ± 0.06 | 3.50 ± 0.49 | 16.5 ± 2.31 |
| PG A17 | 22.40 ± 1.12 | 3.60 ± 0.43 | 1.90 ± 0.29 | 4.10 ± 0.57 | 27.7 ± 3.88 |
| PG 204-215 | 25.20 ± 1.26 | 1.20 ± 0.14 | 0.60 ± 0.09 | 1.30 ± 0.18 | 25.2 ± 3.53 |
| PG 203-214 | 25.20 ± 1.26 | 0.01 ± 0.00 | 0.70 ± 0.11 | 3.10 ± 0.43 | 108.3 ± 15.16 |
| EVE | 21.50 ± 1.08 | 4.80 ± 0.58 | 2.50 ± 0.38 | 5.40 ± 0.76 | 27.2 ± 3.81 |
| PG 201-212 | 25.50 ± 1.28 | 0.30 ± 0.04 | 0.50 ± 0.08 | 0.60 ± 0.08 | 60.7 ± 8.50 |
| PG 200-211 | 23.40 ± 1.17 | 1.30 ± 0.16 | 2.90 ± 0.44 | 3.20 ± 0.45 | 65.6 ± 9.18 |
| PG 205-216 | 24.40 ± 1.22 | 0.01 ± 0.00 | 0.00 ± 0.00 | 3.20 ± 0.45 | 42.9 ± 6.01 |
| PG 202-213 | 24.20 ± 1.21 | 1.00 ± 0.12 | 1.80 ± 0.27 | 2.00 ± 0.28 | 60.9 ± 8.53 |
| PG 206-217 | 16.70 ± 0.84 | 6.40 ± 0.77 | 2.50 ± 0.38 | 6.90 ± 0.97 | 21.6 ± 3.02 |

**Table 3.** Correlation Matrix (Spearman Test) Conducted on Data Obtained from Aril's Juices of 29 Accessions[a]

| | $L^*$ | $a^*$ | $b^*$ | $C$ | $H$ | anthocyanins | week's score |
|---|---|---|---|---|---|---|---|
| TA | −0.51** | 0.60** | 0.41* | 0.63** | −0.14 | 0.51** | 0.48* |
| citric acid | −0.53** | 0.58** | 0.57** | 0.62** | 0.02 | 0.53** | 0.51** |
| TSS | −0.78** | 0.55** | 0.32 | 0.61** | −0.30 | 0.70** | 0.55** |
| glucose | −0.58** | 0.31 | 0.22 | 0.40* | −0.16 | 0.47** | 0.44* |
| fructose | −0.59** | 0.31 | 0.22 | 0.41* | −0.17 | 0.48** | 0.44* |
| $L^*$ | 1.00 | −0.61** | −0.33 | −0.67** | 0.36 | −0.87** | −0.42* |
| $a^*$ | | 1.00 | 0.49** | 0.97** | −0.42* | 0.45* | 0.44* |
| $b^*$ | | | 1.00 | 0.56** | 0.41* | 0.03 | 0.53** |
| $C$ | | | | 1.00 | −0.35 | 0.50** | 0.50** |
| $H$ | | | | | 1.00 | −0.48** | 0.16 |
| anthocyanins | | | | | | 1.00 | 0.29 |
| week's score | | | | | | | 1.00 |

[a] The $r$ value of the correlation is given, and its significance ($p < 0.05$) is identified by one asterisk, while ($p < 0.01$) is identified by two asterisks.

harvested during two months, from the end of August to the end of October (**Table 1**). During this period, climatic conditions varied significantly, exhibited mainly by a reduction in temperature and radiation (see Figure 1 in ref *30*). In general, we got the impression that fruits obtained in the late season had a more red color and their taste was sourer than those obtained in the early season, although some accessions such as PG 130-31and PG 128-29 that have a red color were harvested early in the season (**Table 1**). To determine if this is indeed the case, the accessions harvested at the end of August (the first week of harvesting) received a score of 1, those harvested one week later a score of 2, and so on, up to week 9. Next, correlation tests were performed between these scores and the parameters characterizing fruit quality, TA, TSS and color. The correlation tests demonstrate a significant positive correlation between the week's score and TA, citric acid, TSS, fructose and glucose contents, as well as aril color parameters $a^*$, $b^*$ and $C$ (**Table 3**). A significant negative correlation was found to color parameter $L^*$, and no correlation was found to parameter $H°$, as well as to anthocyanins levels. This indicates that fruits obtained in the late season, when temperature and radiation were significantly lower, have more TSS, TA and red color than fruits obtained in the early season. The results were in agreement with a previous study demonstrating that TSS and TA, as well as the aril's red color, in fruits of pomegranates grown in a desert climate in Israel are affected by climatic conditions (*52*). Three accessions, PG 128-29, PG 119-20, PG 101-2, which were also examined in the current study, were grown in this climate. In this habitat, the harvest season is prolonged, meaning that fruits from the same tree can be obtained for a period of over 10 weeks (*52*). During this period, the TSS, TA and the red color (defined as $a^*$ parameter) increase in the arils of these fruits, correlating to a reduction in radiation and temperature (*52*). Lower temperature and radiation levels are important factors leading to an increase in taste parameters (TSS and TA), the contents of organic acids and sugars, as well as in color parameters, as shown in fruits obtained from two different climates, desert and Mediterranean, having the same genetic background (*21*). These findings suggest that, in general, fruits ripening late in the season (when temperature and radiation are significantly lower) develop more citric and soluble sugars and thus have higher TSS and TA values. In addition, they have more of a red color (**Table 3**).

**Levels of Sugar and Organic Acids Differ in Different Peel Homogenates of Various Accessions.** Peel compounds were found to contribute significantly to the health benefits of pomegranate juice. The peels contain a high concentration of phenolic compounds (*3*, *13*, *53*, *54*), which were found to possess antioxidant activity and anticarcinogenic and antiatherosclerosi abilities. A strong correlation was found between antioxidant activity and the level of hydrolyzable tannins, such as punicalagin and punicalin (*13*, *19*, *21*, *54*). Despite their contribution, the peels are inedible since they have a bitter taste and a difficult unjuicy texture. However, some pomegranate juice industries squeeze the fruits in such a way that, in addition to the arils, the compounds found in the peels are also extracted (*53*). Therefore, it is

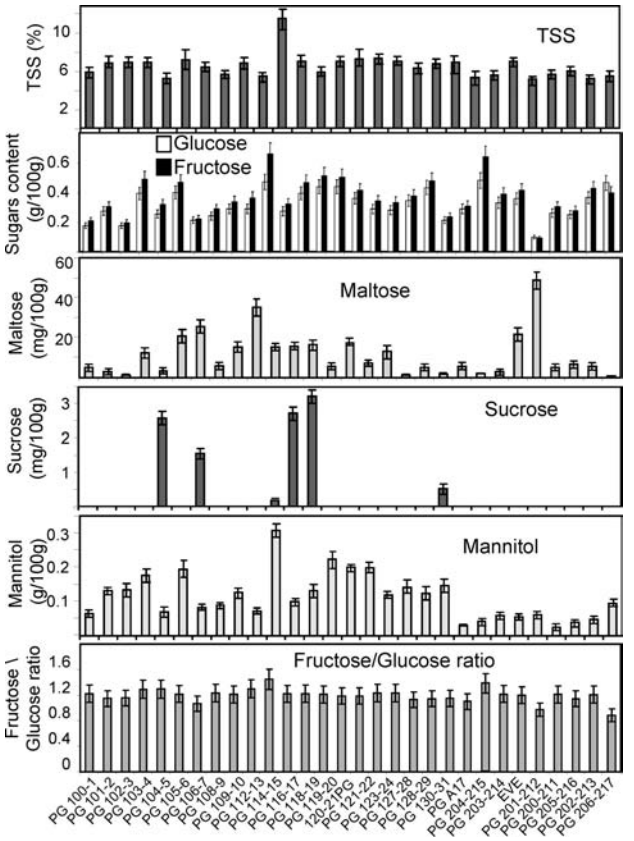


**Figure 4.** TSS, levels of soluble sugars (measured by GC−MS), and the fructose and glucose ratio in peel homogenates of 29 accessions. The data represent the mean ± SD of five replicates from each accession.

important to study the contents of sugars and organic acids in the peels and to determine if they could also contribute to the taste of pomegranate juice. In addition, it would be interesting to determine if those accessions having high TSS and TA levels in the aril juice also have high levels in the peels. This may lead to better knowledge about the relationship between these two fruit tissues. Unlike the TSS values found in the aril juice that varied from 13.7 ± 2.2 to 17.8 ± 1.8 g/100 g, the values obtained for peel homogenates varied significantly, ranging from 4.8 ± 1.3 g/100 g in PG 201-212 to 10.8 ± 1.2 g/100 g in PG 116-17 accession (**Figure 4**). In general, these values were less than those found in aril juices.

The TA level varied in peel homogenates, from 0.25 ± 0.05% in PG 203-214 to 1.1 ± 0.11% in PG 101-2 (about 5.5-fold) (**Figure 5**). These values are within the range found in aril juices, which varied from 0.22% to 1.97%, and were similar to those reported for peels of Georgian accessions, exhibiting TA values ranging from 0.4% to 1.8% (*33*). Notably, the PG 203-214 accession having the lowest TA level in the aril juice also has the lowest TA level in the peel homogenates.

Next, the levels of organic acids and soluble sugars were determined. Determination of sugars and organic acid contents could not be made using HPLC since a high level of polyphenolic compounds was detected at the same wavelength (210 nm) as the organic acids and sugars, thus masking the peaks of the desired compounds. Therefore, in order to identify sugars and organic acids in peel homogenates, we used GC−MS. The predominant sugars detected were glucose and fructose (**Figure 4**). Unlike the values found in aril juices, in which the levels of glucose and fructose did not vary significantly in the different accessions, that of the peel homogenates was found in the range of 0.9 ± 0.05 g/100 g in PG 200-211 to 4.8 ± 0.28 in PG 203-214 for glucose

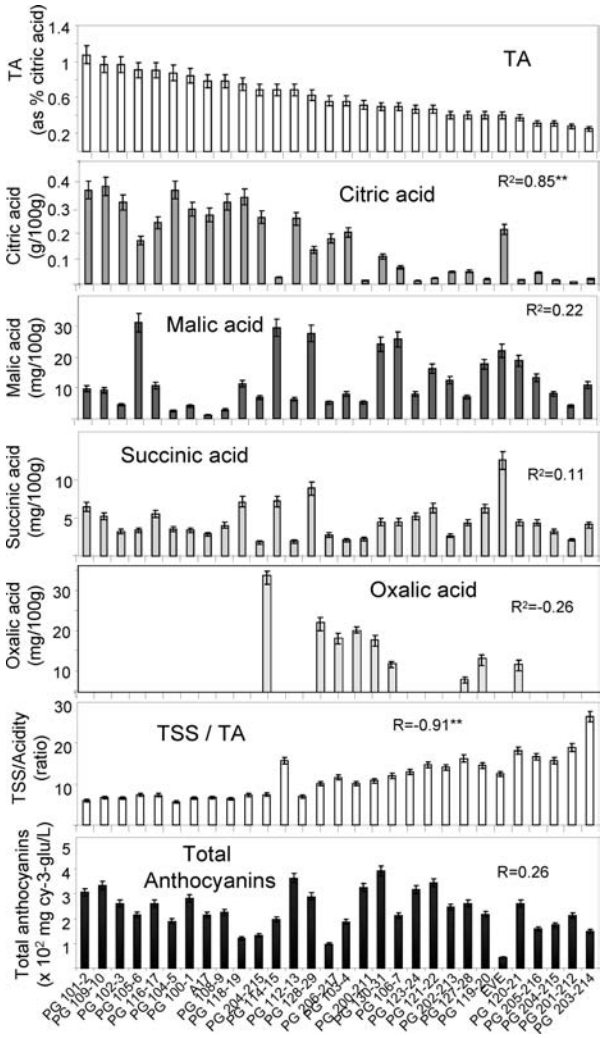


**Figure 5.** Titratable acidity (TA), organic acids levels (measured by GC−MS), the TSS and TA ratio, and total anthocyanins level in mg cyanidin 3-glucoside equivalents per L juice in peel homogenates prepared from 29 pomegranate accessions. The data represent the mean ± SD of five replicates from each accession. The $R^2$ value was calculated against the TA. Significance of the $R^2$ value ($p < 0.01$) is identified by two asterisks.

(5.3-fold), and 0.9 ± 0.07 in PG 200-211 to 6.6 ± 0.65 in PG 114-15 for fructose (6.6-fold). The level of fructose was higher than that of glucose in most of the accessions, and as a result, the fructose-to-glucose ratio exceeded 1 (**Figure 4**), inconsistent with the level found in the aril juices of these accessions. In addition to these two sugars, maltose was also found in a range of 0.8 ± 0.04 mg/100 g in accession PG 206-217 to 48.9 ± 4.6 mg/100 g in accession PG 201-212, as well as sucrose, which was detected in six accessions at relatively low levels (**Figure 4**). The low sucrose content may be explained by the rationale that it may be converted by invertase to glucose and fructose during the ripening process. Mannitol, an alcoholic sugar, was also detected in peel homogenates in a similar amount to glucose and fructose (**Figure 4**).

Citric, malic and succininc acids were detected in peel homogenates. Eight out of 12 accessions having oxalic acid in their aril juices also have oxalic acids in their peel homogenates. As for aril juice, citric acid correlated significantly to TA (**Figure 5**); it was also found to be the major organic acid in the peels, followed by malic, oxalic and succinic acids. Citric acid was also found to be the major organic acid in the peels of six accessions grown in Georgia, followed by malic and succinic acids, while oxalic acid

**Table 4.** Correlation Matrix (Spearman Test) Conducted on Data Obtained from Peel's Homogenates of 29 Accessions in the Season of 2006[a]

| | TA | citric acid | malic acid | succinic acid | oxalic acid | TSS | glucose | fructose | *L** | *a** | *b** | *C* | *H* | anthocyanins | week's score |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| TA | 1.00 | 0.85** | −0.22 | 0.11 | −0.36 | −0.01 | −0.33 | −0.28 | −0.45* | 0.40* | −0.31 | 0.51** | −0.35 | −0.03 | 0.41* |
| citric acid | | 1.00 | −0.28 | 0.05 | −0.19 | −0.29 | −0.28 | −0.28 | −0.48** | 0.39* | −0.37 | 0.48** | −0.38* | −0.16 | 0.52** |
| malic acid | | | 1.00 | 0.62** | 0.22 | 0.58** | 0.58** | 0.51** | 0.19 | 0.06 | −0.13 | −0.17 | −0.02 | 0.58** | −0.60** |
| succinic acid | | | | 1.00 | 0.16 | 0.55** | 0.32 | 0.25 | −0.02 | 0.10 | −0.29 | −0.10 | −0.13 | 0.53** | −0.22 |
| oxalic acid | | | | | 1.00 | 0.37 | 0.35 | 0.32 | 0.08 | 0.11 | −0.24 | −0.14 | −0.16 | 0.21 | 0.19 |
| TSS | | | | | | 1.00 | 0.52** | 0.54** | −0.01 | 0.01 | −0.12 | −0.10 | −0.10 | 0.69** | −0.20 |
| glucose | | | | | | | 1.00 | 0.96** | −0.08 | 0.22 | −0.18 | 0.04 | −0.24 | 0.59** | −0.39* |
| fructose | | | | | | | | 1.00 | −0.15 | 0.26 | −0.24 | 0.09 | −0.30 | 0.61** | −0.34 |
| *L** | | | | | | | | | 1.00 | −0.77** | 0.68** | −0.68** | 0.93** | −0.28 | −0.44* |
| *a** | | | | | | | | | | 1.00 | −0.58** | 0.84** | −0.83** | 0.19 | 0.10 |
| *b** | | | | | | | | | | | 1.00 | −0.25 | 0.79** | −0.31 | −0.13 |
| *C* | | | | | | | | | | | | 1.00 | −0.61** | −0.02 | 0.17 |
| *H* | | | | | | | | | | | | | 1.00 | −0.44* | −0.27 |
| anthocyanins | | | | | | | | | | | | | | 1.00 | −0.10 |
| week's score | | | | | | | | | | | | | | | 1.00 |

[a] The *r* value of the correlation is given, and its significance ($p < 0.05$) is identified by one asterisk, while ($p < 0.01$) is identified by two asterisks.

**Table 5.** Color Determination in Pomegranate Peel's Skin of 29 Accessions

| name | *L** | *a** | *b** | *C* | *H* |
|---|---|---|---|---|---|
| PG 100-1 | 48.10 ± 5.00 | 40.00 ± 7.00 | 26.40 ± 1.50 | 48.10 ± 5.60 | 34.05 ± 5.79 |
| PG 101-2 | 57.67 ± 6.98 | 30.50 ± 14.60 | 31.00 ± 4.00 | 45.11 ± 5.96 | 48.56 ± 8.26 |
| PG 102-3 | 59.50 ± 6.20 | 31.60 ± 8.00 | 35.70 ± 3.60 | 46.60 ± 4.40 | 49.10 ± 8.35 |
| PG 103-4 | 56.20 ± 5.60 | 39.80 ± 8.80 | 26.00 ± 3.20 | 48.00 ± 5.40 | 33.13 ± 5.63 |
| PG 104-5 | 46.60 ± 7.10 | 47.60 ± 4.80 | 23.00 ± 3.40 | 53.00 ± 3.60 | 25.96 ± 4.41 |
| PG 105-6 | 40.09 ± 4.85 | 48.50 ± 3.60 | 18.90 ± 2.70 | 52.11 ± 6.89 | 21.15 ± 3.60 |
| PG 106-7 | 68.90 ± 4.40 | 0.10 ± 6.30 | 41.00 ± 3.40 | 41.50 ± 2.90 | 91.22 ± 15.51 |
| PG 108-9 | 44.00 ± 8.50 | 43.80 ± 5.10 | 23.50 ± 3.60 | 49.90 ± 4.20 | 31.88 ± 5.42 |
| PG 109-10 | 20.60 ± 2.49 | 5.30 ± 1.45 | 2.00 ± 0.33 | 5.70 ± 0.75 | 54.17 ± 9.21 |
| PG 112-13 | 56.20 ± 8.40 | 38.60 ± 10.50 | 27.00 ± 4.40 | 48.00 ± 6.10 | 36.26 ± 6.16 |
| PG 114-15 | 65.60 ± 7.50 | 25.70 ± 11.90 | 29.70 ± 6.20 | 40.80 ± 5.10 | 51.34 ± 8.73 |
| PG 116-17 | 38.40 ± 3.90 | 45.40 ± 5.40 | 18.60 ± 3.40 | 49.10 ± 6.20 | 22.13 ± 3.76 |
| PG 118-19 | 41.08 ± 4.97 | 47.00 ± 5.20 | 19.00 ± 2.60 | 50.50 ± 6.68 | 21.60 ± 3.67 |
| PG 119-20 | 61.74 ± 7.47 | 34.60 ± 12.10 | 25.30 ± 5.80 | 43.29 ± 5.72 | 41.31 ± 7.02 |
| PG 120-21 | 59.90 ± 10.10 | 35.20 ± 14.70 | 28.50 ± 4.90 | 45.80 ± 8.60 | 42.82 ± 7.28 |
| PG 121-22 | 61.10 ± 9.70 | 35.70 ± 13.30 | 26.20 ± 3.70 | 44.90 ± 9.30 | 39.58 ± 6.73 |
| PG 123-24 | 59.50 ± 10.10 | 37.10 ± 15.30 | 27.00 ± 3.10 | 47.20 ± 10.60 | 40.58 ± 6.90 |
| PG 127-28 | 26.60 ± 2.40 | 16.00 ± 6.90 | 2.60 ± 1.90 | 16.20 ± 7.10 | 8.34 ± 1.42 |
| PG 128-29 | 48.31 ± 5.85 | 45.80 ± 6.70 | 25.90 ± 3.40 | 52.95 ± 7.00 | 29.92 ± 5.09 |
| PG 130-31 | 49.94 ± 6.05 | 44.30 ± 12.14 | 28.00 ± 4.63 | 52.23 ± 6.90 | 28.34 ± 4.82 |
| PG A17 | 59.90 ± 12.30 | 24.80 ± 13.40 | 38.40 ± 5.70 | 47.50 ± 3.70 | 57.97 ± 9.85 |
| PG 204-215 | 68.40 ± 7.20 | 16.70 ± 10.80 | 34.20 ± 5.00 | 39.70 ± 3.10 | 64.58 ± 10.98 |
| PG 203-214 | 64.10 ± 4.80 | 3.70 ± 12.10 | 42.10 ± 4.80 | 44.60 ± 2.70 | 84.59 ± 14.38 |
| EVE | 53.10 ± 5.20 | 38.50 ± 7.00 | 30.10 ± 3.50 | 49.30 ± 5.10 | 38.48 ± 6.54 |
| PG 201-212 | 62.30 ± 10.30 | 26.70 ± 15.30 | 34.50 ± 6.70 | 46.20 ± 5.60 | 53.85 ± 9.15 |
| PG 200-211 | 65.40 ± 4.40 | −2.00 ± 7.30 | 44.40 ± 2.60 | 45.00 ± 2.60 | 92.56 ± 15.74 |
| PG 205-216 | 66.23 ± 6.98 | 0.001 ± 0.00 | 0.001 ± 0.00 | 42.34 ± 5.60 | 71.24 ± 12.11 |
| PG 202-213 | 66.10 ± 10.80 | 23.60 ± 15.80 | 28.70 ± 4.80 | 40.10 ± 7.00 | 53.90 ± 9.16 |
| PG 206-217 | 39.40 ± 4.50 | 44.00 ± 4.70 | 18.70 ± 3.90 | 47.90 ± 5.80 | 22.82 ± 3.88 |

was found at a low amount (*33*). Malic and succinic acids were also found to correlate significantly to TSS content ($R^2 = 0.58$ and $R^2 = 0.55$, respectively, $p < 0.01$), suggesting that they contribute to its content.

Next, the ratio between TSS and TA was calculated, showing that this value varied between 5.6 in PG 101-2 to 26.4 in accession PG 203-214. These values are less than those found in aril juices (6.1 to 64.6).

**Lower Correlations Were Found between the Parameters of the Peel Color and TA and TSS.** The color parameters of the peels were studied (**Table 5**) to determine if they correlated to the other parameters of the peels (Table **4**). If such correlations will be found, it will suggest that fruit color could provide a good indication of peel quality and the content of its compounds. The results show, as revealed by the naked eye, that the peel's skin color differs significantly between the various accessions (**Table 5**). As found for the aril juice, the *a** parameter correlated to TA and citric acid, although at lower values (**Table 4**). Total anthocyanins content did not exhibit a significant correlation to TA and citric acid, but significant correlations were found between total anthocyanins content to malic and succinic acids. Significant negative correlations were found between harvesting dates to the *L** parameter, but not to the other color parameters. Similar to aril juice, the anthocyanins level correlated significantly to TSS, glucose and fructose levels, and had a significant negative correlation to color parameter *H*°. The low correlation values can be explained by the fact that the color parameters were only measured on the external peel, whereas the other parameters were

**Table 6.** Correlation Matrix (Spearman Test) Conducted on Data Obtained from the Peels and Aril Juices of 29 Accessions in the 2006 Season[a]

| | peels | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| arils | TA | TSS | citric acid | glucose | fructose | *L** | *a** | *b** | *C* | *H* | anthocyanin |
| TA | 0.62** | −0.21 | 0.78** | −0.10 | −0.08 | −0.21 | 0.20 | −0.048** | 0.25 | −0.31 | −0.28 |
| TSS | 0.75** | 0.22 | 0.61** | −0.08 | −0.08 | −0.55** | 0.32 | −0.62** | −0.003 | −0.56** | 0.36 |
| citric acid | 0.67** | −0.33 | 0.88** | −0.19 | −0.15 | −0.35 | 0.40* | −0.30 | 0.40* | −0.38* | −0.36 |
| glucose | 0.53** | 0.26 | 0.32 | -0.15 | −0.16 | −0.34 | 0.07 | −0.038* | −0.08 | −0.27 | 0.37* |
| fructose | 0.52** | 0.26 | 0.31 | −0.15 | -0.15 | −0.34 | 0.07 | −0.38* | −0.08 | −0.28 | 0.37* |
| *L** | −0.60** | −0.003 | −0.56** | 0.06 | 0.07 | 0.76** | −0.58** | 0.60** | −0.20 | 0.73** | −0.28 |
| *a** | 0.55** | 0.10 | 0.54** | 0.03 | 0.06 | −0.49** | 0.51** | −0.62** | 0.09 | −0.65** | 0.25 |
| *b** | 0.50** | −0.33 | 0.54** | −0.68** | −0.62** | −0.17 | −0.06 | -0.09 | 0.05 | 0.01 | −0.29 |
| *C* | 0.51** | 0.12 | 0.54** | 0.04 | 0.10 | −0.46* | 0.33 | −0.51** | 0.10 | −0.49** | 0.22 |
| *H* | −0.37* | −0.22 | −0.28 | −0.18 | −0.13 | 0.39* | −0.64** | 0.56** | −0.08 | 0.72** | −0.39* |
| Anthocyanins | 0.41* | 0.07 | 0.39* | 0.24 | 0.17 | −0.74** | 0.64** | −0.46* | 0.34 | −0.69** | 0.26 |

[a] The *r* value of the correlation is given, and its significance ($p < 0.05$) is identified by one asterisk, while ($p < 0.01$) is identified by two asterisks.

measured in the peel homogenate, which also includes the internal part of the peels.

**High Correlations Were Found between Arils and Peels in TA Content and Some Color Parameters.** It is important to establish correlations between the external fruit's skin color and that of the aril juice in order to achieve fruit quality determinations by colorimetric online nondestructive methods. It was previously reported that such correlations were found to one of the two accessions that were examined, PG 121-2, while less correlations were found to the second accession, PG 101-2 (*19*). In addition, the relationship between the peels and the arils to the parameters that are important for fruit taste qualities, TA and TSS, could lead to better knowledge about the factors regulating pomegranate juice qualities and, in the future, to gain more information about regulating these parameters. To gain more knowledge about the connections between arils and peels in the color parameters and on the relationship of TSS and TA values, a correlation matrix was performed. It was found that the levels of TA and citric acid are significantly positively correlated, but no correlations were found in the levels of TSS and soluble sugars (**Table 6**). The color parameters *L**, *a** and *H* were found to be highly correlated, but such a correlation was not found to total anthocyanins content and to the *b** and *C* color parameters (**Table 6**). Since correlations were found between the aril and peel color parameters of *L** (defining lightness), *a** (defining red−greenness transition) and *H*°, which are the major parameters defining color, we suggest that, in general, the fruit's skin color could give some indication about aril color. However, the color parameters and total anthocyanins content of the peels cannot predict aril quality, and the levels of TA, TSS, organic acids and sugars of the aril juices.

All in all, our major findings are (i) in aril juices, the TSS and the sugars do not differ considerably among the 29 accessions, implying that they are not the main contributors to taste; (ii) the levels of TA and citric acid differ significantly among these accessions, suggesting that they play a major role in determining juice taste; (iii) citric acid is predominant in the sour accession, while oxalic and succinic acids are major in sweet accessions; (iv) positive and significant correlation was found between TA and TSS, suggesting that sour accessions contain more sugars than sweet accessions; (v) significant positive correlations were found between TA values and the red color parameters in aril juices, and these two parameters, as well as TSS, appeared to be higher in the juices of accessions harvested late in the season; (vi) peel homogenates exhibited lower levels of TSS and TA than aril juices; and (vii) positive correlations were found between aril juices and peel homogenates in the red color parameters, TA and citric acid.

In general, the results of this study could lead to acquiring better knowledge about the diversity of different accessions in parameters relating to taste, color and size. In addition to basic knowledge, such data will enable breeders to select and breed genotypes having higher color and desirable taste, and will help industries to produce better pomegranate juices based on consumer demand.

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## LITERATURE CITED

(1) Bell, C.; Hawthorne, S. Ellagic acid, pomegranate and prostate cancer—a mini review. *J. Pharm. Pharmacol.* **2008**, *60*, 139–44.

(2) Reddy, M. K.; Gupta, S. K.; Jacob, M. R.; Khan, S. I.; Ferreira, D. Antioxidant, antimalarial and antimicrobial activities of tannin-rich fractions, ellagitannins and phenolic acids from *Punica granatum* L. *Planta Med.* **2007**, *73*, 461–467.

(3) Kotwal, G. J. Genetic diversity-independent neutralization of pandemic viruses (e.g. HIV), potentially pandemic (e.g. H5N1 strain of influenza) and carcinogenic (e.g. HBV and HCV) viruses and possible agents of bioterrorism (variola) by enveloped virus neutralizing compounds (EVNCs). *Vaccine* **2007**, *26*, 3055–3058.

(4) Aviram, M.; Rosenblat, M.; Gaitini, D.; Nitecki, S.; Hoffman, A.; Dornfeld, L.; Volkova, N.; Presser, D.; Attias, J.; Liker, H.; Hayek, T. Pomegranate juice consumption for 3 years by patients with carotid artery stenosis reduces common carotid intima-media thickness, blood pressure and LDL oxidation. *Clin. Nutr.* **2004**, *23*, 423–433.

(5) Seeram, N. P.; Zhang Y.; Heber, D. Commercialization of pomegranates: fresh fruit, beverages, and botanical extract. In *Pomegranates: Ancient Roots to Modern Medicine*; Seeram, N. P., Heber, D., Eds.; Taylor and Francis Group: New York, 2006; pp 187−198.

(6) Holland, D.; Hatib, K.; Bar-Ya'akov, I. Pomegranate: Botany, Horticulture, Breeding. *Hortic. Rev.* **2008**, *35*, 127–191.

(7) Poyrazolua, E.; Gökmen, V.; Artık, N. Organic Acids and Phenolic Compounds in Pomegranates (*Punica granatum* L.) Grown in Turkey. *J. Food Compos. Anal.* **2002**, *15*, 567–575.

(8) Melgarejo, P.; Salazar, D.; Artes, F. Organic acids and sugars composition of harvested pomegranate fruits. *Eur. Food Res. Technol.* **2000**, *211*, 185–190.

(9) Cemeroglu, B.; Artik, N.; Erbas, S. Gewinnung von Granatapfelsaft und seine Ausammensetzung. *Fluess. Obst.* **1992**, *59*, 335–340.

(10) Unal, C.; Velioglu, S.; Cemeroglu, B. Turk nar sularinin bilesim ogeleri. *Gida* **1995**, *20*, 339–345.

(11) Al-Kahtani, H. A. Intercultivar differences in quality and postharvest life of pomegranates influenced by partial drying. *J. Am. Soc. Hortic. Sci.* **1992**, *117*, 100–104.

(12) Gabbasova, L. B.; Abdurazakova, S. K. Chemical composition of pomegranate juice. *Pishch. Tekhnol.: Nauchno-Tekh. Z.* **1969**, *4*, 30–31.

(13) Tzulker, R.; Glazer, I.; Bar-Ilan, I.; Holland, D.; Aviram, M.; Amir, R. Antioxidant activity, polyphenol content and related

compounds in different fruit juices and homogenates prepared from 29 different pomegranate accessions. *J. Agric. Food Chem.* **2007**, *55*, 9559–70.
(14) Giusti, M. M.; Wrolstad, R. E. Characterization and measurement of anthocyanins by UV-visible spectroscopy, in Current Protocols in Food Analytical Chemistry, Schwartz, S. J., Wrolstad, R. E., Eds.; John Wiley & Sons, Inc.: New York, 2001; pp F1.2.1−F1.2.13.
(15) Han, K. H.; Sekikawa, M.; Shimada, K.; Hashimoto, M.; Hashimoto, N.; Noda, T.; Tanaka, H.; Fukushima, M. Anthocyanin-rich purple potato flake extract has antioxidant capacity and improves antioxidant potential in rats. *Br. J. Nutr.* **2006**, *96*, 1125–1133.
(16) Solomon, A.; Yablowicz, Z.; Grossman, S.; Bergman, M.; Gottlieb, H. E.; Altman, A.; Kerem, Z.; Flaishman, M. A. Antioxidant activities and anthocyanin content of fresh fruits of common fig (*Ficus carica* L.). *J. Agric. Food Chem.* **2006**, *54*, 7717–7723.
(17) Fanciullino, A. L.; Cercós, M.; Dhuique-Mayer, C.; Froelicher, Y.; Talón, M.; Ollitrault, P.; Morillon, R. Changes in carotenoid content and biosynthetic gene expression in juice sacs of four orange varieties (*Citrus sinensis*) differing in flesh fruit color. *J. Agric. Food Chem.* **2008**, *56*, 3628–3638.
(18) Aaby, K.; Wrolstad, R. E. Phenolic composition and antioxidant activities in flesh and achenes of strawberries (Fragaria ananassa). *J. Agric. Food Chem.* **2005**, *53*, 4032–4040.
(19) Shwartz, E.; Glazer, I.; Bar-Ya'akov, I.; Matityahu, I.; Bar-Ilan, I.; Holland, D.; Amir, R. Changes in chemical constituents during the maturation and ripening of two commercially important pomegranate cultivars. *Food Chem.* **2009**, *115*, 965–973.
(20) Fait, A.; Angelovici, R.; Less, H.; Ohad, I.; Urbanczyk-Wochniak, E.; Fernie, A. R.; Galili, G. Arabidopsis seed development and germination is associated with temporally distinct metabolic switches. *Plant Physiol.* **2006**, *142*, 839–854.
(21) Shwartz, E.; Revital, T.; Glazer, I.; Bar-Ya'akov, I.; Tripler, E.; Wiesman, Z.; Bar-Ilan, I.; Fromm, H.; Borochov-Neori, H.; Holland, D.; Amir, R. Environmental conditions affect the color, taste and antioxidant capacity of 11 pomegranate accessions' fruits. *J. Agric. Food Chem.* **2009**, *57*, 9197–9209.
(22) Ben-Arie, R.; Segal, N.; Guelfat-Reich, S. The maturation and ripening of the 'wonderful' pomegranate. *J. Am. Soc. Hortic. Sci.* **1984**, *109*, 898–902.
(23) Gil, M. I.; Artes, F.; Tomas-Barberan, F. A. Changes in pomegranate juice pigmentation during ripening. *J. Sci. Food Agric.* **1995**, *68*, 77–81.
(24) Sezer, E. D.; Akçay, Y. D.; Ilanbey, B.; Yildirim, H. K.; Sözmen, E. Y. Pomegranate wine has greater protection capacity than red wine on low-density lipoprotein oxidation. *J. Med. Food.* **2007**, *10*, 371–374.
(25) Veres, M. Mechanical and chemical composition of cultivated pomegrante. *Huana Isharna* **1976**, *17*, 426–432.
(26) Casini, C.; Dardanelli, J. L.; Martínez, M. J.; Balzarini, M.; Borgogno, C. S.; Nassetta, M. Oil quality and sugar content of peanuts (*Arachis hypogaea*) grown in Argentina: their relationship with climatic variables and seed yield. *J. Agric. Food Chem.* **2003**, *51*, 6309–6313.
(27) Fauconnier, M. L.; Beltrán, R.; Delcarte, J.; Dejaeghere, F.; Marlier, M.; du Jardin, P. Lipoxigenase pathway and membrane permeability and composition during storage of potato tubers (*Solanum tuberosum*. L. Cv. Bintje and Désirée) *in different conditions*. *Plant Biol.* **2002**, *4*, 77–85.
(28) Pereira, G. E.; Gaudillere, J. P.; Pieri, P.; Hilbert, G.; Maucourt, M.; Deborde, C; Moing, A.; Rolin, D. Microclimate influence on mineral and metabolic profiles of grape berries. *J. Agric. Food Chem.* **2006**, *54*, 6765–6775.
(29) Doty, T. E. fructose sweetness: a new dimension. *Cereal Food World* **1976**, *21*, 62–63.
(30) Stanhope, K. L.; Schwarz, J. M.; Keim, N. L.; Griffen, S. C.; Bremer, A. A.; Graham, G.; Hatcher, B.; Cox, C. L.; Dyachenko, A.; Zhang, W.; McGahan, J. P.; Seibert, A.; Krauss, R. M.; Chiu, S.; Schaefer, E. J.; Ai, A.; Otokozawa, S.; Nakajima, K.; Nakano, T.; Beysen, C.; Hellerstein, M. K.; Berglund, L.; Havel, P. J. Consuming fructose-sweetened, not glucose-sweetened, beverages increases visceral adiposity and lipids and decreases insulin sensitivity in overweight/obese humans. *J. Clin. Invest.* **2009**, *119*, 1322–1334.
(31) Legua, P.; Melgarejo, P.; Martnez, M.; Hernández, F. Evolution of sugars and organic acid content in three pomegranate cultivars (*Punica granatum L*). *Options Mediterr.* **2000**, *42*, 99–104.
(32) Al-Maiman, S. A.; Ahnad, D. Changes in physical and chemical properties during pomegranate (*Punica granatum L.*) fruit maturation. *Food Chem.* **2002**, *76*, 437–441.
(33) Pande, G.; Akoh, C. C. Antioxidant Capacity and Lipid Characterization of Six Georgia-Grown Pomegranate Cultivars. *J. Agric. Food Chem.*, in press.
(34) Khodade, M. S.; Wavhal, K. N.; Kale, P. N. Physico-chemical changes during growth and development of pomegranate fruit. Indian. *J. Hortic.* **1990**, *47*, 21–27.
(35) Gautier, H.; Bénard, C.; Reich, M.; Buret, M.; Bourgaud, F.; Poëssel, J. L.; Caris-Veyrat, C.; Génard, M. How does tomato quality (sugar, acid, and nutritional quality) vary with ripening stage, temperature, and irradiance? *J. Agric. Food Chem.* **2008**, *56*, 1241–1250.
(36) Aarabi, A.; Barzegar, M.; Azizi, M. H. Effect of Cultivar and Cold Storage of Pomegranate (*Punica granatum L.*) Juices on Organic Acid Composition. *ASEAN Food J.* **2008**, *15*, 45–55.
(37) Dumlu, M. U.; Gürkan, E. Elemental and nutritional analysis of *Punica granatum* from Turkey. *J. Med. Food.* **2007**, *10*, 392–5.
(38) Sadka, A.; Dahan, E.; Or, E.; Cohen, L. NADP(+)-isocitrate dehydrogenase gene expression and isozyme activity during citrus fruit development. *Plant Sci.* **2000**, *158*, 173–181.
(39) Silva, B. M.; Andrade, P. B.; Valentao, P.; Ferreres, F.; Seabra, R. M.; Ferreira, M. A. Quince (Cydonia oblonga Miller) fruit (pulp, peel, and seed) and Jam: antioxidant activity. *J. Agric. Food Chem.* **2004**, *52*, 4705–4712.
(40) Rapisarda, P.; Lo Cascio, R.; Bonina, F.; De Pasquale, A.; Saija, A. Antioxidant effectiveness as influenced by phenolic content of fresh orange juices. *J. Agric. Food Chem.* **1999**, *47*, 4718–4723.
(41) Cisse, M.; Vaillant, F.; Acosta, O.; Dhuique -Mayer, C.; Dornier, M. Thermal Degradation Kinetics of Anthocyanins from Blood Orange, Blackberry, and Roselle Using the Arrhenius, Eyring, and Ball Models. *J. Agric. Food Chem.* **2009**, *57*, 6285–6291.
(42) Kim, D. O.; Heo, H. J.; Kim, Y. J.; Yang, H. S.; Lee, C. Y. Sweet and sour cherry phenolics and their protective effects on neuronal cells. *J. Agric. Food Chem.* **2005**, *53*, 9921–9927.
(43) Bordonaba, J. G.; Terry, L. A. Biochemical profiling and chemometric analysis of seventeen UK-grown black currant cultivars. *J. Agric. Food Chem.* **2008**, *56*, 7422–7430.
(44) Laleh, G.; Frydoonfar, H.; Heidary, R.; Jameei, R.; Zare, S. The effect of light, temperature, pH and species on stability of anthocyanin pigments in four Berberis species. *Pak. J. Nutr.* **2006**, *5*, 90–92.
(45) Zheng, Y.; Tian, L.; Liu, H.; Pan, Q.; Zhan, J.; Huang, W. Sugars induce anthocyanin accumulation and flavanone 3-hydroxylase expression in grape berries. *Plant Growth Regul.* **2009**, *58*, 251–260.
(46) Moalem-Beno, D.; Tamari, G.; Leitner-Dagan, Y.; Borochov, A.; Weiss, D. Sugar-Dependent Gibberellin-Induced Chalcone Synthase Gene Expression in Petunia Corollas. *Plant Physiol.* **1997**, *113*, 419–424.
(47) Vitrac, X.; Larronde, F.; Krisa, S.; Decendit, A.; Deffieux, G; Mérillon, J. M. Sugar sensing and Ca2+-calmodulin requirement in Vitis vinifera cells producing anthocyanins. *Phytochemistry* **2000**, *53*, 695–665.
(48) Hara, M.; Hoshino, K.; Oki, K.; Kuboi, T. Induction of anthocyanin accumulation by sugar in radish hypocotyl. *Plant Cell Physiol.* **2003**, *44*, S120.
(49) Sheng, T.; Leónie, B.; Maarten, K.; Sjef, S. Sucrose-Specific Induction of Anthocyanin Biosynthesis in Arabidopsis Requires the MYB75/PAP1 Gene. *Plant Physiol.* **2005**, *139*, 1840–1852.
(50) Yanjun, Z.; Hongtao, L.; Qiuhong, P.; Jicheng, Z.; Weidong, H. Sugars induce anthocyanin accumulation and flavanone 3-hydroxylase expression in grape berries. *Plant Growth Regul.* **2009**, *58*, 251–260.

(51) Gollop, R.; Farhi, S.; Perl, A. Regulation of the leucoanthocyanidin dioxygenase gene expression in Vitis vinifera. *Plant Sci*. **2001**, *161*, 579–588.

(52) Borochov-Neori, H.; Judeinstein, S.; Tripler, E.; Harari, M.; Greenberg, A.; Shomer, I.; Holland, D. Seasonal and cultivar variations in antioxidant and sensory quality of pomegranate (*Punica granatum L.*) fruit. *J. Food Compos. Anal*. **2009**, *22*, 189–195.

(53) Gil, M. I.; Hess-Pierce, B.; Holcroft, D. M.; Kader, A. A. Antioxidant activity of pomegranate juice and its relationship with phenolic composition and processing. *J. Agric. Food Chem*. **2000**, *48*, 4581–4589.

(54) Li, Y.; Guo, C.; Yang, J. J.; Wei, J.; Xu, S.; Cheng, S. Evaluation of antioxidant properties of pomegranate peel extract in comparison with pomegranate pulp extract. *Food Chem*. **2006**, *96*, 254–260.

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