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Environmental Conditions Affect the Color, Taste, and Antioxidant Capacity of 11 Pomegranate Accessions' Fruits

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The well-established health beneficial value of pomegranate juice is leading to increased demand for pomegranate products and to the expansion of pomegranate orchards worldwide. The current study describes differences in the chemical composition of major ingredients of the arils and peels of 11 accessions grown in Mediterranean and desert climates in Israel. In most of the accessions, the levels of antioxidant activity and content of total phenolics, total anthocyanins, total soluble solids, glucose, fructose, and acidity were higher in the aril juice of fruit grown in the Mediterranean climate compared to those grown in the desert climate. However, the peels of fruit grown in the desert climate exhibited higher antioxidant activity, and the levels of total phenolics, including the two hydrolyzable tannins, punicalagin and punicalin, were higher compared to those in the peels of fruit grown in the Mediterranean climate. The results indicate that environmental conditions significantly affect pomegranate fruit quality and health beneficial compounds.

KEYWORDS: Anthocyanin; antioxidant activity; climatic conditions; growth conditions; total phenolics; *Punica granatum* L.

INTRODUCTION

Fruits and vegetables contain high levels of antioxidant compounds that protect against harmful free radicals and have been associated with a lower incidence of cancer and heart disease in addition to other health benefits (*1*, *2*). Pomegranate (*Punica granatum* L.) is known to be one of the healthiest fruits due to its high antioxidant activity (*3*−*6*) and high content of anticarcinogenic compounds (*7*−*12*). Recent biological studies have established that certain compounds in pomegranate juice that significantly reduce low-density lipoproteins (LDL) oxidation can also reduce blood pressure and possess antiatherosclerotic effects (*4*).

By screening 29 pomegranate accessions, we recently found that the antioxidant activity in aril juice correlated significantly to total polyphenol (*13*, *14*) and anthocyanin contents (*13*). However, the antioxidant activity of homogenates prepared from the peels were approximately 40-fold higher than that measured in aril juice, demonstrating that the peels contain compounds having high antioxidant activity, as previously suggested (*5*, *15*). The antioxidant level in the homogenates correlated significantly to the contents of four hydrolyzable tannins, of which punicalagin was the predominant component, whereas no correlation was found to the level of anthocyanins (*13*).

Extensive knowledge of pomegranate's health attributes and increasing public awareness of functional foods has led in recent years to a large increase in the demand for pomegranate fruit and its byproducts in the Western world (*16*). As a result, the land area devoted to pomegranate orchards has increased significantly, including plantations in different geographic regions having diverse growth conditions (*17*). It is suggested that Mediterranean-like climates are optimal for pomegranate fruit growth. These climatic conditions include intense sunlight, mild winters with minimal temperatures not lower than −12 °C, and hot dry summers without precipitation during the last stages of the fruit's development (*18*). Under such conditions, the fruit can reach its optimal size, color, and sugar accumulation, without the danger of peel splitting (*18*). Pomegranates are cultivated today throughout the world in subtropical and tropical areas in many different microclimatic zones (*17*). Commercial orchards of pomegranate trees are currently grown in the Mediterranean basin (e.g., North Africa, Egypt, Israel, Syria, Lebanon, Greece, Italy, and Spain) and Asia (e.g., Iran, Iraq, Turkey, Turkmenistan, Georgia, India, China, and Thailand). Pomegranates are also grown in the New World, and North and South America, and new orchards are currently being established in South Africa, Australia, Argentina, and Brazil (*17*).

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Knowledge of how environmental conditions affect the pomegranate fruit's nutritional value, quality, and health properties has not yet been established. We recently studied fruit quality parameters in three identical pomegranate accessions grown in Yizre'el Valley (Mediterranean climate) (*13*) and in the southern Arava Valley (desert climate) (*14*). However, a detailed comparison between the two habitats with regard to antioxidant activity and major chemical compositions was not made, and this is our main objective in the current study. Additional objectives in this research were (i) to study whether the growth habitat affects harvest dates and (ii) to examine whether the different pomegranate accessions having different genetic backgrounds are influenced similarly by exposure to different environmental conditions.

MATERIALS AND METHODS

Meteorological Measurements. Data were collected from standard meteorological stations situated on the southern Arava R&D and Newe Ya'ar experimental farms and are presented for the period of the fruit's development and ripening. Temperature and relative humidity (RH) (maximum/minimum) were measured using electronic RH and temperature sensors (MP101A-T7-W4W probe, Rotronic A.G., Switzerland). Global radiation was measured using a Pyranometer (Campbell Scientific Inc., Logan, UT) and given in megajoules per square meter (MJ/m^2). The values for the 2007 season are presented in **Figure 1**; similar data were obtained during the 2006 season.

Plant Materials and Fruit Processing. Eleven pomegranate accessions (P.G.101-2, P.G.104-5, P.G.105-6, P.G.106-7, P.G.114-15, P.G.116-17, P.G.118-19, P.G.119-20, P.G.127-28, P.G.128-29, and P.G.130-31) were chosen for this study. These accessions were chosen because they exhibit high variability with regard to ripening date, peel and aril colors, and taste. Cuttings from pomegranate accessions [registered in Israel Plant Bank (IGB, Website http://igb.agri.gov.il)] (*17*) were taken from the experimental orchard of the Newe Ya'ar Research Center, ARO, and transferred to the southern Arava R&D experimental farm in 2001.

The Newe Ya'ar experimental farm is located in the western Yizre'el Valley in northern Israel (latitude 32° 42′ N, longitude 35° 11′ E) and is characterized by a Mediterranean temperate to subtropical climate (*17*). The experimental farm is located 100 m above sea level, the soil is heavy clay grumusol (vertisol) (**Table 1**), and the average annual rainfall is 580 mm, which occurs mostly between November and March. Irrigation was applied using drinking quality water, 1.2 $Ds \cdot m^{-1}$. Daily irrigation began in March and ended in October (about 6000 m^3/ha), varying slightly between the years. Soil and water compositions are given in **Table 1**.

The southern Arava R&D experimental farm is situated in Israel's southern Arava Valley (latitude 29° 53′ N, longitude 35° 3′ E) and is characterized by a desert climate (*14*). Arava sandy loam soil (*Typic Torrifluvent*) properties and local irrigation water composition are given in **Table 1**. Year-round irrigation was applied on a daily basis, according to local practice (12000 m^3 of water/ha and 1.008 ton/ha of nitrogen). Horticultural management (pest control, pruning, thinning, and harvesting) was based on recommended practices specified for southern Israel by the Ministry of Agriculture Extension Service.

Trees in the experimental farms were planted at a 3 × 5 m distance, four replicates per accession. Ten fruits from each accession (three to four fruits per tree) were harvested when fully matured from the first wave of ripening of each accession. Intact fruits of the same size, shape, and color were selected. The fruits were transported under ventilated conditions to the laboratory. In the laboratory, fruits were cut, and intact arils were separated from the pith by hand; ripeness was further assessed by tasting. Only nonastringent edible fruits were analyzed further.

In the 2006 season, each of the 10 fruits obtained per pomegranate accession was divided into halves. One half was squeezed with an orange juice extractor to produce juice originating from the arils, the membranous walls (septum), and the inner part of the peel. The arils from the second half were hand-separated and squeezed using a nylon sieve to produce the aril juice. In the 2007 season, the peels and arils were separated from each fruit. Peel homogenate was prepared by homogenizing 10 g of the peels

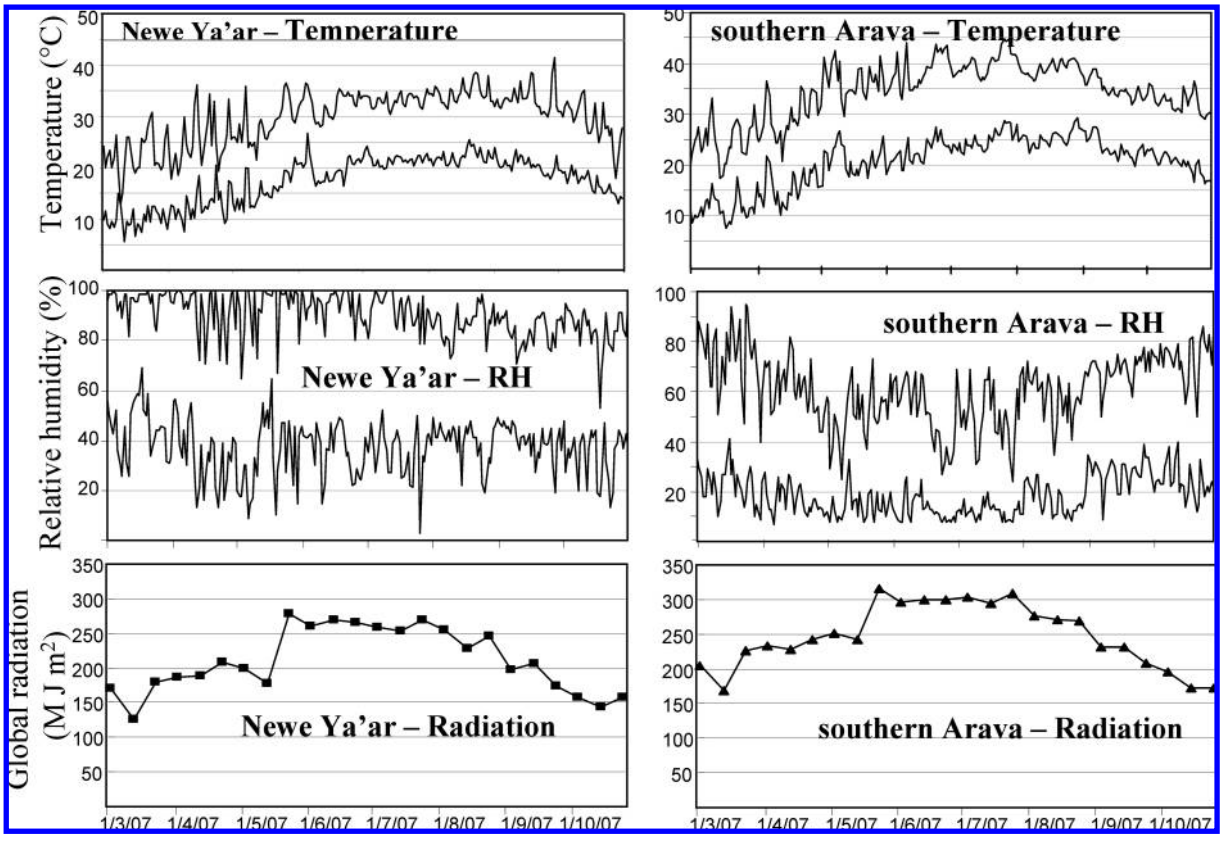

Figure 1. Climatic data of the Newe Ya'ar experimental farm (western Yizre'el Valley, latitude 32° 42′ N, longitude 35° 11′ E) (left panels) and the southern Arava R&D experimental farm (Israeli southern Arava Valley, latitude 29° 53′ N; longitude 35° 3′ E) (right panels). Maximal (upper lines) and minimal (lower lines) air temperature (upper panels), maximal and minimal relative humidity (RH) (middle panels), and global radiation are shown for the 2007 season.

Table 1. Physical Properties of Soil (**A**) and Chemical Composition of the Irrigation Water (**B**) from Newe Ya'ar and the Southern Arava Valley

(A) Physical Properties of Soil

	value (%)	
soil parameter	Newe Ya'ar	southern Arava
particle size distribution		
sand	13	83
silt	27	8
clay	60	9
organic matter content	1.28	1.3

(B) Chemical Composition of Irrigation Water

	value	
ion	Newe Ya'ar	southern Arava
Ca^{2+} (mmol L^{-1})	7	11
Na^{+} (mmol L^{-1})	4	6
Cl^{-} (mmol L^{-1})	7	30
SO_4^{2-} (mmol L^{-1})	62	1
HCO^{3-} (mmol L^{-1})	3	2.29
EC (dS m^{-1})	1.2	3

with 40 mL of distilled water (*19*, *20*) in a Retsch mill (model MM301). Aril juice was prepared as described for the 2006 season. The different juices and peel homogenates were centrifuged (15 min at 4000 rpm), and the supernatants were collected and stored frozen at −20 °C for further analysis.

Physical Analysis. Each fruit from every accession was weighed to 1 mg accuracy on a balance. Fruit volume was measured using the liquid displacement method.

Determination of Total Soluble Solids (TSS) and Titratable Acidity (TA). The measurements were made on fresh aril juice. TSS in the juice was measured using a digital refractometer (ATAGO RR-1 serial no. 602055 Tokyo, Japan; calibrated with distilled water). The instrument was set to measure %TSS with the temperature compensation mode. A Metrohm titration unit (Brinkmann, Metrohm ch-9101, Herisau, Switzerland) equipped with a 719S Titrino titration assembly was used to determine the total TA. 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 and expressed as grams of citric acid per 100 g of fresh weight or percentage of citric acid. Measurements were repeated twice for each aril juice sample.

Determination of Total Phenolic Content. For total phenolic compounds content determination, the juices were diluted 1:10 and the colorimetric method, which modified the Singleton method for small volumes (*19*, *21*), was employed. Half a milliliter of the juice sample, or quercetin (4−20 μM) standard, was mixed with 2.5 mL of diluted Folin−Ciocalteu reagent (1:10 in water). After a short vortexing, 20% Na_2CO_3 was added and the tubes were mixed for 5 min at 50 °C. The absorbance of the cooled (25 °C) samples was measured at 760 nm.

Antioxidant Activity Evaluation. Antioxidant activity was measured, as previously recommended, using the FRAP method (*5*, *13*). This method was developed to measure the ferric reduction ability of plasma at a low pH (*22*). An intense blue color is formed when the ferric−tripyridyltriazine (Fe^{3+}−TPTZ) complex is reduced to the ferrous (Fe^{2+}) form, which was recorded at 593 nm. Standard solution of 1 mM Trolox (6-hydroxy-2,3,7,8-tetramethylchroman-2-carboxylic acid) in methanol was prepared. The pomegranate juices were diluted 1:50 in water. Fifty microliters of diluted samples or standards (0.1−0.7 μM) was mixed with 950 μL of FRAP solution freshly prepared by mixing 25 mL of acetate buffer (pH 3.6), 2.5 mL of TPTZ, and 2.5 mL of $FeCl_3 \cdot 6H_2O$ solutions. These solutions were left to react for 4 min under continuous stirring. Changes in absorbance at 593 nm were then measured at 25 °C. The results were expressed as Trolox equivalent antioxidant capacity (TEAC) (*15*, *22*).

Determination of Total Anthocyanins Content. Anthocyanin pigments undergo reversible structural transformations with a change in pH manifested by strikingly different absorbance spectra (*23*). 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 later used to dilute the sample with the sodium acetate buffer. The absorbance of each sample, diluted in the two different buffers, was recorded at two wavelengths, 510 and 700 nm, after 15 min of incubation (four measurements per sample). Anthocyanin absorbance was then calculated according to the equation $A = (A_{510} - A_{700})_{\text{pH 1.0}} - (A_{510} - A_{700})_{\text{pH 4.5}}$ and used to estimate total anthocyanin content. A molar extinction coefficient (ε) of 26900 M^{-1} cm^{-1} and a molecular weight of 449.2 were employed to express the results as milligrams of cyanidin-3-glucoside per liter of juice.

Determination of Aril Juice and Peel Color. Pomegranate skin color was measured using a colorimeter (Chroma Meter CR-301, Minolta, Ramsey, NJ) (*24*). 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, and a^* and b^* define red-greenness and blue-yellowness, respectively; 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.

Arils from each of the 10 fruits were pooled and pressed through nylon sieve to extract the juice. Juice color was measured using a Minolta CR-301 colorimeter. An aliquot of each juice was analyzed using a plate (diameter of 5 cm) and a white background. Blank measurements were made with the plate filled with distilled water (*25*).

LC-MS Analysis of Hydrolyzable Tannins. The squeezed juice (2006 season) and peel homogenate (2007 season) were diluted with doubled-distilled water 1:1000. The samples were further diluted at 1:1 with acetonitrile (Merck catalog no. 30) to achieve a final dilution of 1:2000. The samples were filtered through a 0.45 μm filter into testing vials and analyzed by an LC-MS instrument using a Waters 2790 HPLC system equipped with a Micromass triple-quadrupole Quatro-Ultima mass spectrometer in series consisting of a HPLC quaternary pump, an autosampler, and a vacuum degasser. The system was controlled by Micromass MassLynx ver. 4.0 software. The chromatographic separations and solvent gradient were the same as previously described for punicalagin and punicalin (*13*, *20*). The solute was inserted into the mass spectrometer using an electrospray ionization probe in the negative mode. The high selectivity identity of the compound was obtained using the multiple-reaction monitoring (MRM) method according to their mother and daughter ions. The mother ion (precursor ion) was fragmented by argon using different collision energies, and the daughter ion areas of the standard solutions were compared to those obtained from the pomegranate samples (*13*, *20*).

Organic Acid and Sugar Contents. The contents of organic acids and sugars were determined in the aril juice. Aril juice was diluted 1:10 with distilled water and then filtered through a 0.45 μm 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, whereas the detection of sugars was obtained by a refractive index detector. A standard curve of pure organic acids and sugars (glucose, fructose, sucrose, and sorbitol) purchased from Sigma (Poole, Dorest, U.K.) was used for quantification. Results were expressed as milligrams of ascorbic acid per 100 g (*26*) and those for sugars as parts per million (ppm) sugars of the juices (*20*).

Determination of Oil Content in the Pomegranate Seed. The seeds left after the aril juice preparation were cleaned manually of pulp and juice, washed carefully, and then dried for 48 h in an oven heated to 70 °C. The resulting dried seeds were used as raw material for oil extraction. The oil was extracted by a pressurized solvent extraction (PSE) technique using the onePSE machine (Applied Separation, Inc.) (*27*). The samples were prepared according to the onePSE user's manual, version 1.91. Dried seeds (1.5 g) were crushed by a mortar and pestle with an added 3 g of onePSE

matrix. Extraction conditions were as follows: operating temperature and pressure were set at 110 °C and 100 bar, respectively. The onePSE was programmed to perform the extraction in two consecutive 25 min cycles using hexane as a solvent. After these cycles, the hexane was evaporated in a rotary evaporator at a reduced temperature (below 50 °C).

Statistical Analysis. Data obtained from this study were analyzed statistically using JMP software, ver. 5. This software used the Student's *t* test. Principal component analysis (PCA) was performed on the data sets obtained from different measurements with the software TMEV (*28*) using the default weighted covariance-estimation function. The data were log-transformed and normalized to the median of the entire sample set for each parameter before analysis. This transformation reduced the influence of outliers.

RESULTS AND DISCUSSION

The 11 pomegranate accessions chosen for the current study differed in their peel and aril colors (**Figure 2**), taste, and size (*13*, *14*, *17*). Copies of these accessions were grown in Newe Ya'ar and the southern Arava Valley, which differ significantly in terms of their temperature and humidity (minimum/maximum) as well as radiation, as shown in **Figure 1**. Newe Ya'ar is characterized by a Mediterranean temperate to subtropical climate (*17*), whereas the southern Arava is characterized by a hot and dry desert climate (*14*). The different climates in both locations affect the flowering and harvest dates. Observations performed in the past five years demonstrated that the flowering season begins in early March in southern Arava and mid-April in Newe Ya'ar. This probably affects the harvest dates that were generally earlier in southern Arava than in Newe Ya'ar (shown for the 2007 season in **Table 2**). The generally earlier ripening date of pomegranates in the southern Arava valley indicates the potential of extending the fruit season by growing pomegranate cultivars in this location.

Fruits Obtained from Newe Ya'Ar Were Juicier than Those Obtained from Southern Arava. Fruit sizes from the two growth habitats were measured in both seasons. In general, fruit weight and volume were higher in the fruits grown in southern Arava than in Newe Ya'ar in 2006; however, in 2007, the fruits obtained from Newe Ya'ar were heavier than those from southern Arava (**Figure 3**; Supporting Information Table A). Although fruit sizes differed in both years, the volume of aril juices was higher in Newe Ya'ar in both seasons. This suggests that more arils were produced or that the arils were juicier in fruits grown in Newe Ya'ar. To gain better knowledge about this finding, we next counted the number of white membranous walls inside each fruit. We found that their numbers were 1.14−1.25-fold higher in fruits obtained from Newe Ya'ar than those from southern Arava (see also **Figure 2**). Because the number of membranous walls affects the number of arils and peel thickness (*14*), we further suggest that the environmental conditions at Newe Ya'ar promote a higher formation of these membranous walls, and this is the main reason for the higher juice content found in fruits from this habitat. These results are in agreement with our previous study demonstrating that the arils per fruit and juice content in fruits of pomegranate grown in the southern Arava are affected by climatic conditions (*14*). This was found in three accessions, P.G. 128-29, P.G.119-20, and P.G. 101-2, which were also examined in the current study. In this habitat, the harvest season is prolonged (*14*) and aril weights increases with harvest season progression. Because water and soil qualities do not change in this habitat during the harvesting period, we further suggest that climate, mainly radiation, temperature, and humidity, which vary significantly during this period (*14*), affects aril numbers and their juice content.

Oil Content in Seeds. Oil content in the fruit seeds was measured in the 2007 season (**Table 3**). The values were in the range from 7.76 to 17.96 g/100 g of dried seeds, varying for the different accessions. Four accessions (P.G.114-15, P.G.116-17, P.G.128-29, and P.G.130-31) exhibited significantly higher oil contents when grown in southern Arava compared to Newe Ya'ar. These results suggest that fruits grown in a hot dry climate may have higher oil content in the seeds. In both habitats, the oil content was higher than that reported previously in 20 pomegranate accessions from Turkey, the contents of which were 2.41−3.73 g/100 g in the dry seeds (*29*). The differences between the Turkish and Israeli collections could most probably stem from the different analysis tools and/or genetic backgrounds; however, they might be also explained by the growth conditions that could have enhanced oil content under the hotter conditions found in Israel compared to Turkey during fruit development and ripening.

Table 2. Harvest Days of Pomegranate Accessions Grown in Two Geographical Regions in Israel (Newe Ya'ar and Southern Arava Valley) Examined in 2007

accession	Newe Ya'ar	southern Arava
P.G.101-2	October 10	October 1
P.G.105-6	August 26	July 25
P.G.106-7	September 30	August 20
P.G.114-15	August 26	August 6
P.G.116-17	October 7	August 6
P.G.118-19	September 23	July 25
P.G.119-20	August 26	August 6
P.G.127-28	October 28	October 1
P.G.128-29	August 26	July 22
P.G.130-31	August 26	July 22

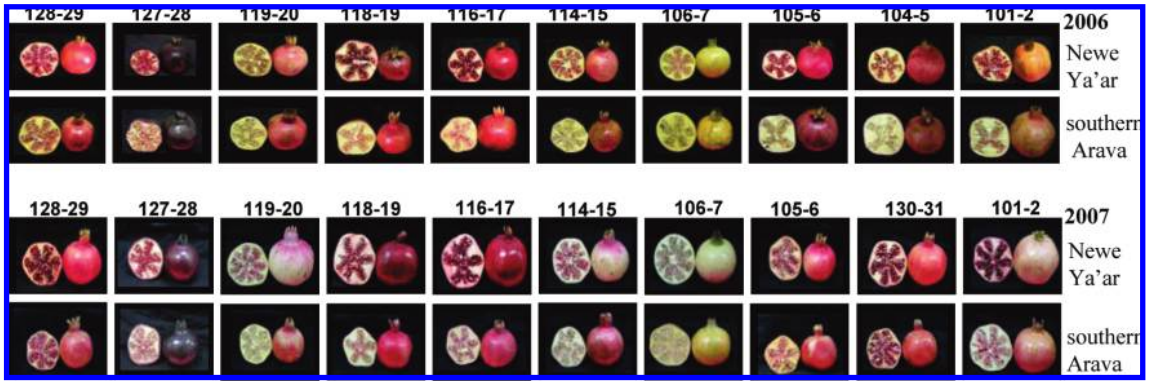

Figure 2. Fruits of the 11 pomegranate accessions used in this study as photographed in the 2006 and 2007 seasons.

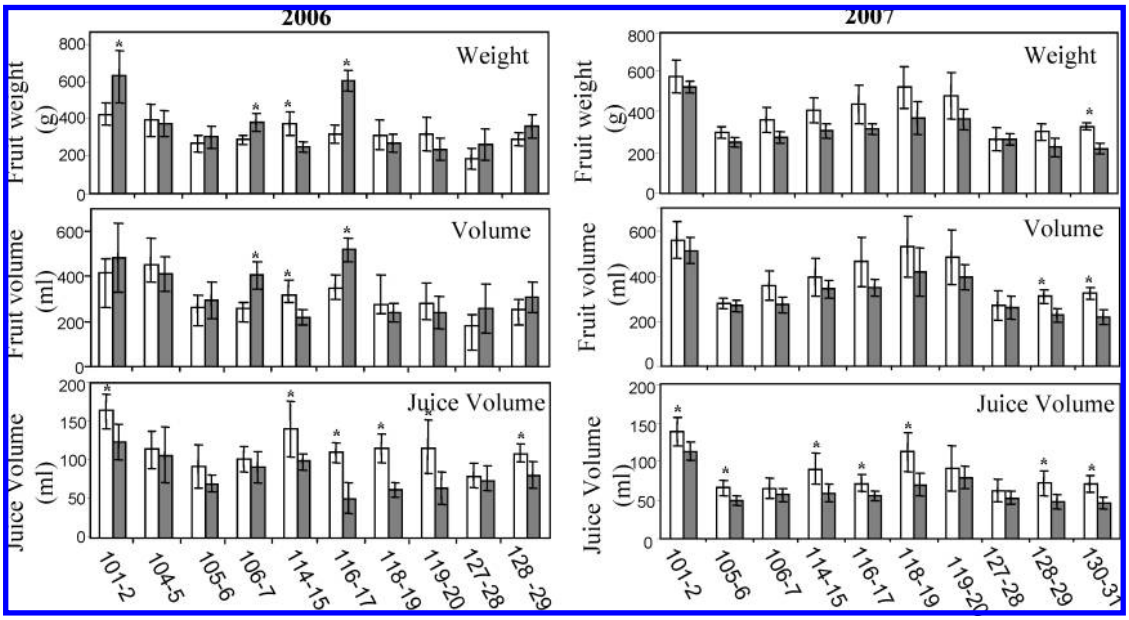

Figure 3. Fruit size, weight, and volume, as well as aril juice volume, of the 11 pomegranate accessions grown in Newe Ya'ar (white bars) and southern Arava (gray bars) in the 2006 and 2007 seasons. The data represent the mean ± SD of 10 replicates from each accession. Independent sample Student's *t* test was used to determine statistically significant differences in each accession grown in the two habitats ($p < 0.05$), identified by an asterisk.

Table 3. Oil Content in Pomegranate Seeds of Fruit from Different Accessions Grown in Two Locations in Israel (Newe Ya'ar and Southern Arava Valley) in the 2007 Season[a]

	oil (g/100 g of dry seed)	
accession	Newe Ya'ar	southern Arava
P.G.101-2	11.81 ± 0.56	12.14 ± 0.65
P.G.105-6	10.97 ± 0.44	9.96 ± 0.51
P.G.106-7	11.09 ± 0.33	10.99 ± 0.20
P.G.114-15	8.74 ± 0.37	9.76 ± 0.80 *
P.G.116-17	9.84 ± 0.88	11.88 ± 0.60 *
P.G.119-20	8.64 ± 0.78	7.76 ± 0.48
P.G.128-29	16.17 ± 0.19	17.96 ± 0.27 *
P.G.130-31	16.43 ± 0.45	17.45 ± 0.35 *

[a] Each value is the mean of four ± SD. Statistically significant changes ($p < 0.05$, using Student's *t* test) are identified with an asterisk.

Levels of Antioxidant Capacity, Total Phenolic, and Total Anthocyanin Contents in Aril Juice Were Generally Higher in Fruit Grown in Newe Ya'ar than in Southern Arava. Pomegranate fruit's beneficial health effects are related, at least in part, to its antioxidant activity (*30*). Isolated pomegranate arils are the edible part of the fruit; therefore, we first examined the level of antioxidant activity in aril juice. In both years and in most of the accessions, the aril juice obtained from Newe Ya'ar fruits had a higher antioxidant activity than those from southern Arava (exceptions are P.G.114-15 from 2006 and P.G.116-17 and P.G.118-19 in both seasons) (**Figure 4**; Supporting Information Table B).

Because in pomegranates as well as in many other fruits and vegetables, the antioxidant activity level can be attributed to total phenolic content (*13*, *20*), the latter was measured. As described in **Figure 4**, total phenolic content is generally consistent with antioxidant activity, as expected. In many berry fruits, as well as in pomegranates, total phenolic content is also correlated to the level of total anthocyanins. Anthocyanins are water-soluble polyphenolic pigments, the primary source of the attractive red-violet-blue colors of many fruits, including pomegranate arils and peels (*13*, *31*, *32*), and exhibit considerable antioxidant activity (*33*−*36*). Indeed, we previously found high and significant correlations between antioxidant activity and the level of total anthocyanins in pomegranate aril juices (*13*, *20*). Hence, the levels of total anthocyanins in aril juice were then measured. It was found that the level of total anthocyanins was significantly higher in fruits obtained from Newe Ya'ar in both years compared to those from southern Arava (**Figure 4**; Supporting Information Table B). The level of anthocyanins correlated highly to the antioxidant levels and to total phenolic content in Newe Ya'ar ($r = 0.84$, $p < 0.01$; $r = 0.70$, $p < 0.01$, respectively), but less in southern Arava ($r = 0.34$, $p < 0.01$; $r = 0.61$, $p < 0.01$, respectively). This suggests that anthocyanins are the major contributors to the antioxidant activity detected in aril juices of the Newe Ya'ar fruit but less so in the southern Arava fruit, where the major contributors to antioxidant activity are likely phenolics other than anthocyanins.

The higher level of anthocyanins in Newe Ya'ar fruits could be attributed to the relatively lower temperatures in this habitat compared to southern Arava during the time of fruit development and ripening (**Figure 1**). Temperature is an important factor that affects anthocyanin accumulation in plants. The expression of anthocyanin biosynthetic genes has been induced by low temperature and repressed by high temperature in various plants, such as apple (*37*), *Arabidopsis* (*38*), grape (*39*, *40*), red orange (*41*), and rose (*42*). However, additional studies have shown that chemical or enzymatic degradation also plays a role in anthocyanin accumulation (*39*, *40*). In red wine grape, high temperatures (maximum 35 °C) reduced total anthocyanin content to less than half of that in the control berries (maximum = 25 °C) (*39*). The decrease in anthocyanin accumulation at the high temperature was attributed to factors such as enhanced anthocyanin degradation as well as the inhibition of mRNA transcription of anthocyanin biosynthesis genes (*39*). Degradation is also affected by pH, light, oxygen, and anthocyanin structure (*39*). In addition, some of the low anthocyanin levels found in grape berries grown at high temperatures could also be attributed to the oxidative stress occurring under such conditions, which induced peroxidase that degraded anthocyanins in the skin of the berries (*39*). Peroxidase was also found to be involved in the degradation of anthocyanin in *Brunfelsia* flowers (*43*). Therefore, we hypothesize that the lower anthocyanin level in the arils of pomegranates grown in southern Arava is mainly due to the effect

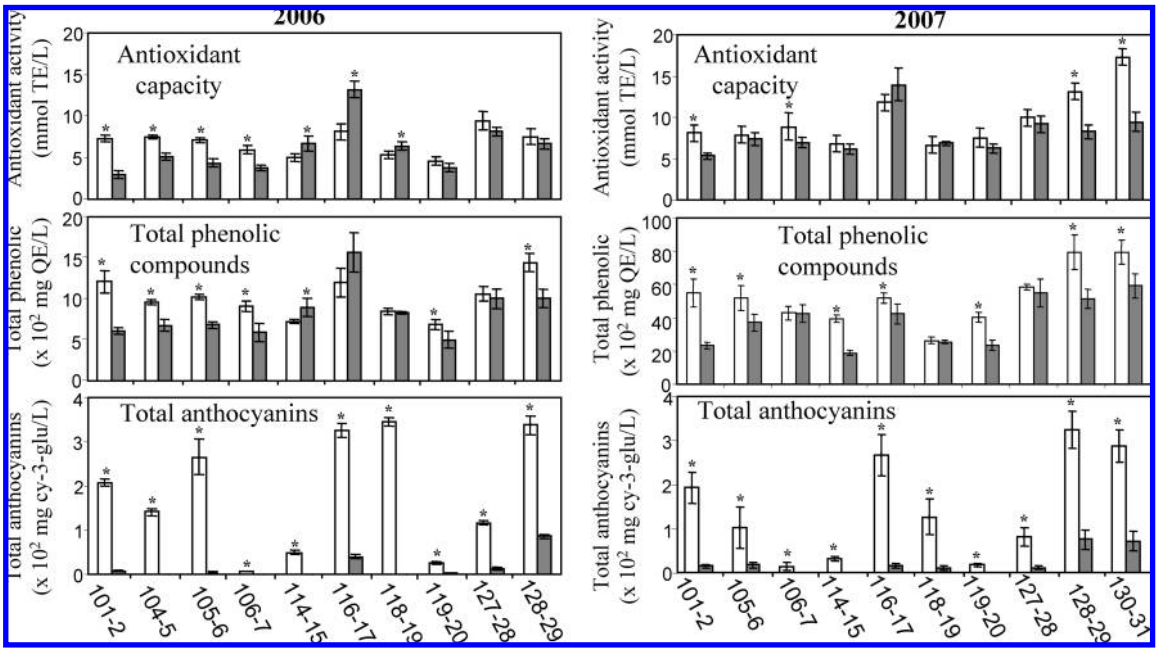

Figure 4. Antioxidant activity (in mmol of Trolox equivalents (TE)/L of juice), total soluble phenolic compounds content (in mg of quercetin equivalents (QE)/L of juice), and total anthocyanin level (in mg of cyanidin 3-glucoside equivalents/L of juice) in aril juices prepared from the pomegranate accessions grown in Newe Ya'ar (white bars) and southern Arava (gray bars) in the 2006 and 2007 seasons. The data represent the mean ± SD of 10 replicates from each accession. Independent sample Student's *t* test was used to determine statistically significant differences in each accession grown in the two habitats ($p < 0.05$), identified by an asterisk.

Table 4. Color Determination in Pomegranate Aril Juices of Different Accessions Grown in Newe Ya'ar and the Southern Arava Valley

	*L**		*a**		*b**		*C*		*H*	
accession	Newe Ya'ar	southern Arava	Newe Ya'ar	southern Arava	Newe Ya'ar	southern Arava	Newe Ya'ar	southern Arava	Newe Ya'ar	southern Arava
					2006					
P.G.101-2	20.1 ± 0.6	24.1 ± 3.2	3.3 ± 0.8	1.9 ± 0.2	1.7 ± 0.3	1.5 ± 0.2	3.7 ± 0.2	2.5 ± 2.4	28.4 ± 3.6	43.6 ± 7.1
P.G.104-5	19.7 ± 0.3	25.4 ± 4.3	3.9 ± 0.4	0.1 ± 0.2	2.2 ± 0.6	1.5 ± 0. 4	5.1 ± 0.5	1.5 ± 0.6	26.1 ± 5.2	100.4 ± 8.2
P.G.105-6	19.9 ± 0.4	23.5 ± 2.6	4.1 ± 0.9	0.6 ± 0.1	1.4 ± 0.2	2.1 ± 0.2	4.3 ± 0.4	3.5 ± 0.9	19.1 ± 5.2	79.1 ± 13.1
P.G.106-7	24.1 ± 0.8	24.3 ± 1.9	0.5 ± 0.1	0.7 ± 0.3	2.3 ± 0.6	2.2 ± 0.3	2.3 ± 0.2	2.3 ± 0.3	78.6 ± 10.7	72.5 ± 10.2
P.G.114-15	23.3 ± 0.2	26.1 ± 2.1	4.4 ± 0.7	0.1 ± 0.1	1.1 ± 0.1	0.8 ± 0.1	4.5 ± 0.8	0.9 ± 0.1	12.8 ± 1.6	121.9 ± 20.3
P.G.116-17	19.0 ± 0.8	27.6 ± 1.2	3.1 ± 0.7	4.7 ± 0.9	1.8 ± 0.6	0.6 ± 0.2	3.6 ± 0.3	5.9 ± 1.2	30.1 ± 5.2	331.1 ± 23.4
P.G.118-19	21.6 ± 3.1	27.1 ± 0.4	3.3 ± 0.9	4.9 ± 0. 6	1.5 ± 0.4	1.7 ± 0.1	3.6 ± 0.9	2.1 ± 0.2	25.1 ± 1.4	306.4 ± 18.3
P.G.119-20	23.2 ± 1.5	25.3 ± 3.1	3.4 ± 1.2	0.9 ± 0.2	1.1 ± 0.2	0.2 ± 0.1	3.5 ± 0.5	1.1 ± 0.4	17.7 ± 1.4	11.6 ± 0.8
P.G.127-28	19.9 ± 3.2	22.2 ± 0.4	4.4 ± 0.6	3.1 ± 0.5	1.8 ± 0.2	0.1 ± 0.1	4.8 ± 0.7	3.1 ± 0.4	22.4 ± 3.5	1.1 ± 0.2
P.G.128-29	19.6 ± 2.4	22.1 ± 1.1	3.5 ± 0.5	3.2 ± 0.3	1.3 ± 0.3	1.8 ± 0.5	3.7 ± 0.3	3.7 ± 0.6	20.1 ± 5.4	29.6 ± 2.8
					2007					
P.G.101-2	15.8 ± 0.3	20.8 ± 1.2	5.2 ± 0.7	2.9 ± 0.6	2.5 ± 0.6	3.5 ± 0.4	5.8 ± 0.8	4.6 ± 0.5	25.5 ± 3.4	49.6 ± 6.6
P.G.105-6	18.5 ± 0.6	21.1 ± 0.6	3.4 ± 0.5	1.9 ± 0.5	1.7 ± 0.5	3.1 ± 0.7	3.8 ± 0.6	3.6 ± 0.6	25.8 ± 5.3	58.7 ± 9.6
P.G.106-7	22.1 ± 0.5	23.4 ± 0.5	2.7 ± 1.1	0.4 ± 0.1	2.2 ± 0.4	2.1 ± 0.4	3.6 ± 0.7	2.1 ± 0.4	41.9 ± 15.2	80.6 ± 3.1
P.G.114-15	20.1 ± 0.5	25.1 ± 0.6	5.4 ± 0.4	0.2 ± 0.1	1.1 ± 0.2	0.3 ± 0.1	5.5 ± 0.5	0.4 ± 0.1	11.7 ± 1.6	62.2 ± 25.6
P.G.116-17	18.3 ± 1.1	22.2 ± 0.7	3.4 ± 0.7	3.6 ± 0.7	1.5 ± 0.3	0.8 ± 0.2	3.7 ± 0.8	3.7 ± 0.7	23.7 ± 2.1	13.4 ± 5.5
P.G.118-19	19.1 ± 0.8	23.1 ± 0.5	6.1 ± 0.4	3.1 ± 0.9	2.9 ± 0.3	0.8 ± 0.1	6.8 ± 0.4	3.1 ± 0.8	25.9 ± 2.1	17.1 ± 6.6
P.G.119-20	21.7 ± 0.5	23.8 ± 0.4	3.8 ± 0.7	0.1 ± 0.1	0.7 ± 0.1	0.9 ± 0.1	3.9 ± 0.7	0.9 ± 0.1	10.5 ± 1.8	98.5 ± 12.5
P.G.127-28	20.0 ± 2.6	17.4 ± 0.7	3.9 ± 0.9	4.6 ± 0.9	2.1 ± 0.5	1.2 ± 0.6	4.4 ± 1.1	4.7 ± 1.1	28.4 ± 3.7	15.0 ± 6.2
P.G.128-29	17.8 ± 1.1	20.9 ± 0.4	2.6 ± 0.5	3.7 ± 0.5	1.1 ± 0.2	1.5 ± 0.3	2.8 ± 0.6	4.1 ± 0.4	21.9 ± 1.5	22.4 ± 3.6
P.G.130-31	20.1 ± 0.6	20.6 ± 1.1	1.8 ± 0.4	3.9 ± 0.3	0.4 ± 0.2	1.7 ± 0.2	1.8 ± 0.4	4.2 ± 0.4	11.4 ± 4.7	24.7 ± 2.9

of the high temperatures in this habitat on the biosynthesis pathway as well as anthocyanin degradation but is also due to the high oxidative stress that can induce peroxidase activities.

The results obtained from the anthocyanin content measurements were in accordance with the color measurements (**Table 4**), showing significant differences in aril color between fruits from the two locations; those originating from Newe Ya'ar were more colorful than those from southern Arava in both harvest seasons (see also **Figure 2**). The *L** value was higher in fruit from southern Arava than from Newe Ya'ar. Because $L^* = 0$ denotes black and $L^* = 100$ indicates diffuse white, the results indicate that aril juices from fruits of southern Arava have a lighter color than those from Newe Ya'ar. The *a** and *b** values were higher in aril juices from fruits of Newe Ya'ar, indicating that these fruits have more of the magenta-red and yellow color components, respectively (**Table 4**). The *C* value (chroma), which represents the

"purity" of a color, with lower chroma being less pure (*44*), was higher in aril juices from Newe Ya'ar fruits.

To gain more knowledge of the effects of year, location and accession on aril color, a PCA was performed. In this analysis, the different parameters measured in aril juice colors were grouped into three components according to their contribution to the variance. The results (Supporting Information Figure A) have shown that in general in both years, fruits grown in Newe Ya'ar were located at the center of the figure, whereas fruits obtained from southern Arava can be found more at the periphery of this figure. This indicates that fruits grown in Newe Ya'ar can achieve their full colors and, thus, are more similar to each other. However, fruits grown in southern Arava exhibit a relatively great variation between the three different components, which leads to major differences among them. The growth conditions in this habitat expose more the genetic differences between accessions in terms of aril color.

Taken together, the results demonstrate that the arils obtained from Newe Ya'ar had a more intense color; this could be due to the higher temperatures in southern Arava, which negatively affects anthocyanin content and consequently color. Indeed, studies conducted in southern Arava on three of the pomegranate accessions that are also examined in the current investigation have shown that fruits ripening during July and August when the temperature was extremely high (about 40 °C, **Figure 1**) had a less intense aril color than fruits from the same trees that ripened in October when the temperature decreased to about 30 °C (*14*). As mentioned above, the irrigation regimen and the water and soil qualities in the southern Arava did not significantly change throughout the harvest season, thus allowing the separation of the effects of these environmental factors and those of the climatic conditions. The results imply that temperature is the primary factor affecting total anthocyanin content and aril color. The effect of temperature on aril color intensity was also observed when shade nets were employed in the southern Arava plot to reduce air temperature during ripening in July and August, resulting in a significantly darker red color of the arils (*14*).

Acidity and Sugar Levels Were Higher in Aril Juices Obtained from Newe Ya'ar than in Those from Southern Arava. To further study how environmental conditions affect aril quality, we measured the parameters commonly used to evaluate fruit taste, that is, titratable acidity (TA) and total soluble solids (TSS). The ratio between TSS and TA is commonly used to define the "taste" of a fruit, and the values for the 'Wonderful' accession varied from 11 to 16 (*45*). The TSS (**Figure 5**; Supporting Information Table C) was found to be higher in fruits obtained from Newe Ya'ar compared to those from southern Arava in both seasons examined. These were in accordance with the levels of glucose and fructose, two sugars that were detected by HPLC in the juices, which are the main TSS components. Next, the fructose-to-glucose ratio was calculated (**Figure 5**). Determination of this ratio is important because a high ratio may cause diarrhea or abdominal pain due to excess fructose fermentation 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, whereas a high level of glucose did not show this effect (*46*). 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 have equal amounts of glucose and fructose, as is recommended (**Figure 5**).

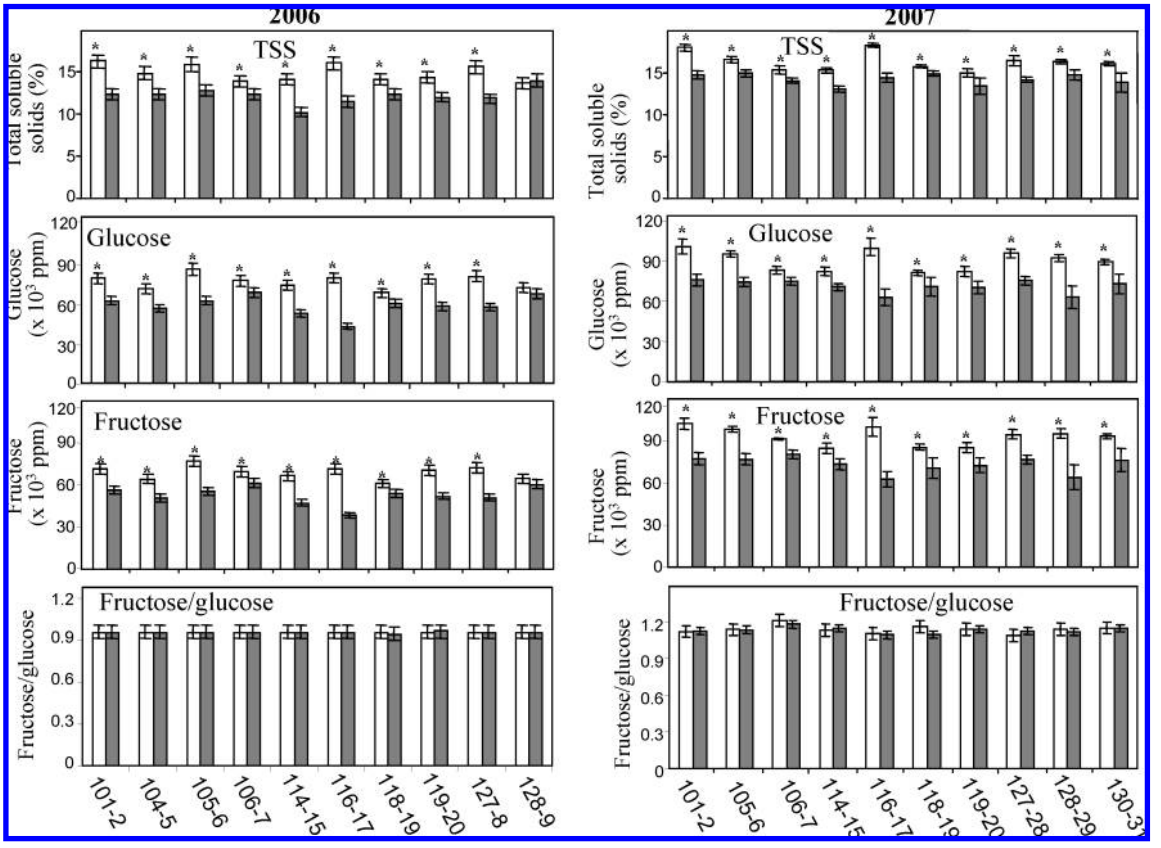

Figure 5. TSS, glucose, and fructose contents, as well as the ratio between fructose/glucose, in aril juices prepared from pomegranate accessions grown in Newe Ya'ar (white bars) and southern Arava (gray bars) in the 2006 and 2007 seasons. The data represent the mean ± SD of 10 replicates from each accession. Independent sample Student's *t* test was used to determine statistically significant differences in each accession grown in the two habitats ($p < 0.05$), identified by an asterisk.

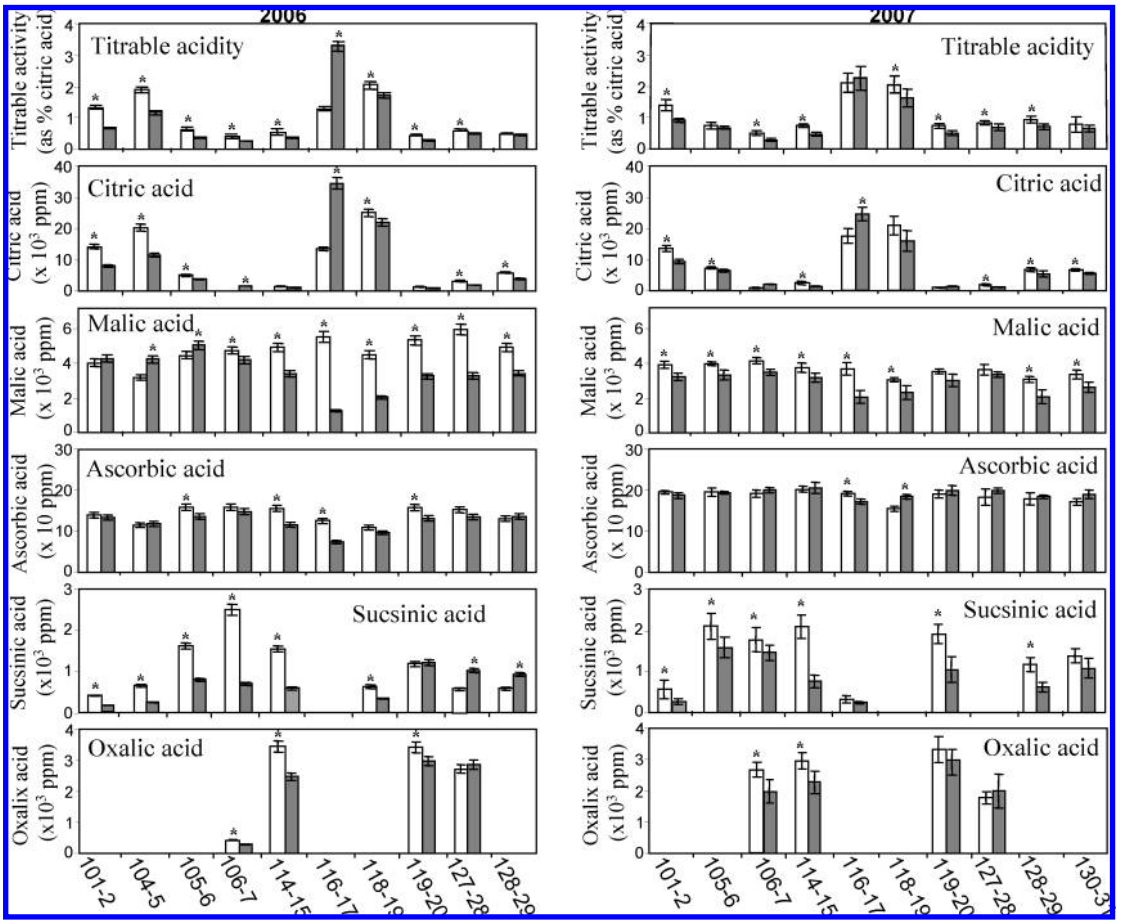

Figure 6. Titratable acidity and organic acid composition in aril juices prepared from pomegranate accessions grown in Newe Ya'ar (white bars) and southern Arava (gray bars) in the 2006 and 2007 seasons. The data represent the mean ± SD of 10 replicates from each accession. Independent sample Student's *t* test was used to determine statistically significant differences in each accession grown in the two habitats ($p < 0.05$), identified by an asterisk.

A comparable effect of climate on TSS levels of pomegranate fruits was also reported for 'Wonderful' accession fruits grown in two distinct locations in Israel; one was grown in a hotter climate than the other (*45*). The relatively cooler temperatures in Newe Ya'ar most likely promoted an increase in glucose and fructose, as previously shown for peanuts (*47*) and potatoes (*48*), whereas higher temperatures exceeding 40 °C (as prevailed in southern Arava) can decrease sugar content, as observed for grape berries (*49*).

Acidity also plays 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 sugars. As mentioned above, overall consumer appreciation is related more to TSS/TA ratio than to soluble sugars content alone (*50*). TA level was determined in fruits obtained from both habitats and found to be higher in accessions grown in Newe Ya'ar compared to those grown in southern Arava in both seasons (with the exception of P.G.116-17, **Figure 6**; Supporting Information Table D). Similar results were also obtained for 'Wonderful' fruits grown in two distinct locations in Israel, where fruits grown in a hotter climate had a lower TA than fruits grown in a temperate climate (*45*). This is also in accordance with results obtained for tomatoes, in which TA levels decreased to 25% when the temperature increased from 21 to 26 °C (*51*).

To gain more knowledge about the effect of habitat on the level and composition of organic acids that contributed to the TA of these juices, we next analyzed organic acids content using HPLC. It was previously suggested that although several organic acids were found in pomegranate aril juices, the major acid accounting for TA is citric acid (*52*). The results obtained by HPLC analysis show that this is also the case for some of the accessions examined (**Figure 6**), for which citric acid level significantly correlated to TA levels ($r = 0.72$, $p < 0.01$). In general, the citric acid level was higher in fruits obtained from Newe Ya'ar compared to those from southern Arava. These results were consistent with those previously obtained for peach fruit, suggesting that an increased temperature in the final weeks prior to harvesting decreased citrate production (*50*). The level of malic acid, the second most frequent organic acid in aril juice, was also found to be lower in the fruit of all of the accessions examined from southern Arava compared to those from Newe Ya'ar. A reduction in malic acid levels due to high temperature was reported for peach fruit: vacuolar malic acid decreased by about 50% when the temperature increased from 15 to 25 °C (*53*). Similarly, decreased malic acid content was reported when grape berry temperature was increased (*49*).

The levels of succinic and oxalic acids, which were not detected in all of the accessions examined, were also in general lower in fruits obtained from southern Arava. The ascorbic acid level did not differ significantly between the two habitats. This was inconsistent with data reported for ascorbic acid in tomatoes, showing that increasing fruit temperature to 32 °C reduced total ascorbate accumulations (*51*).

Taken together, the results show that environmental conditions affect some of the organic acids found in pomegranate accessions, but not others. These results are in accordance with other published data showing that location and climate significantly affect the levels of organic acids in two native black mulberry fruits (*54*) and in strawberry (*55*), as well as in quince fruits grown in seven different geographic regions in Portugal, where the total organic acids and the level of individual organic

acids were shown to differ significantly (*56*). The reason for these changes is not yet clear.

Antioxidant Activity and Total Phenolic Levels of Pomegranate Peels Were Higher in Fruits Obtained from Southern Arava than from Newe Ya'ar. It was previously found that pomegranate peels contain a high concentration of antioxidant and phenolics compounds (*4*, *5*, *13*, *15*). The high correlation between antioxidant activity and the level of hydrolazable tannins, such as punicalagin and punicalin, which are found mostly in peels, suggests that the latter compounds contribute significantly to the antioxidant activity of peels (*13*, *20*). Thus, we next examined whether antioxidant activity and total phenolics, punicalagin, and punicalin contents in peels differ in fruits obtained from the two habitats. To this end, we examined the juices obtained in 2006 using a juice extractor, which include the aril juice and the extract of inner parts of the peels, as well as peel homogenates from the 2007 season. The results (**Figure 7**; Supporting Information Table E) show that antioxidant activity and total phenolic content were significantly higher in fruits obtained from southern Arava in both seasons, as well as the levels of punicalagin, the major compound of hydrolyzable tannins found in the peels, and punicalin (**Figure 7**). Consistent with our previous results (*13*), the levels of punicalagin and punicalin in both locations correlated significantly to the levels of antioxidant and total phenols (Supporting Information Table F).

The reasons for the higher phenolics compounds in the peels of pomegranates grown in southern Arava are not yet clear but could be attributed to the higher temperatures and radiation found in southern Arava compared to Newe Ya'ar (**Figure 1**). It was previously reported that the major phenolic compounds (rutin and caffeic acid) in tomatoes significantly increased when fruit temperature increased from 27 to 32 °C to protect the fruit from oxidative stress induced by a temperature increase (*51*). In field experiments, it is not easy to separate temperature from photochemical effects, and net solar radiation absorbed by fruits is mainly converted into heat (*49*). The soluble phenol content of tomato plants grown under high light intensity is approximately double the content of low-light plants (*51*, *57*). In addition, the high flavonol content measured in the skin of sun-exposed grape berries (*49*) was attributed by the authors to a photochemical rather than a temperature effect. We assume that the higher phenolic compounds content in the peels of pomegranates grown in southern Arava is mainly due to the high temperature and radiation found in this habitat during fruit development and ripening (**Figure 1**). The higher phenolic compounds content might protect the fruits against higher oxidative stress occurring under the prevailing climatic conditions.

The levels of total anthocyanins, a distinct family within the phenolic compounds, were measured during both seasons. Anthocyanin content in the fruit peels obtained from Newe Ya'ar

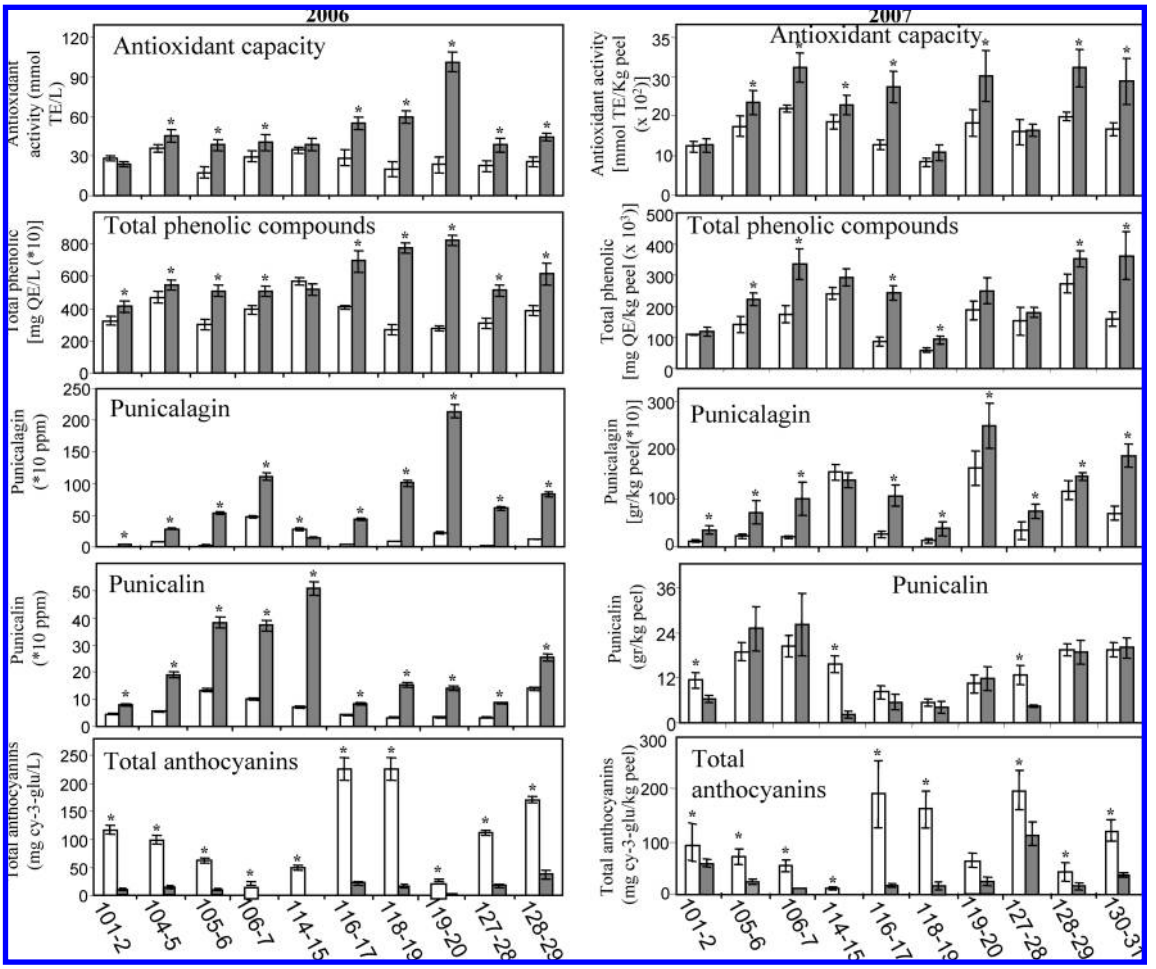

Figure 7. Antioxidant activity (in mmol of Trolox equivalents (TE)/kg of peel), total soluble phenolic compounds content (in mg of quercetin equivalents (QE)/kg of peel), total anthocyanin level (in mg of cyanidin 3-glucoside equivalents/kg of peel), and contents of punicalagin and punicalin in juice prepared by a juice extractor (in 2006), containing aril juice and the inner part of the peel, and in peel homogenates (in 2007) of pomegranate fruit from accessions grown in Newe Ya'ar (white bars) and southern Arava (gray bars). The data represent the mean ± SD of 10 replicates from each accession. Independent sample Student's *t* test was used to determine statistically significant differences in each accession grown in the two habitats ($p < 0.05$), identified by an asterisk.

was, in general, higher than that obtained from southern Arava. The total anthocyanin content in juices obtained using a juice extractor (2006 season) includes anthocyanins from the aril juice. However, total anthocyanins measured in the peel homogenates (2007 season) clearly demonstrated that the levels were higher in the peels of fruits obtained from Newe Ya'ar. These results are similar to those obtained for aril juice (**Figure 4**), indicating that higher temperatures in southern Arava reduced the levels of total anthocyanins in both the peels and arils of these fruits. Light intensity may also have affected the levels of anthocyanins in the pomegranate peels. It was previously reported that exposure to sunlight affects anthocyanin content in the peels of pomegranate fruit: fruits oriented directly to the sunlight accumulated more anthocyanins in their peels compared to those located on the inner branches, where exposure to direct sunlight was prevented (*31*). The reddish pigmentation of the external fruits could be influenced by the direct incidence of sunlight because UV light is one of the factors affecting the biosynthesis of anthocyanins (*31*). From these results, we can assume that the lower anthocyanin content in the peels obtained from Arava is not due to light intensity, which should increase anthocyanin biosynthesis, but to the higher temperature leading to a decrease in anthocyanins content.

The results obtained from the anthocyanin measurements were in accordance with those obtained from color measurements (**Table 4**), showing significant differences in peel color between fruits from both locations; those originating from Newe Ya'ar were more colorful than those from southern Arava in both seasons (see also **Figure 2**). When fruits from the two environmental conditions were compared, L^* and C values were found to be higher in some accessions grown in southern Arava and lower in others (**Table 5**). However, b^* values were higher in general in southern Arava, indicating that these fruits have more of the yellow color component. The a^* value was higher in Newe Ya'ar, indicating that these fruits have more of the magenta-red color component in their peels. Using the $H°$ parameter also revealed that, in general, fruits obtained from Newe Ya'ar have a more red to magenta color than those obtained from southern Arava.

To gain more knowledge about the variance between the parameters of peel color, a PCA was performed. The results (lower panel, Supporting Information Figure A) showed that the differences between different accessions grown in both locations were less than those found for the aril analyses (compare upper and lower panels in Supporting Information Figure A). In general, fruits obtained from Newe Ya'ar were found along the periphery, whereas those from southern Arava were located in the center (in contradiction to the situation found in the aril color analysis, Supporting Information Figure A, upper panel). This suggests that growth conditions in southern Arava lead to similarity in peel color of the fruits, while growth conditions in Newe Ya'ar lead to greater variations among the accessions, whereby every accession changed differently to the various color parameters analyzed and thus to the components.

Taken together, in both tissues, peels and arils, antioxidant activity correlated significantly with the total phenolic compounds. Because fruit temperature in the arils and peels should be correlated, the results of this study suggest that temperature affects the peels and arils differently. In southern Arava, the high temperature leads most probably to a decrease in anthocyanins levels, which are the main contributors to antioxidant activity of arils juice, whereas in the peels it stimulated together with radiation the synthesis of hydrolyzable tannins, which are the main contributors to antioxidant activity of the peels (Supporting Information Table F). Because antioxidant activity was reduced in aril juice of southern Arava compared to Newe Ya'ar, but not to the same extent as the reduction in anthocyanins, we further suggest that additional phenol compounds (yet unknown) in southern Arava also contributed to the antioxidant activity of the aril juices.

PCA Suggests That Environmental Conditions Affect Metabolite Changes in Several Accessions and Less in Others. To further understand how environmental conditions affect metabolic

Table 5. Color Determination in the Pomegranate Peel Surface of Different Accessions Grown in Newe Ya'ar and the Southern Arava Valley

	L^*		a^*		b^*		C		H	
accession	Newe Ya'ar	southern Arava	Newe Ya'ar	southern Arava	Newe Ya'ar	southern Arava	Newe Ya'ar	southern Arava	Newe Ya'ar	southern Arava
					2006					
P.G.101-2	57.7 ± 3.2	45.1 ± 3.6	30.4 ± 8.2	34.1 ± 5.8	30.9 ± 4.3	34.1 ± 5.8	45.1 ± 2.7	44.5 ± 3.3	48.5 ± 3.4	38.9 ± 6.6
P.G.104-5	46.6 ± 4.2	51.3 ± 4.2	47.5 ± 4.7	39.2 ± 6.8	22.9 ± 3.3	39.2 ± 6.8	53 ± 3.6	47.2 ± 2.9	25.8 ± 5.3	33.65 ± 8.6
P.G.105-6	40.1 ± 2.9	43.6 ± 2.3	48.5 ± 3.7	41.7 ± 5.4	18.8 ± 2.8	41.7 ± 5.4	52.1 ± 2.4	46.6 ± 3.3	21.1 ± 2.2	26.4 ± 3.5
P.G.106-7	69.1 ± 4.6	66.5 ± 4.1	0.9 ± 4.7	12.3 ± 6.5	41.5 ± 2.8	12.3 ± 6.5	41.7 ± 1.2	44.2 ± 5.1	91.2 ± 5.9	73.9 ± 12.3
P.G.114-15	65.6 ± 4.5	46.3 ± 3.7	25.1 ± 8.1	33.7 ± 8.4	29.7 ± 6.2	33.7 ± 8.4	40.7 ± 1.7	45.4 ± 4.2	51.3 ± 3.4	41.2 ± 5.6
P.G.116-17	38.3 ± 2.4	45.7 ± 2.9	45.4 ± 4.6	35.7 ± 8.5	18.6 ± 2.9	35.7 ± 8.5	49.1 ± 3.2	48.8 ± 2.6	22.1 ± 2.1	30.1 ± 6.6
P.G.118-19	41.1 ± 6.1	48.2 ± 3.1	46.9 ± 5.1	34.2 ± 10.4	18.6 ± 2.7	34.2 ± 10.4	50.5 ± 3.7	50.7 ± 4.2	21.6 ± 1.8	29.2 ± 5.7
P.G.119-20	61.7 ± 7.2	46.6 ± 5.7	32.4 ± 9.7	32.1 ± 6.3	25.9 ± 5.1	32.1 ± 6.3	43.3 ± 0.9	40.9 ± 1.6	41.3 ± 3.4	37.5 ± 3.2
P.G.127-28	26.6 ± 2.2	27.6 ± 2.1	15.9 ± 6.9	11.7 ± 6.7	2.6 ± 1.9	11.7 ± 6.7	16.2 ± 1.2	12.2 ± 2.3	8.3 ± 1.4	17.2 ± 3.6
P.G.128-29	48.3 ± 3.4	48.8 ± 1.5	45.8 ± 6.6	39.6 ± 7.5	25.9 ± 3.4	39.6 ± 7.5	52.9 ± 4.2	46.1 ± 6.4	29.9 ± 3.2	30.5 ± 3.9
					2007					
P.G.101-2	50.4 ± 12.3	53.4 ± 9.5	39.6 ± 13.2	30.8 ± 9.1	29.1 ± 2.3	31.8 ± 7.1	48.8 ± 9.5	45.6 ± 2.9	38 ± 11.7	46.1 ± 14.2
P.G.105-6	50.9 ± 6.2	52.3 ± 5.2	46 ± 6.4	43.3 ± 4.9	27.2 ± 2.9	30.3 ± 3.5	53.6 ± 5.8	56.3 ± 6.7	30.9 ± 4.4	36.3 ± 3.8
P.G.106-7	68.9 ± 9.6	59.1 ± 4.1	13.3 ± 11.1	7.2 ± 3.8	43.1 ± 4.1	37.9 ± 1.5	46.1 ± 4.6	38.8 ± 1.6	73.6 ± 13.7	79.3 ± 5.6
P.G.114-15	68.3 ± 6.7	52.4 ± 7.3	24.7 ± 11.4	36.1 ± 9.4	29.5 ± 4.2	25.7 ± 7.7	39.7 ± 5.8	45.5 ± 4.4	51.4 ± 15.5	36 ± 14.4
P.G.116-17	37.4 ± 3.3	49.1 ± 1.8	47.3 ± 4.5	33.9 ± 8.4	21.1 ± 4.5	23.6 ± 1.1	51.8 ± 5.8	41.6 ± 6.7	23.7 ± 3.1	35.9 ± 7.1
P.G.118-19	40.7 ± 7.9	65.2 ± 6.8	46.9 ± 3.2	25.2 ± 8.1	22.5 ± 3.3	28.7 ± 2.7	52.1 ± 4.1	38.8 ± 4.8	25.5 ± 2.6	49.6 ± 10.5
P.G.119-20	68.2 ± 8.4	59.1 ± 4.9	22.1 ± 11.4	25.9 ± 5.1	33.8 ± 3.4	27.2 ± 3.9	41.4 ± 6.6	38.0 ± 1.5	58.4 ± 14.3	46.5 ± 9.4
P.G.127-28	24.4 ± 1.1	25.4 ± 1.4	19.1 ± 2.6	17.1 ± 1.9	3.5 ± 0.6	3.3 ± 0.9	19.4 ± 2.7	17.3 ± 2.0	10.3 ± 1.0	10.7 ± 2.1
P.G.128-29	51.6 ± 11.5	54.9 ± 5.47	42.3 ± 11.2	30.6 ± 5.1	30.2 ± 3.1	28.1 ± 4.3	52.5 ± 8.4	41.9 ± 2.5	36.7 ± 9.6	42.6 ± 8.5
P.G.130-31	53.4 ± 5.2	49.5 ± 7.1	44.3 ± 5.9	27.5 ± 4.5	28.1 ± 2.9	30.3 ± 0.9	52.5 ± 5.4	41.0 ± 3.3	32.6 ± 4.3	48.0 ± 4.4

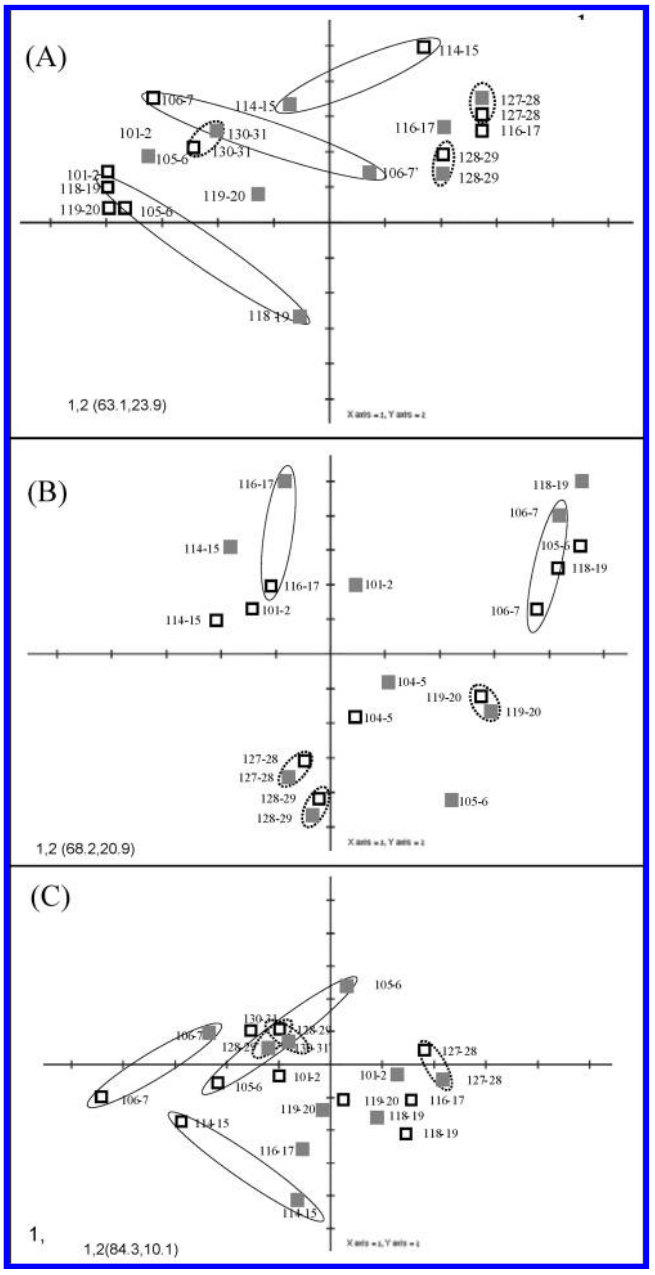

Figure 8. PCA of different parameters and metabolites measured in pomegranate fruit from accessions grown in Newe Ya'ar (white square) and southern Arava (gray squares) in aril juice for data collected (**A**) in 2006 and (**B**) in 2007; and in peel homogenates (**C**) for data collected in 2007. The variance explained by each component is shown in parentheses. The black lines show accessions that have relatively large distances between the two habitats; the broken lines shown accessions that have relatively short distances.

changes in the 11 accessions, we analyzed the data sets by performing a PCA. In this analysis, the different parameters measured in aril juices were grouped into three components according to their contribution to the variance. This analysis was performed on aril juices from both seasons (**Figure 8A**,**B**) and on peel homogenates (in 2007, **Figure 8C**) for each of the 11 accessions. The results obtained for the aril juices showed that in some accessions (e.g., P.G.114-15, P.G.106-7, and P.G.118-19, marked with black ellipses in **Figure 8A**,**B**) the distance between fruits grown in Newe Ya'ar and southern Arava is relatively large, proving that the effects of environmental conditions on fruit quality were significantly higher than in the other accessions, such as P.G.130-31, P.G.128-29, P.G.127-28 (marked with dashed line ellipses in **Figure 8A**,**B**), for which distances are relatively short between the two growth habitats. This analysis was used to further study the distances between the different accessions according to parameters measured in the peel homogenates (**Figure 8C**). In general, accessions having short distances between the two habitats in aril juices also had short distances in peel homogenates, and two accessions, P.G114-15 and P.G.106-7, having larger distances between the two habitats in aril juices also had large distances in peel homogenates (**Figure 8C**).

Additionally, the results of the PCA could provide more knowledge about the question of which factor is more dominant in determining pomegranate fruit quality, the genetic background or the environmental conditions? The results of the current study suggest that the genetic background of each accession plays a critical role in controlling its sensitivity to different environmental conditions. Some accessions demonstrate a relatively "strong" genetic background (such as P.G.130-31, P.G.128-29, and P.G. 127-28), in which the environmental conditions do not significantly affect fruit quality, whereas other accessions exhibit a "weak" genetic background and thus were significantly modified under different environmental conditions.

In general, the results of the current study showed that growing pomegranates in relatively hot and dry conditions led to a higher antioxidant activity in the peels of these fruits, but their color lessened in intensity compared to those grown in a Mediterranean climate. Moreover, lower antioxidant activity and anthocyanin levels, as well as lower levels of sugars and acidity, were found in the aril juices of these fruits. These parameters, color and taste, determine fruit attractiveness to the consumer, which is a vital factor in marketing considerations. Therefore, the results show that pomegranate fruits grown in Mediterranean conditions are of higher quality than those grown in desert climate conditions. On the other hand, the high content of health-promoting components in the peels of fruit grown in a desert climate are advantageous to the byproduct health ingredients industry. The results of this study also demonstrated that of the 11 accessions grown in southern Arava, accessions P.G.128-29 and P.G.130-31 harvested earlier compared to Newe Ya'ar and their fruit quality parameters did not change significantly. These two accessions can enlarge and extend the period during which fresh fruit can be supplied to the markets.

ABBREVIATIONS USED

LDL, low-density lipoprotein; RH, relative humidity; TSS, soluble solids; TA, titratable acidity; TEAC, Trolox equivalent antioxidant capacity; CIE, Commission International del'Eclairage; MRM, multiple-reaction monitoring; PSE, pressurized solvent extraction; PCA, principal component analysis.

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Supporting Information Available: Supplementary Tables A−F and Supplementary Figure A. This material is available free of charge via the Internet at http://pubs.acs.org.

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