Seasonal and cultivar variations in antioxidant and sensory quality of pomegranate (*Punica granatum* L.) fruit grown in the southern Arava Valley.

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ABSTRACT

Fruit of three diverse pomegranate (*Punica granatum* L.) cultivars grown in the southern Arava Valley were analyzed for soluble phenolics content, antioxidant activity, soluble solid concentration, acidity and internal red color intensity. Analysis was carried out on several dates along the harvest season, corresponding to different climatic conditions during fruit ripening. Values obtained varied with cultivar and ripening date. Comparison between late- and early-ripening fruit revealed that arils of fruit ripening later in the season contained more soluble phenolics (1.21-1.71 compare to 0.22-0.88 pyrogallol equivalents, gl^{-1}) and exhibited a higher antioxidant activity, as measured by the ferric reducing ability (FRAP) assay (1.22-2.37 compared to 0.86-1.95 vitamin C equivalents, gl^{-1}). Arils' red color intensity inversely related (R^2=0.89-0.94) to the sum of heat units accumulated during fruit ripening. The results imply that in the southern Arava Valley pomegranate fruit antioxidant and sensory quality traits can be enhanced by choice of cultivar and controlled-climate cultivation management.

INTRODUCTION

The pomegranate (*Punica granatum* L.) fruit is highly valued for its health promoting effect in reducing the risk of cardiovascular and other chronic disorders. The latter is supported by the results of an increasing number of clinical studies in both humans and animals (Lee and Watson, 1998; Aviram et al., 2000; Aviram and Dornfeld, 2001; Kaplan et al, 2001; Aviram et al., 2004; de Nigris et al, 2005) and *in vitro* experiments in tumor and macrophage cell cultures (Kim-NamDeuk et al., 2002; de Nigris et al, 2005; Fuhrman et al, 2005; Seeram et al., 2005). The beneficial health qualities were attributed to the exceptionally high antioxidative capacity of the fruit juice (Gil et al., 2000; Akay et al., 2001), seemingly effected by the remarkably high content and unique composition of soluble phenolic compounds (Gil et al., 2000; Poyrazoglu et al, 2002; Seeram et al, 2005). Phenolics' concentration and composition in the pomegranate fruit are cultivar dependent; the most abundant components are anthocyanins, catechins, ellagic tannins, gallic and ellagic acids (El-Nemr, et al, 1990; de Pascual-Teresa et al, 2000; Gil et al., 2000; Poyrazoglu et al, 2002).

The *in vivo* and *in vitro* studies described in the scientific literature were conducted with pomegranate juice prepared from fruit of the more popular cultivars, typified by an intense internal red color. Thus, a special significance was proposed for the anthocyanins (Noda et al., 2002), the molecular red color origin of the fruit juice (Gil et al., 1995; Hernandez et al., 1999). It appears, however, that anthocyanin bioavailability is lower than that of other soluble polyphenolics, such as phenolic acids, isoflavones and catechins (Scalbert and Williamson, 2000; Perez-Vincente et al., 2002; Manach et al, 2004, 2005). To date, no comparative studies were reported on the health promoting effects of pomegranate juice from cultivars of a less intense internal red color. In addition, fruit physical and chemical properties are highly dependent on the season of development and ripening (Ben-Arie et al., 1984; Badenes et al., 1998; Borochov-Neori and Shomer, 2001; Dumas et al., 2003; Toor et al., 2006; Raffo et al., 2006). The reported *in vivo* and *in vitro* studies employed pomegranate juice prepared from commercial harvests, where cultivar, level of ripening, agricultural practices and harvest date reflect grower and producer preferences that do not necessarily match the health promoting incentive. To accurately assess the health value in pomegranate fruit and juice consumption it is important to examine cultivar and seasonal variations in antioxidant content and activity.

The present study aimed to develop knowledge on cultivar and seasonal differences in pomegranate fruit antioxidant and sensory quality traits. To achieve this objective, three pomegranate cultivars differing in fruit internal color (from white to deep red), taste (from sour to sweet) and ripening season (from early summer to late autumn) were examined on

several ripening dates along the harvest season (mid July - end of October); arils' size and color as well as juice content, soluble phenolics concentration, antioxidative capacity, pH and total soluble solid (TSS) content were measured. The results were used to explore the role of climate factors in pomegranate fruit antioxidant and sensory related parameters.

MATERIALS AND METHODS

Fresh ripe pomegranate (*Punica granatum* L.) fruit were analyzed. The fruit were sampled from the pomegranate orchard at the Experimental Farm of the southern Arava R&D situated in the Israeli southern Arava Valley (lat. 29º53'N; long. 35º3'E), which is characterized by desert climate (Figure 1) and inferior water quality (electrical conductivity of ~3.5 dSm^{-1}).

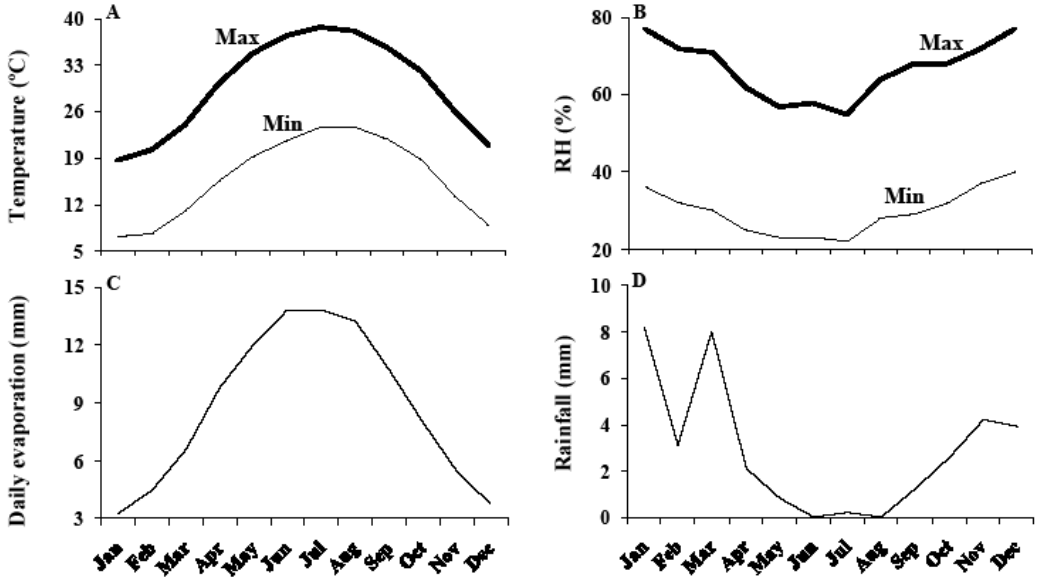

Figure 1: Climatic data for the Israeli southern Arava Valley (lat. 29º53'N; long. 35º3'E). The values are the long term averages obtained from the local meteorological station during the years 1995-2005. A. Maximal and minimal air temperature. B. Maximal and minimal relative humidity (RH). C. Daily evaporation. D. Rainfall.

The pomegranate plot accommodates trees of cultivars originally from the collection of Assaf *et al*, Newe Ya'ar Research Center, ARO [registered in the Israel Gene Bank for Agricultural Crops (IGB, web site: http://igb.agri.gov.il)]. On each sampling date newly ripen fruit were selected by external criteria according to customary practices, including external

color, size and shape measures. The fruit were cooled and studied within 24h. Each measurement was repeated on five fruit of a similar size from different trees and locations in the orchard, i.e. 5 replicates. Analytical assays were carried out in triplicates.

Intact arils were separated from the pith and carpellary membranes by hand and ripeness was further assessed by tasting; only non-astringent edible fruit were analyzed. The separated arils were counted and weighed. Surface color measurements were performed on uniform 3-cm thick layers of separated arils using a chromameter equipped with a glass light projection tube (CR-300 and CR-A33e, Minolta, Japan). The color was expressed in CIELAB coordinates, where positive **"a*"** and **"b*"** represent the red and yellow components, respectively, and **"L*"** conveys the luminosity dimension, ranging from 0 (pure black) to 100 (white, calibrated against the white reference plate provided with the chromameter).

Juice was prepared from isolated arils by a solid fruit juice extractor (Juice Extractor, Model Le Duo, Magimix, France), weighed and immediately analyzed. pH was measured using a specialized food electrode (pH 211 microprocessor pH meter and FC 200B food electrode, Hanna Instruments, Romania). Total soluble solid (TSS) concentration in % was evaluated with a hand refractometer (ATAGO, ATC-1E, Brix 0-32%, Japan). Pomegranate juice was extracted (1:3, v/v) with 80% methanol supplemented with 2mM NaF, centrifuged (10,000 rpm for 10 min at 4°C, Sorvall Instruments RC5C) and the supernatant diluted 10-fold with double distilled water (DDW). Concentration of total soluble phenolics was measured colorimetrically with Folin-Ciocalteau 2N phenol reagent (SIGMA Chemical Co, USA) according to Singleton and Rosssi (1965). Aliquots of 100 µL were added to 900 µL reaction solution consisting of 200 µL freshly prepared 10-fold diluted Folin-Ciocalteau reagent, 100 µL Na_2CO_3 and 600 µL DDW. Pyrogallol (SIGMA Chemical Co, USA) was used for the calibration curve (0-100 µg mL^{-1}). The absorbance at 765 nm was measured with a spectrophotometer (SHIMADZU Corporation, UV–1650PC, Kyoto, Japan) after 1-hour incubation, and the results were expressed in pyrogallol equivalents. Antioxidative capacity (AOC) was measured by the colorimetric test originally developed to assess the ferric reducing ability of plasma (FRAP) (Benzie and Straino, 1996); the assay was shown to be appropriate for AOC estimation in pomegranate juice (Gil et al., 2000). Clear methanolic extract was prepared as described earlier and diluted 10 to 20-fold with DDW. Fifty µL were added to 950 µL freshly prepared FRAP working solution [50 mL 300 mM acetate buffer + 5 mL 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) + 5 ml 20 mM ferric chloride] in a 37°C water bath. Absorbance at 593 nm was measured with a spectrophotometer (SHIMADZU Corporation, UV–1650PC, Kyoto, Japan) after 4 min. Vitamin C (Fluka, Switzerland) was

used for the calibration curve (0-100µg mL^{-1}), and the results were expressed in terms of vitamin C equivalents.

The experimental values for each sampling date are the average and standard deviation of measurements performed on five fruit from different trees and repeated on two or three harvest seasons.

RESULTS

Fruit analysis along the harvest season

Ripe pomegranate fruit of three cultivars (CVs) were studied on several dates along the harvest season in the years 2002, 2004 and 2005 (Figures 2 - 5). The cultivars, identified here by Newe Ya'ar code system (IGB, web site: http://igb.agri.gov.il), represent three distinct types of the crop: P.G. 128-29 – internally red, sweet, early ripening (early-CV); P.G. 119-20 – internally pink, sweet, early to mid-season ripening (mid-CV); P.G. 101-2 – internally red, sweet and sour, late ripening (late-CV). The early-CV started yielding ripe fruits on July 12, the first sampling date. Fruit of the mid- and late-CVs reached ripeness approximately one and two months later, respectively. Once ripening began, newly ripened fruit were available on the selected sampling dates along the study period. In the early-CV, fruit juice content (Figure 2) was considerably lower in early July and October compare to end of July to end of September. Also, with the progression in harvest season the aril's weight increased; concomitantly, arils' number per fruit decreased. Unlike the early-CV, in the mid- and late-CVs, fruit juice content as well as aril's weight and number (Figure 2) did not significantly change during the entire sampling period.

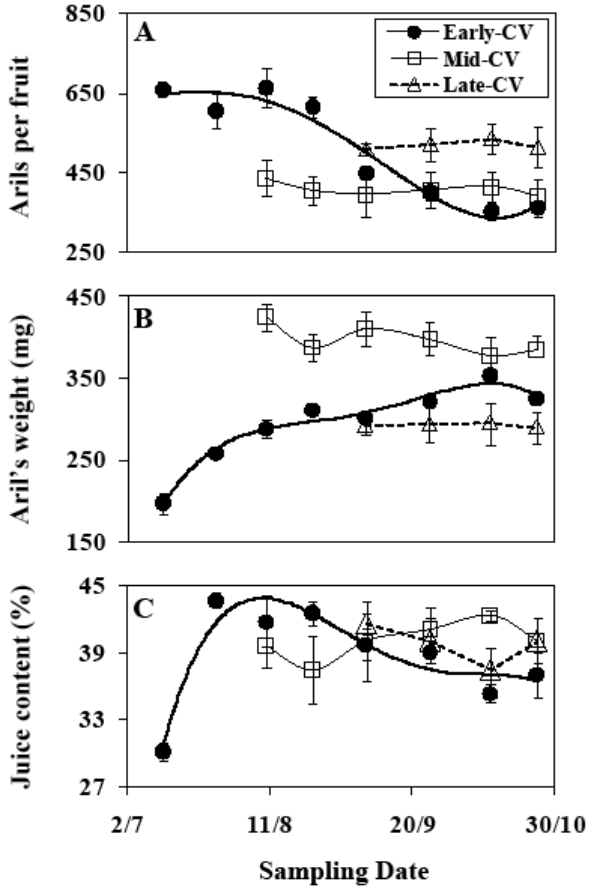

Figure 2: Physical parameters along the harvest season of the edible portion of ripe pomegranate fruit in three CVs: P.G. 128-29 (Early-CV, ●), P.G. 119-20 (Mid-CV, □), P.G. 101-2 (Late-CV, Δ). A. Number of arils per fruit; B. Weight of a single aril; C. Weight fraction of arils' juice.

Fruit arils' color varied along the season (Figure 3). Red color intensity (**"a*"**) of arils from the early-CV decreased from July to September and increased on later sampling dates; the luminosity (**"L*"**) declined concurrently. Arils of the mid-CV were initially white and gradually changed to pink during October as reflected by the small increase in **"a*"** and decrease in **"L*"**. In arils of the late-CV **"a*"** developed slowly in September and faster in October, with **"L*"** decreasing concomitantly.

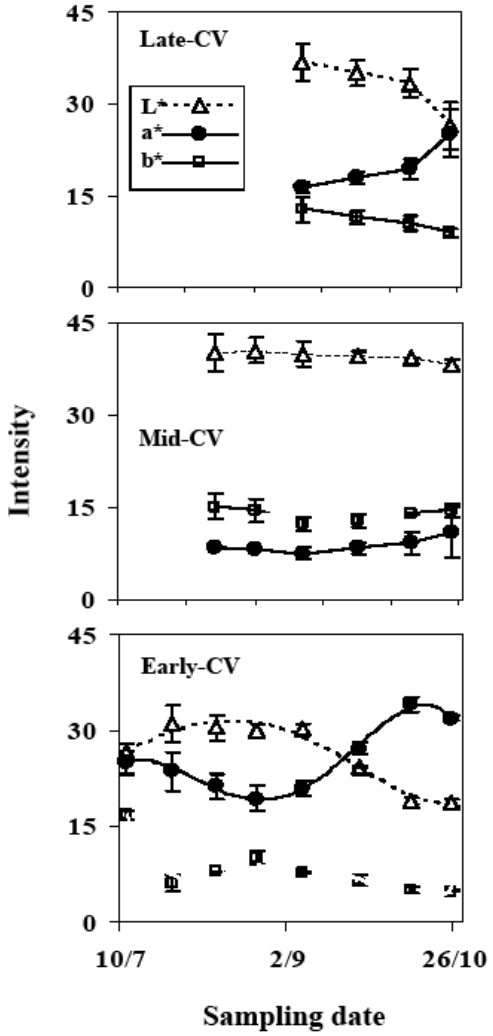

Figure 3: Arils' color measurements along the harvest season in ripe fruit of three pomegranate CVs. A. Late-CV (P.G. 101-2); B. Mid-CV (P.G. 119-20); C. Early-CV (P.G. 128-29). **"L*"**- luminosity, **"a*"**- red component, **"b*"**- yellow component.

The extent of internal red color development in relation to the local temperatures during fruit development and ripening was examined in the early- and late-CVs (Figure 4). In ripe fruit arils, **"a*"** inversely related to the sum of heat units (Wang, 1960) accumulated during six weeks prior to harvest. In calculating the accumulated heat, daily heat units were defined as the difference, in Celsius degrees, between the daily average temperature and 25ºC. A heat unit value of zero was assigned when the average daily temperature was less than 25ºC. Regression analysis of the data gave best fit to inverse linear (R^2=0.89) and reciprocal (R^2=0.94) correlations for the early- and late-CV, respectively.

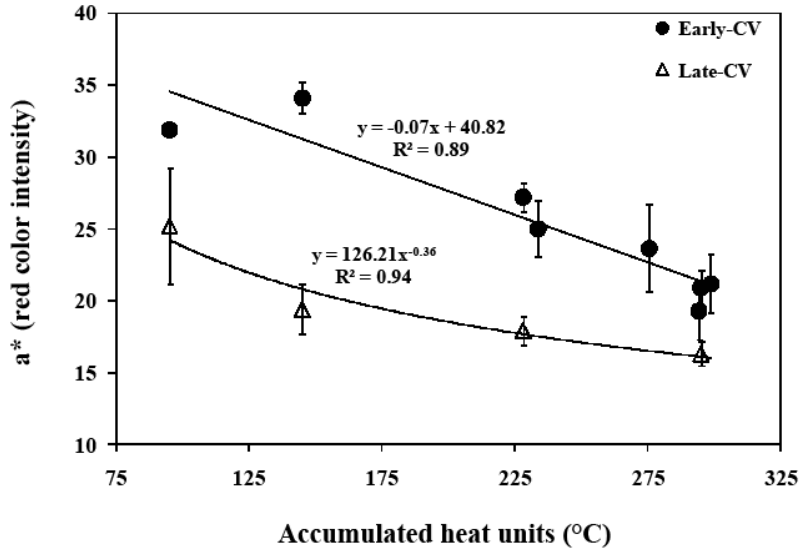

Figure 4: Correlation analysis between aril's red color intensity, "a*", and the sum of heat units accumulated during the last six weeks of fruit development and ripening. Lines are the best fit curves obtained by linear (Early-CV, P.G. 128-29) and reciprocal (Late-CV, P.G. 101-2) regression analyses.

Similar inverse correlations were obtained when shorter periods of heat accumulation were considered (Table 1).

Table 1: Correlation analysis between the aril's red color intensity, "**a***", and heat unit accumulation during fruit development and ripening. Correlation coefficients (R^2) derived from linear [early-CV (P.G. 128-29)] and reciprocal [late-CV (P.G. 101-2)] regression analyses are presented.

Period of heat unit accumulation	Correlation coefficient (R^2)	
(weeks prior to harvest)	P. G. 128-29	P. G. 101-2
2	0.84	0.78
4	0.89	0.91
6	0.89	0.94

The chemical parameters of fresh juice extracted from the separated arils changed along the sampling season (Figure 5). TSS (Figure 5A) increased with the advancement in ripening date, reaching approximately 15.5% in the three CVs. Juice pH increased from 3.8 to 4.2

between July and August in the early-CV; in all three CVs the pH (Figure 5B) was constant in fruit that ripened during August and September and decreased in fruit ripening in October. Highest and lowest pH values were measured in the early- and late-CVs, 3.8-4.2 and 3.2-3.4, respectively. The content of total soluble phenolics (Figure 5C) slightly decreased in the early-CV between mid July and mid August (from ~0.44 to ~0.39 pyrogallol equivalents, gl^{-1}) and was significantly higher in fruits of all three CVs that ripened on later dates, reaching values in the range of 1.2-1.7 pyrogallol equivalents, gl^{-1}, depending on the CV. The antioxidative capacity (Figure 5D) followed a similar trend with a decrease in the early-CV from mid July to mid August (from ~1.95 to ~ 1.54 vitamin C equivalents, gl^{-1}) and an increase later in the season for all three CVs, reaching values in the range of 1.2-2.4 vitamin C equivalents, gl^{-1}, depending on the CV. Both parameters (total phenolics and antioxidative capacity) were lower in the mid-CV compare to both the early- and late-CVs.

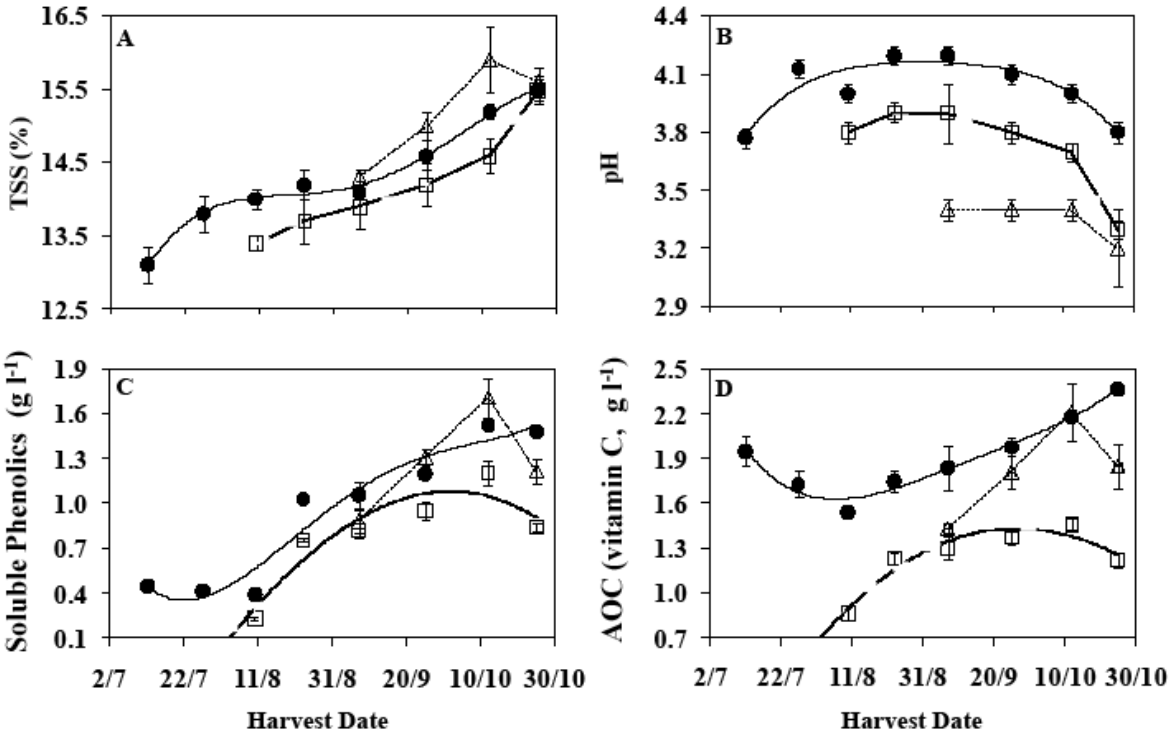

Figure 5: Chemical parameters along the harvest season of freshly extracted arils' juice from ripe fruit of three pomegranate CVs: Early-CV (P.G. 128-29, ●), Mid-CV (P.G. 119-20, □), Late-CV (P.G. 101-2, Δ). The total soluble phenolics content and antioxidative capacity (AOC) are in gl^{-1} pyrogallol and vitamin C equivalents, respectively.

DISCUSSION

The growing number of scientific reports on the health benefits of the pomegranate fruits (Lee and Watson, 1998; Aviram et al., 2000; Aviram and Dornfeld, 2001; Kaplan et al, 2001; Aviram et al., 2004; de Nigris et al, 2005) generated a significant increase in consumer interest and, consequently, agricultural production of the crop. To assure consumer satisfaction and producer profitability, it is of a particular importance to ensure both high content of health ingredients and fruit attractiveness for either fruit or juice consumption. In this context, the present study demonstrates that pomegranate antioxidant and sensory quality depend on cultivar and climatic conditions during fruit maturation and ripening.

The different ripening dates examined throughout the harvest season signify distinct regimes of climatic conditions during fruit development and ripening. In three pomegranate cultivars differing in sensory qualities and ripening season the aril's color as well as the pH, TSS, total soluble phenolics content and antioxidative capacity of the extracted juice varied with ripening date in a similar fashion and on a comparable time scale (when applicable) despite cultivar diversity. The results are in agreement with numerous reports on the major effect of abiotic conditions during development, maturation and ripening on fruit quality and chemistry (Crisosto et al., 1997; Ben-Arie et al., 1984; Badenes et al., 1998; Borochov-Neori and Shomer, 2001; Dumas et al., 2003; Toor et al., 2006; Raffo et al., 2006).

The higher red color intensity of the arils at the beginning (early July) and the end (October) of the sampling period, compared to late July through September, probably reflect the detrimental effect of high temperatures on anthocyanin accumulation (Oren-Shamir and Nissim-Levi, 1999); the extreme temperatures during July and August in the southern Arava Valley (Figure 1) may decrease anthocyanin content by slowing down synthesis (Shvarts et al., 1977) and accelerating degradation (Shaked-Sachary et al., 2002). Indeed, arils' red color intensity was found to be inversely related to the sum of heat units accumulated during fruit development and ripening (Figure 4, Table 1). Moreover, in preliminary experiments that employed shade nets to reduce air temperature in fruit vicinity during ripening, aril's red color intensity was enhanced in fruit of the early-CV that ripened during July and August (Tripler and Borochov-Neori, unpublished results). A comparable climate effect on the red color intensity of pomegranate fruit juice was demonstrated for the CV 'Wonderful' grown in two distinctly different climatic regions in Israel (Ben-Arie et al., 1984).

A general trend of increase in arils' TSS, soluble phenolics content and antioxidative capacity with the progression along the harvest season was established between August and the end of October. Taken together with the seasonal variations in arils' red color intensity,

pomegranate fruit from later harvest dates were of superior sensory and antioxidant value in the three CVs.

CONCLUSIONS

The pomegranate fruit is highly valued for its health promoting benefits. However, the carpometric characteristics of the fruit also play an important role in its consumption. While the health relating quality, i.e. the antioxidative capacity, is mainly dependent on the soluble phenolics content, fruit attractiveness is primarily related to color and taste parameters. Consumer satisfaction and producer profitability require that the fruit excels in both aspects. The findings of this study that the health and attractiveness factors in the pomegranate fruit are not interrelated and vary with cultivar and season of fruit development and ripening, would be important to the current efforts to upgrade pomegranate fruit quality by breeding and agricultural practices. Cultivation approaches that influence the season of fruit production and include climate management during fruit development and ripening will enable growers of the southern Arava Valley to improve fruit sensory qualities and antioxidant value of favorite pomegranate cultivars.

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