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Original Article

Seasonal and cultivar variations in antioxidant and sensory quality of pomegranate (*Punica granatum* L.) fruit

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ABSTRACT

Fruits of diverse pomegranate (*Punica granatum* L.) cultivars were analyzed for soluble phenolics content, antioxidant activity, soluble solid concentration, acidity and internal red color intensity. Analysis was carried out at various dates throughout the harvest season, corresponding to different climatic conditions during fruit ripening. Values obtained varied with cultivar and ripening date. In three cultivars of different sensory properties and harvest season, 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 compared to 0.22–0.88 pyrogallol equivalents, g L^{-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, g L^{-1}). The red color intensity of the arils inversely related (R^2 = 0.89–0.94) to the sum of heat units accumulated during fruit ripening. Multiple linear regression analysis on fruit characteristics in 11 diverse cultivars indicated that juice antioxidative capacity linearly correlated with soluble phenolics content (R^2 = 0.98), but not with the red color intensity of the arils (R^2 = 0.38). Also, no significant correlation was established between aril color and either juice pH or total soluble phenolics content. The results imply that pomegranate fruit antioxidant and sensory quality traits can be enhanced by the choice of cultivar and controlled-climate cultivation management.

1. Introduction

The pomegranate (*Punica granatum* L.) fruit is highly valued for its health-promoting effects in reducing the risk of cardiovascular and other chronic disorders. This claim 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, 2004; Aviram and Dornfeld, 2001; Kaplan et al., 2001; 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 have been attributed to the exceptionally high antioxidative capacity (AOC) of the fruit juice (Gil et al., 2000; Akay et al., 2001), seemingly the result of the remarkably high content and unique composition of soluble phenolic compounds (Gil et al., 2000; Poyrazoglu et al., 2002; Seeram et al., 2005). Phenolic 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 (CVs), 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, the physical and chemical properties of the fruit 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

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cultivar, level of ripening, agricultural practices and harvest date reflect grower and producer preferences that do not necessarily match health-promoting objectives. 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, a diverse collection of 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) was examined on several ripening dates throughout the harvest season (mid-July to end of October); aril dimension 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 test for correlations between fruit antioxidant and sensory-related parameters and explore the role of climate factors.

2. Materials and methods

Fresh ripe pomegranate (*P. granatum* L.) fruits 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 (latitude 29°53′N; longitude 35°3′E), which is characterized by desert climate (Fig. 1) and inferior water quality (electrical conductivity of ~3.5 dS m^{-1}). The pomegranate plot accommodates trees of 11 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)]. The manuscript summarizes studies conducted during 14 August–25 October 2002 and 12 July–25 October in 2004 and 2005, respectively. On each sampling date newly ripened fruit were selected by external criteria according to customary grower practices; the latter include external color, size and shape. The fruit were cooled and studied within 24 h. Each measurement was repeated on five fruits of a similar size from different trees and locations in the orchard, i.e. five 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); it was then 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). 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 2 mM 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 Rossi (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-h incubation, and the results were expressed in pyrogallol equivalents. 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 microliters 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 (0–100 μg mL^{-1}), and the results were expressed in terms of vitamin C equivalents.

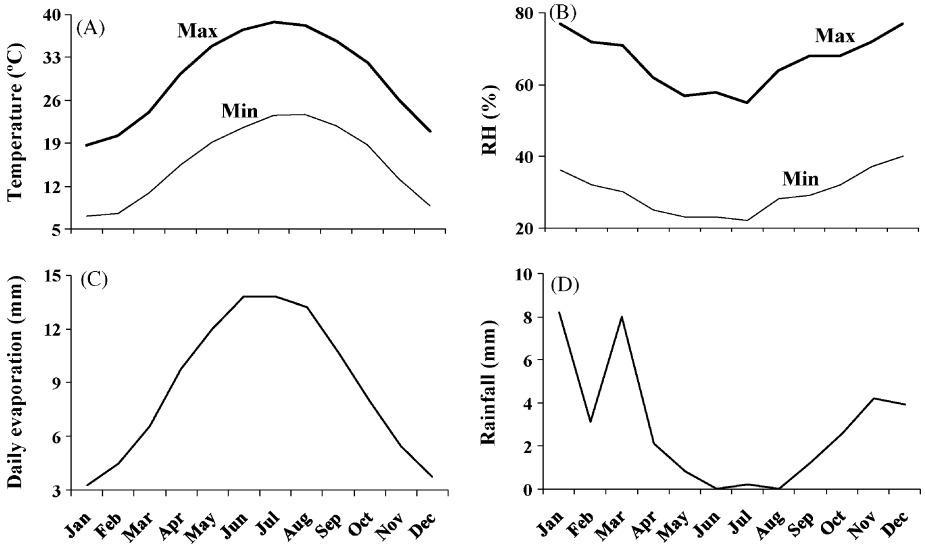

Fig. 1. Climatic data for the Israeli southern Arava Valley (latitude 29°53′N; longitude 35°3′E). The values are the long-term averages obtained from the local meteorological station during the years 1995–2005. (A) Maximal and minimal air temperature. (B) Maximal and minimal relative humidity (RH). (C) Daily evaporation. (D) Rainfall.