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Definition of minimum maturity indices for harvesting of early-season sweet pomegranate (*Punica granatum* L.) fruit

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SUMMARY

There are no defined maturity indices for harvesting early-season "sweet" pomegranate (*Punica granatum* L.) varieties, and this often results in the harvesting and marketing of inedible, immature fruit. We evaluated the changes in various maturity indices, and in sensory quality, during the ripening of two commercial, early-season "sweet" pomegranate varieties: 'Acco' and 'Shani–Yonay'. The results showed that, during the last 5 weeks of fruit ripening, only slight changes occurred in peel and aril colour, and in the total soluble solids contents and titratable acidity of the juice. In contrast, during ripening, we observed gradual increases in fruit and aril fresh weights (FWs), and a decrease in hydrolysable tannin contents. Furthermore, these changes were accompanied by a gradual increase in fruit flavour preference, which mainly reflected a parallel decrease in the undesirable mouth-feel sensation of astringency. Finally, we found a high correlation (r^2 = 0.99) between the observed increases in fruit flavour and aril FW, and therefore suggest that an average mean aril FW of > 0.23 g may serve as a simple and reliable index of maturity for timing the commercial harvesting of these two early-season "sweet" pomegranate varieties.

Over the last few years, and especially since their recently discovered benefits in the prevention of various chronic diseases, there has been a sustainable increase in the global production and marketing of fresh pomegranate (*Punica granatum* L.) fruit and juice (Facial and Ocalhau, 2011; Johanningsmeier and Harris, 2011; Rymon, 2011). Overall, more than 500 pomegranate varieties are known worldwide, including several dozen commercially cultivated varieties (Still, 2006; Holland *et al*., 2009). Pomegranate varieties are generally categorised into three major groups according to their average titratable acidity (TA) level: "sweet", "sweet-sour", or "sour", with average TA values of 0.32, 0.78, and 2.72% (w/v), respectively (Melgarejo *et al*., 2000; Dafny-Yalin *et al*., 2010). In Spain, for example, out of 40 varieties adapted to the Mediterranean climate, 35 were classified as sweet, three as sweet-sour, and only two varieties were considered sour (Melgarejo *et al*., 2000).

To ensure that fruits and vegetables are harvested at the correct maturity stage, with at least the minimum acceptable quality, optimum maturity indices have been developed and established for each commodity crop (Reid, 2002). The establishment of minimum maturity indices for harvesting is especially important for pomegranate, which is a non-climacteric fruit that does not continue to ripen after harvest (Ben-Arie *et al*., 1984; Kader *et al*., 1984). In the case of pomegranate, maturity indices have been established only for the late-season, California-grown, "sweet-sour" variety 'Wonderful', which is ready for harvest at a total soluble solids content (TSSC) of > 17% (w/v) and a TA of < 1.85% (w/v) (Kader, 2006; Crisosto *et al*., 2013). As yet, there are no defined maturity indices for the harvesting of early-season, sweet pomegranate varieties which are characterised by having much lower TA and TSSC values. It is therefore necessary to define specific maturity indices for various "sweet", "sweet-sour", and "sour" pomegranate varieties. The establishment of minimum maturity indices is especially important for early-season varieties, because growers often tend to harvest these fruit too early, in order to achieve a higher price and a marketing advantage.

In previous studies, it was found that pomegranate fruit underwent various biochemical and physiological changes during ripening, including the accumulation of anthocyanins and consequent changes in fruit colour, increases in TSSC, and decreases in TA values, and changes in total phenolics contents and anti-oxidant activity (Ben-Arie *et al*., 1984; Gil *et al*., 1995; 1996; Al-Maiman and Ahmad, 2002; Kulkarni *et al*., 2005; Shwartz *et al*., 2009; Zarei *et al*., 2009). However, most studies on the biochemical composition of pomegranate have addressed the earliest stages of development, shortly after fruit set, and were less focussed on the changes that occurred during the final stages of fruit ripening, just before commercial harvest.

The present study focussed on two commercial, early-season sweet pomegranate varieties: 'Acco' and 'Shani–Yonay' (Holland *et al*., 2007; 2009). We examined the changes that occurred in various maturity indices,

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and in flavour and fruit quality, every 7 d during the last 5 weeks of the ripening process, from the unripe to the fully-ripe stage. Our findings demonstrated that the main factor limiting the early harvest of these sweet pomegranate varieties was a low flavour preference, which resulted from the incomplete removal of the undesirable mouth-feel sensation of astringency. Furthermore, we observed a high correlation between the increase in fruit-flavour preference and the average fresh weight (FW) of the arils during ripening. Therefore, we suggest that aril FW may serve as an appropriate, simple and reliable maturity index to determine the timing of commercial harvesting of these early-season sweet varieties of pomegranate.

MATERIALS AND METHODS

Plant material

Pomegranate (*Punica granatum* L.) fruit of the early-season varieties 'Acco' and 'Shani–Yonay' were harvested in the Arava Valley in the southern part of Israel once a week from the beginning of July until mid-August during three growing seasons (2010 – 2012). To ensure that all fruit were of the same age and ripening stage, fruitlets were labelled with ribbon tape immediately after fruit set. Any fruit that set early or late were discarded. The arils from five different fruit were separated manually and squeezed between four layers of miracloth to extract the juice for chemical analysis.

Determinations of TSSC and TA

The TSSC values of the juice were determined using a Model PAL-1 digital refractometer (Atago, Tokyo, Japan). Titratable acidity (TA, as a percentage) was measured by titration to pH 8.3 with 0.1 M NaOH in a Model CH-9101 automatic titrator (Metrohm, Herisau, Switzerland). Each measurement was repeated five times using the juice collected from three fruit (i.e., a total of 15 fruit per measurement).

Determination of total phenolic compound concentrations

Phenolics were extracted by stirring 1.0 ml of each juice sample with 9 ml of 80% (v/v) methanol for 30 min at room temperature, followed by centrifugation at 10,000 $\times$ *g* for 10 min at 4ºC. Total phenolics contents were determined by the Folin-Ciocalteu method (Singleton *et al*., 1999). Briefly, each reaction mixture included 0.2 ml of the methanol extract of each juice sample, 0.2 ml of Folin-Ciocalteu reagent, and 7 ml of 7% (w/v) Na_2CO_3. The reaction mixtures were incubated for 90 min at room temperature, after which their absorbance values were measured at 750 nm using a spectrophotometer against a blank containing reagents without a juice sample. Total phenolics contents were expressed as gallic acid equivalents (in mg l^{-1}) by reference to a standard curve.

Determination of hydrolysable tannin concentrations

Hydrolysable tannins were extracted from pomegranate juice according to Hagerman and Butler (1978). Briefly, each 500 µl sample of juice was stirred with 1.0 ml of 1.0 mg ml^{-1} bovine serum albumin (BSA) dissolved in 200 mM acetic acid, 170 mM NaCl buffer, pH 4.9, for 15 min with slow agitation at room temperature, then centrifuged at 10,000 $\times$ *g* for 5 min. The pellet was washed twice with 1.0 ml of the same buffer, after which 875 µl of a buffer containing 5% (v/v) triethanolamine and 5% (w/v) sodium dodecyl sulphate, pH 9.4, plus 125 µl of ferric chloride reagent (10 mM $FeCl_3$ dissolved in 0.01 M HCl) were added, and the sample was allowed to incubate for a further 10 min at room temperature. Hydrolysable tannin concentrations were measured by reading the absorbance at 510 nm in a spectrophotometer, and were expressed in catechin equivalents (in mg l^{-1}) by reference to a standard curve.

In other experiments, hydrolysable tannin concentrations were measured either by dipping the cut surface of each fruit in 5% (w/v) $FeCl_3$ solution, or by pressing the fruit (or injecting 100 µl of juice) onto a dry filter paper that had previously been soaked in 5% (w/v) $FeCl_3$ solution, as described for persimmon fruit by Gazit and Levi (1963).

Sensory evaluations

The sensory quality of each sample of separated arils was evaluated by acceptance and descriptive tests (Lawless and Heymann, 1999). In all cases, a mixture of separated arils from five different fruit were placed in plastic cups that were identified by randomly assigned three-digit codes. Sensory acceptance was evaluated according to a nine-point hedonic scale ranging from "very strong dislike" to "very strong like". This acceptance test was conducted by 40 members of staff or students working in the Department of Postharvest Science at the ARO, Volcani Center.

Quantitative descriptive analyses were performed by a trained sensory panel consisting ten members: five males and five females, aged 25 – 62. Each panelist assessed the various attributes of each sample according to an unstructured 100-mm linear scale for each attribute, ranging from "very weak" to "very strong". The sensory data were recorded as distances (mm) from the origin. The sensory attributes evaluated by the trained panel were: taste (sweet, sour, or bitter), odour (red wine, or pomegranate fruity notes), and mouth-feel sensations (astringency, juiciness, or seed hardness). The panel was trained to evaluate astringency sensation by tasting a reference 0.2% (w/v) tannic acid solution, as described by Cadwallader *et al*. (2010).

Statistical analysis

One-way analysis of variance (ANOVA) and Tukey's honestly significant difference (HSD) pairwise comparison tests were applied using the JMP statistical software programme, Version 7 (SAS Institute Inc., Cary, NC, USA). Correlation curves (bivariate fits) and r^2 values were calculated using Microsoft Office Excel and JMP Version 7 statistical programmes.

RESULTS

Visual appearance, and fruit and aril fresh weights

Regarding fruit appearance and colour, it can be seen (Figure 1) that fruit from both 'Acco' and 'Shani-Yonay' had attained their typical pink-reddish internal and external colour by the first evaluation date (10 July), long before full maturity (14 August). Therefore, fruit colour did not change significantly during the last stages of ripening.

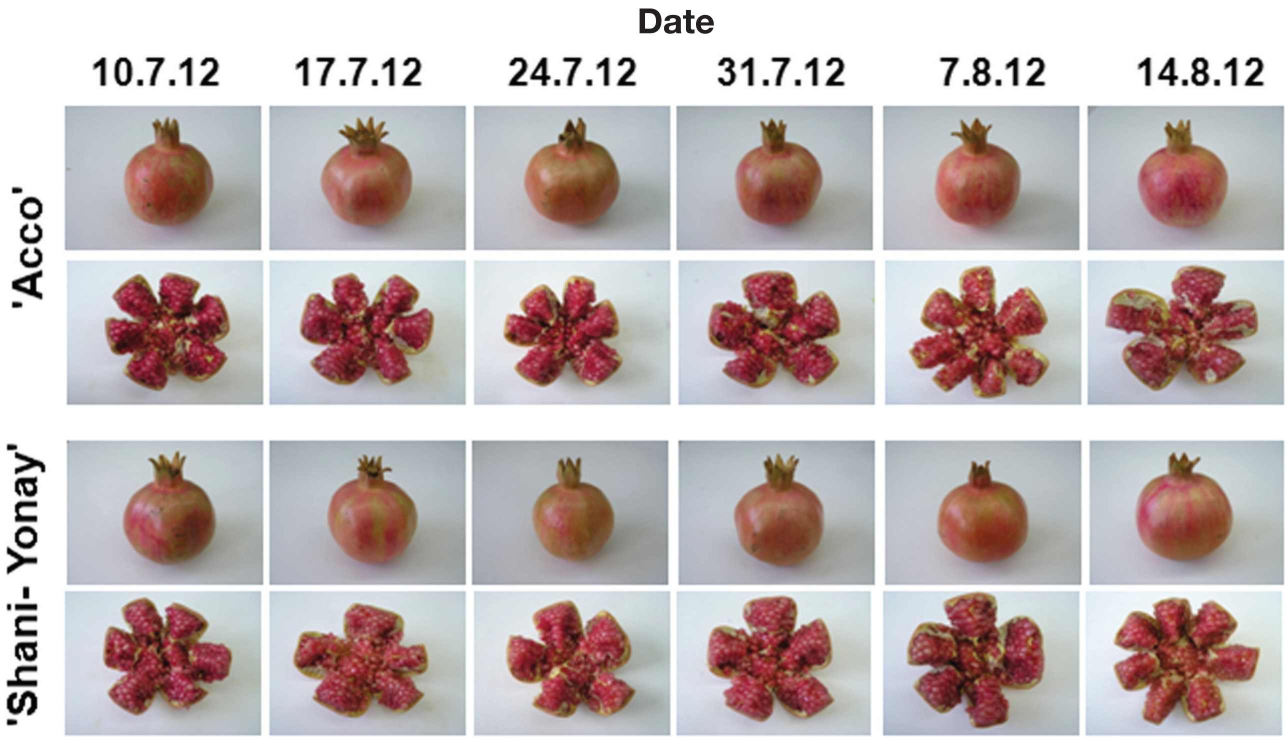

FIG. 1
Visual appearance of 'Acco' and 'Shani-Yonay' pomegranate fruit and arils during the last 5 weeks of the ripening process in 2012, showing little or no change in colour.

In contrast to fruit colour, we observed gradual and continuous increases in fruit and aril FWs over the last 5 weeks of ripening (Figure 2). The average FWs of 'Acco' and 'Shani-Yonay' fruit on the first evaluation date (10 July) were 195 g and 225 g, respectively. Subsequently, the FWs of both fruit increased to approx. 270 g at full-ripeness (14 August; Figure 2). Similarly, aril FWs of 'Acco' and 'Shani-Yonay' fruit increased from approx. 0.18 g on 10 July to 0.27 g at full-ripeness (14 August; Figure 2).

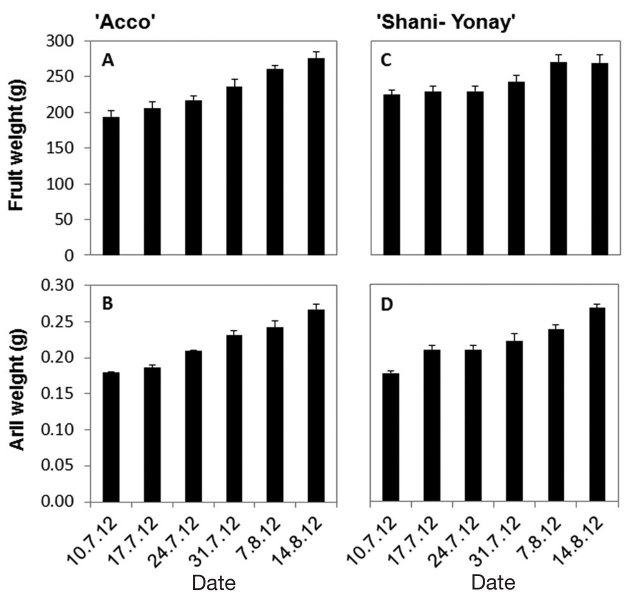

FIG. 2
Changes in 'Acco' (Panels A, B) and 'Shani–Yonay' (Panels C, D) pomegranate fruit (Panels A, C) and aril fresh weights (FWs; Panels B, D) during the last 5 weeks of the ripening process in 2012. Data are means + SE of ten measurements on ten different fruit (n = 10).

TSSC and TA values

The TSSC of juice extracted from 'Acco' and 'Shani-Yonay' arils increased slightly from 12.4 – 12.8% (w/v) on the first evaluation date (10 July), to 14.0 – 14.2% on the last evaluation date (14 August; Figure 3). However, most of the observed increase in juice TSSC occurred between the first two evaluation dates, approx. 4 – 5 weeks before full-ripeness. TSSC values did not change

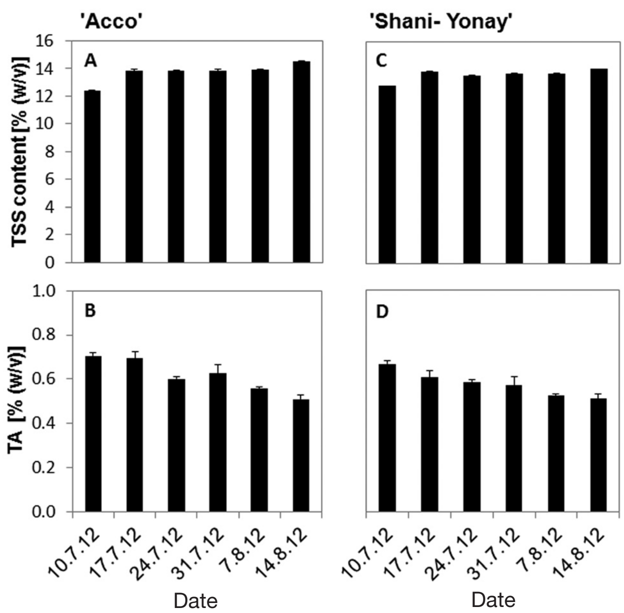

FIG. 3
Changes in total soluble solids content (TSSC; Panels A, C) and titratable acidity (TA; Panels B, D) values of the juice extracted from 'Acco' (Panels A, B) and 'Shani-Yonay' (Panels C, D) pomegranate arils during the last 5 weeks of the ripening process in 2012. Data are means + SE of three separate measurements on the juice from five different fruit (n = 15).

significantly during the last 4 weeks of the ripening process (Figure 3). In parallel, the TA of the juice decreased slightly from approx. 0.7% (w/v) on the first evaluation date (10 July), to approx. 0.5% (w/v) at full-ripeness (14 August; Figure 3).

Sensory analysis and flavour preferences

Flavour preference tests on successive dates during the ripening of 'Acco' and 'Shani-Yonay' fruit revealed that the flavour preference ratings of both varieties were extremely low on the first two evaluation dates (10 and 17 July) at 3.4 and 3.9, respectively, on a scale from 1 – 9. Subsequently, and until full-ripeness, we observed continuous, gradual increases in fruit flavour preference scores, which finally reached 6.4 and 6.1 for 'Acco' and 'Shani-Yonay' fruit, respectively (14 August; Figure 4). In parallel with the observed increases in fruit flavour preference scores during ripening, observations by the trained sensory panel revealed a gradual decline in the sensation of astringency, which decreased from a relatively high score of approx. 3.2 – 3.3 (on a scale of 0 – 10) on the first evaluation date (10 July), to a low score of just 0.9 – 1.2 at full-ripeness (14 August; Figure 4). Thus, we observed high correlations ($r^2 > 0.9$) between the increase in fruit flavour preference score and the decrease in astringency during fruit ripening in both varieties (data not shown). In addition to a significant decrease in the astringency sensation, the descriptive sensory analysis further revealed slight increases in 'sweet' taste and in 'fruity' odour during ripening (data not shown).

Total phenolics and hydrolysable tannin concentrations

During the last 5 weeks of fruit ripening we did not observe any significant change in total phenolics

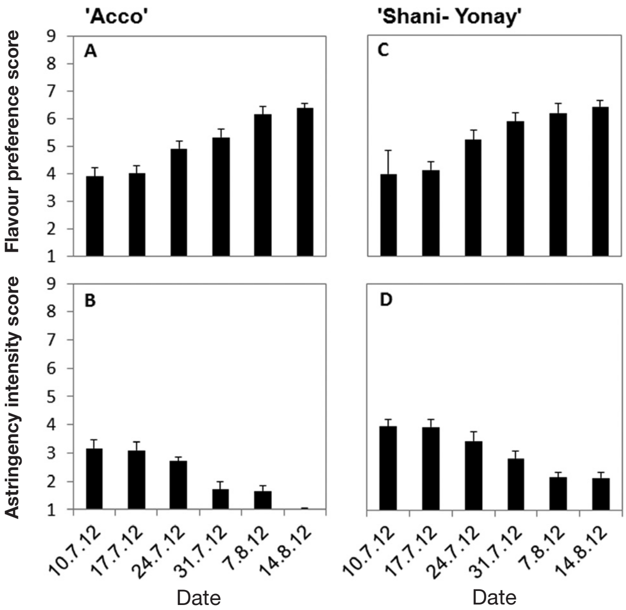

Fig. 4

Changes in flavour preference score (Panels A, C) and astringency sensation score (Panels B, D) on a scale from 1 – 9 of 'Acco' (Panels A, B) and 'Shani-Yonay' (Panels C, D) pomegranate fruit during the last 5 weeks of the ripening process in 2012. Flavour preferences were evaluated according to a nine-point hedonic scale ranging from "very strong dislike" to "very strong like". Flavour preference data are means + SE of the marks ascribed by 40 untrained testers. Astringency sensation was evaluated by a trained panel, and the data are the means of the ratings of ten testers.

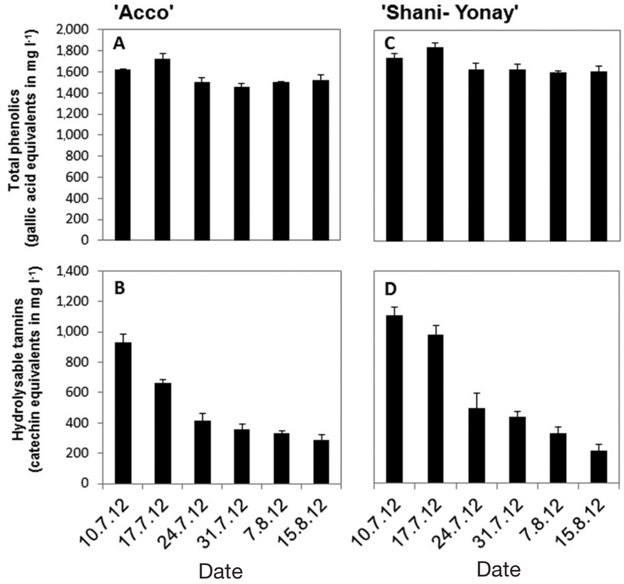

Fig. 5

Changes in total phenolics (Panels A, C) and hydrolysable tannin (Panels B, D) concentrations in the juice extracted from 'Acco' (Panels A, B) and 'Shani-Yonay' (Panels C, D) pomegranate arils during the last 5 weeks of the ripening process in 2012. Data are means + SE of three separate measurements on the juice from five different fruit (n = 15).

contents (Figure 5). However, in parallel with the observed decrease in astringency during ripening, we observed a significant and specific decrease in hydrolysable tannin concentration in the juice of both 'Acco' and 'Shani-Yonay' (Figure 5). Hydrolysable tannin concentrations in 'Acco' and 'Shani-Yonay' juice were 930 mg l^{-1} and 1,100 mg l^{-1}, respectively, on the first evaluation date (10 July), and decreased gradually to 290 mg l^{-1} and 210 mg l^{-1}, respectively, at full-ripeness (14 August; Figure 5).

In addition to spectrophotometric measurements of hydrolysable tannin concentrations, we also measured hydrolysable tannin concentrations in pomegranate fruit and juice using filter papers soaked in $FeCl_3$, according to protocols previously developed for persimmon fruit (Gazit and Levy, 1963). However, this procedure was found to be unsuitable for pomegranate fruit, because no changes in staining level were observed during fruit ripening (Figure 6).

Correlation between aril fresh weight and flavour preference

In order to define a simple and reliable maturity index to determine the harvesting period for early-season, sweet pomegranate fruit, we examined possible correlations between the increases in flavour preference scores and in aril FW during ripening. There was a high correlation ($r^2 = 0.99$) for both 'Acco' and 'Shani-Yonay' (Figure 7). Thus, aril FW may serve as a reliable and appropriate ripening index to determine the commercial harvesting dates of early-season pomegranate fruit.

DISCUSSION

To ensure that fruits and vegetables are harvested at optimum maturity, and when they exhibit at least the minimum acceptable quality, it is necessary to define

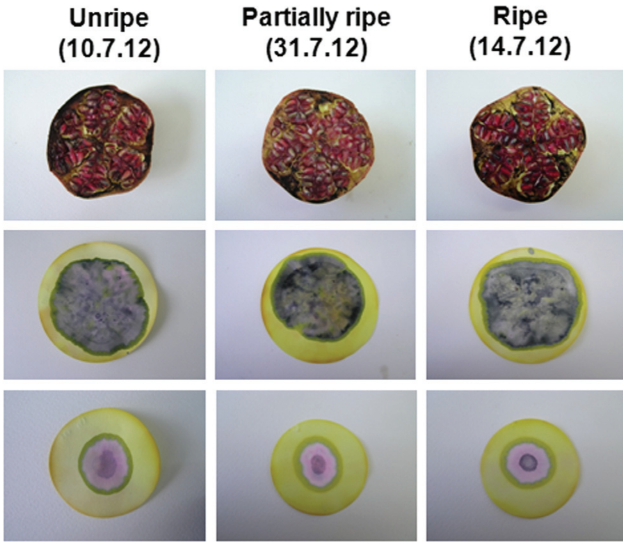

FIG. 6
Analysis of hydrolysable tannin concentrations in 'Acco' pomegranate fruit and juice, determined by the ferric chloride detection method (Gazit and Levi, 1963). The upper three Panels show cut fruit dipped in 5% (w/v) $FeCl_3$. The middle Panels show filter papers previously soaked with 5% (w/v) $FeCl_3$ after contact with the cut fruit surface. The bottom Panels show filter papers previously soaked with 5% (w/v) $FeCl_3$ after spotting a 50 µl sample of aril juice on each.

appropriate maturity indices for each commodity. In the case of pomegranate fruit, there are established maturity indices based on juice TSSC and TA [i.e., TSSC > 17% (w/v) and TA < 1.85% (w/v); Kader, 2006; Crisosto *et al.*, 2013] for the late-season, sweet-sour Californian variety, 'Wonderful'. However, these indices are not suitable for early-season sweet pomegranate varieties, which have much lower TSSC and TA values than 'Wonderful'. Furthermore, it is still necessary to define specific maturity indices for sweet, sweet-sour, and sour pomegranate varieties (Melgarejo *et al.*, 2000).

In the present study, gradual increases in fruit and aril FWs occurred during the last 5 weeks of the ripening process of early-season 'Acco' and 'Shani–Yonay' pomegranate fruit (Figure 2), whereas the colour of the peel and arils barely changed (Figure 1). Moreover, we observed only slight increases in TSSC values and slight decreases in TA values, which were already relatively low (< 0.7%) 1 month before full maturity (10 July; Figure 3). Therefore, evaluations of TSSC or TA did not provide an

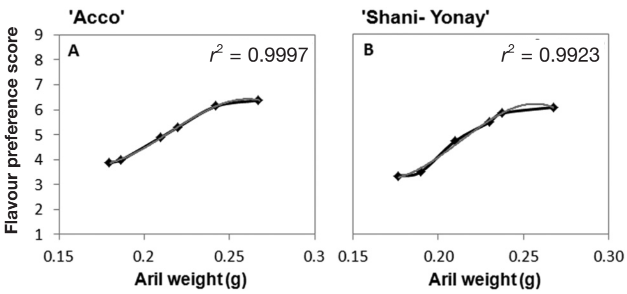

FIG. 7
Correlations between aril fresh weight (FW) and the flavour preference scores for 'Acco' (Panel A) and 'Shani-Yonay' (Panel B) pomegranate fruit. r^2 values were calculated using the Microsoft Office Excel and JMP Version 7 statistical programmes. The data were from the 2012 pomegranate ripening season, but are representative of all three independent growth seasons.

appropriate maturity index for the optimal harvest period of early-season sweet pomegranates. It was therefore necessary to define another more appropriate maturity index.

The main finding of the present study was that the major factor limiting the early harvesting of early-season sweet pomegranate fruit was their low flavour preference score, which largely reflected high rates of astringency (Figure 4). The decrease in astringency sensation during the last period of fruit ripening (Figure 4) was supported by biochemical analyses that demonstrated a gradual decrease in the hydrolysable tannin concentration in juice that imparts an undesirable mouth-feel sensation of astringency (Figure 5). It has been reported that pomegranate fruit are astringent (Bajec and Pickering, 2008). However, to the best of our knowledge, the present study provides the first evidence that astringency is the main limiting factor for the palatability of pomegranate fruit, and that its removal should determine the optimal harvesting period.

Simple and easy-to-use methods to evaluate astringency levels have been developed for other astringent fruit, such as persimmon. They include covering the fruit surface with a dry filter paper previously soaked in 5% (w/v) $FeCl_3$, and observing the resulting level of blue staining (Gazit and Levy, 1963). Unfortunately, this simple and commonly used method was not applicable to pomegranate fruit because the level of staining did not change during the course of fruit ripening. This may have been due to interference by the high concentrations of anthocyanin pigments in the fruit and juice (Figure 6). Therefore, we searched for other appropriate maturity indices specific to pomegranate, instead of direct evaluations of hydrolysable tannin concentrations. We found a high correlation (r^2 > 0.99) between the increase in fruit flavour preference score and the increase in aril FW during ripening (Figure 2; Figure 4; Figure 7). Therefore, we suggest that measurements of mean aril FWs may provide a simple and reliable maturity index for determining the harvest period of early-season, sweet pomegranate fruit.

In the cases of 'Acco' and 'Shani-Yonay' fruit, we found that an appropriate maturity index for harvesting would be a mean aril FW of > 0.23 g, which matched a flavour preference score of ≥ 6, on a scale of 1 – 9 (Figure 7). However, appropriate aril FWs to determine the optimum harvest period for other varieties would have to be determined specifically for each variety.

The present proposal to measure aril FW as an indicator of flavour acceptability is reminiscent of the current use of dry weight (DW) as a maturity index for determining the harvest period of avocado fruit. In the case of avocado, it was found that the improvement in flavour preference ratings during ripening was highly correlated with oil content or DW percentage; therefore, these two indicators have served as reliable and appropriate maturity indices for determining the minimum acceptable quality for harvesting avocado fruit (Lee *et al.*, 1983; Reid, 2002).

We also found a high correlation between the increase in fruit flavour preference score and the increase in total fruit FW during ripening (data not shown). However, we noticed that fruit FW and size are growth-related processes that depend on various parameters such as fruit load, irrigation, fertilisation, and climatic conditions. In contrast, the increase in mean aril FW during ripening was tightly linked to the ripening process, as a similar increase in aril FW during ripening was observed in both small and large fruit (data not shown).

In conclusion, we found that changes in fruit and aril colour, as well as changes in TSSC and TA values did not provide suitable maturity indices for determining the harvesting period of early-season sweet 'Acco' and 'Shani-Yonay' pomegranates. In contrast, the main factor limiting early harvesting of these early-season fruit was their low flavour preference score resulting from a mouth-feel sensation of high astringency. At last, we found a high correlation (r^2 = 0.99) between the observed increase in fruit flavour preference and mean aril FW during ripening, and suggest that a mean aril FW of > 0.23 g may serve as a simple and reliable maturity index for timing the commercial harvesting of these early-season pomegranate varieties.

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