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Seasonal variation in chemical composition, aroma volatiles and antioxidant capacity of pomegranate during fruit development

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This study was conducted to investigate compositional changes and antioxidant capacities of pomegranate fruit ('Bhagwa' and 'Ruby') at five distinct stages of maturity over two growing seasons. Total soluble solids (TSS), pH, titratable acidity (TA), phenolic concentrations, antioxidant capacity and aroma volatile constituents were investigated. Principal component analysis (PCA) and Pearson correlation were used to visualize the changes in major chemical indices and the relationship among them. Results show that major compositional changes in fruit are developmentally regulated. Significant increases in total soluble solids (TSS), coupled with significant decline in titratable acidity (TA) and total phenolics (TP) occurred with advancing maturity. Fruit at advanced maturity stages were characterized by intense pigmentation of peel and aril, which coincided with maximum accumulation of anthocyanins. TSS and TA showed strong relationships with most of the chemical indices, each showing significantly ($p < 0.05$) strong correlations with phenolic components as well as with the antioxidant capacity (FRAP and DPPH) measured. There were no significant ($p < 0.05$) seasonal effects on juice absorbance (colouration) and TA for 'Bhagwa' as well as juice absorbance, TSS, TSS/TA and BrimA for 'Ruby'. In combination, the identified maturity indices (absorbance, TSS, TSS:TA and BrimA level) would account for the evolution of juice colour, flavour and taste. The identified maturity indices for each cultivar could aid the search for reliable maturity markers to determine fruit readiness for harvest.

Key words: Antioxidant capacity, BrimA, ripeness, seasonality, pomegranate, South Africa.

INTRODUCTION

A large number of physiological, biochemical, and structural changes occur during fruit maturation and ripening (Nunes et al., 2009). Biochemical components, volatile constituents and polyphenol concentrations are mostly considered parameters for quality assessment of many types of fruit and vegetables, due to the roles they play in determining nutritive value and making the produce desirable for consumption (Ashoor and Knox, 1982; Glew et al., 2003). In pomegranate fruit handling and marketing, important quality attributes include size, skin and aril colour, juiciness, taste and flavour (Jalikop, 2007; Holland et al., 2009; Fawole and Opara, 2013).

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Abbreviations: TSS, Total soluble solids; **TA,** titratable acidity; **PCA,** principal component analysis; **TP,** total phenolics; **PJ,** pomegranate juice; **TGC,** total gallotannins concentration; **TAC,** total anthocyanin concentration.

The edible part of the fruit (aril), which is usually consumed as fresh aril or as processed products, contains considerable amounts of acids, sugars, polyphenol and important minerals (Al-Maiman and Ahmad, 2002).

Pomegranate fruit peel colour is not a reliable indicator of the degree of ripening or readiness for consumption (Holland et al., 2009), therefore, harvesting fruit at optimum maturity is crucial for maintaining high sugar content, good colour and overall fruit flavour for fresh market and juice industries. Fruit maturity status of some pomegranate cultivars has previously been assessed based on a combination of indices including external (peel) colour, aril pigmentation, total soluble solids contents and titratable acidity (Ben-Arie et al., 1984; Cristosto et al., 2000; Martinez et al., 2006). Aside from the influence of phenolics on the sensory quality of the fruit colour (anthocyanins) and taste (tannins), phenolic compounds in pomegranate have beneficial health effects. In recent years, due to an increasing consumer interest and an awareness on the health benefits of the fruit juice, the importance of evaluating fruit health-benefiting phenolic compounds as part of the target traits for accurate fruit quality evaluation is well recognised (Holland et al., 2009). The health benefits of consuming pomegranates have been attributed to the exceptionally high antioxidant capacity that strongly correlates with high concentration and unique composition of phenolic compounds (Gil et al., 2000; Borochov-Neori et al., 2011; Fischer et al., 2011; He et al., 2011). However, the phytochemicals differ in concentration and are dependent on cultivar types (Gil et al., 2000; Shwartz et al., 2009; Tezcan et al., 2009; Elfalleh et al., 2011).

Commercial production of pomegranates is fairly new in South Africa, and the cultivars ‘Bhagwa’ and ‘Ruby’ are among the most widely grown in the country and globally. Practically, it is the responsibility of the fruit industry to establish a minimum maturity indices based on available scientific information and its needs (Cristosto et al., 2000). Unfortunately, at present there are no established quality and maturity standards in the South African pomegranate industry. Furthermore, information is lacking on seasonal evolution of compounds responsible for fruit organoleptic and health-promoting properties during fruit development and ripening of South African grown pomegranate cultivars. In the absence of objective maturity indices, calendar dates are commonly used to determine harvest periods by farmers (Brodie, 2009; Olivier, F., pers. comm., 2011). Unfortunately, maturity indices established in other pomegranate growing countries may not be directly applicable under South Africa conditions due to differences in cultivar types and the agro-climatic regions. For instance, Chace et al. (1981) established a maturity standard for ‘Wonderful’ pomegranate grown in California based on the conclusion that 1.8% titratable acidity (TA) level and total soluble solids (TSS) content above 17% was the most satisfactory maturity standard. From the point of view of flavor, these values are too high for most of the pomegranate cultivars grown in South Africa. More so, that the choice of a reliable harvest index should reflect consumer sensory quality requirements of harvested fruit that permits the postharvest delivery of the fruit to consumers to meet their demand for organoleptic, nutritional and antioxidant attributes (Kader, 2008).

To determine the optimal accumulation period of desirable compounds, it was imperative to study the changes that occur in fruit metabolites such as soluble solids, acidity and volatile constituents as well as health-benefiting phenolic content. This could be useful for two major reasons; first, to provide information on the behavior of the fruit cultivars during development and ripening, and second, it will enable the identification of most consistent indices relating to desirable organoleptic attributes, which are those with the best potential for the development of a reliable maturity index. Based on these considerations, the aim of this work was to study the evolution of fruit biochemical properties, volatile constituents and antioxidant capacity during development and ripening over two growing seasons.

MATERIALS AND METHODS

Fruit sample

The study was carried out over two seasons during 2010/2011 and 2011/2012 pomegranate seasons. Pomegranate fruit (cvs ‘Ruby’ and ‘Bhagwa’) grown in commercial orchards located in the Porterville regions (South Africa, 33°01′00″S, 18°58′59″E) were studied. ‘Ruby’ is an early cultivar and considered sweeter than ‘Bhagwa’ which is a mid cultivar. The orchards were located on sandy loam soil, and the trees received the same fertilizer program and irrigation delivering about 32 $L.ha^{1}.day^{-1}$. The trees were about six years old at planting distance of 5 x 3 m, with same row orientation and tree training. The rainfall and temperature data were recorded at a nearby local meteorological station during the growing seasons. A sample of twenty fruits of the same size and without physical defect was randomly collected from different positions of 10 randomly selected trees per orchard, and transferred to the laboratory in an air-conditioned car. Sampling was done monthly, with the first samples being in January (54 days after full bloom; DAFB) when it was possible to squeeze juice from the arils till April for ‘Ruby’; 139 DAFB or May for ‘Bhagwa’; 165 DAFB. Each harvest date corresponded to a different maturity stage (S) ranging from S1 to S5 during fruit development. A detailed description of fruit at these stages is presented in Table 1. Arils were removed manually from fruits, and then followed by extraction of juice from the arils (without crushing the kernels) using a blender (Mellerware, South Africa).

Chemical composition

Juice absorbance, pH, titratable acidity and total soluble solids

Pomegranate juice (PJ) colour absorbance was measured at 520 nm using a Helios Omega UV-vis spectrophotometer (Thermo Scientific technologies, Madison, USA). The pH of PJ was determined at room temperature by using a pH meter (Crison, Barcelona, Spain). All measurements were made on individual fruit

Table 1. Description of fruit maturity at different sampling stages along days after full bloom (DAFB).

Stage	DAFB (Month)		Description of maturity stage
	'Bhagwa'	'Ruby'	
S1	54 (Jan)	54 (Jan)	Immature: Green skin, immature white arils with immature kernels
S2	82 (Feb)	82 (Feb)	Mature/unripe: Light-red skin, mature white arils with mature kernels
S3	110 (Mar)	110 (Mar)	Mature/semi-ripe: Red skin, mature pink arils with mature kernels
S4	140 (Apr)	132 (Apr)	Mature/ripened/ harvest 1: Red skin, mature red arils with mature kernels
S5	165 (May)	139 (Apr)	Mature/full-ripened/harvest 2: Deep-red skin, deep-red arils with mature kernels

samples. Titratable acidity (TA) was determined by titration using a Metrohm 862 compact titrosampler (Herisau, Switzerland). Fresh juice (2 mL) was diluted with Milli-Q water (70 mL) and titrated with 0.1 N NaOH to an endpoint of pH 8.2 and results expressed as gram (g) tartaric acid/100 mL of juice. Total soluble solids (TSS) were measured using a digital refractometer (Atago, Tokyo, Japan) calibrated with Milli-Q water. BrimA index, a variant of TSS/TA and a criterion for acceptance of fruit juice, which is expressed as BrimA = TSS – k * TA, where k is the tongue's sensitivity index normally ranging from 2 - 10 (Jordan et al., 2001; Jaya and Das, 2003). In this study k value of 2 was used to avoid negative BrimA index (Fawole and Opara, 2013).

Phytochemical composition

Sample preparation

Crude PJ sample (1 mL) was extracted with 29 mL of cold 50% aqueous methanol. The resulting mixture was vortexed, and then sonicated in ice for 20 min in a cold water bath followed by centrifuging at 10000 rpm for 5 min at 4°C (Merk, Eppendorf AG, Germany). The supernatant was subsequently collected and assayed for phenolic components and antioxidant capacity (Fawole and Opara, 2013).

Determination of total phenolic compounds

Total phenolic (TP) content in PJ methanolic extracts was determined using the Folin-Ciocalteu (Folin-C) colourimetric method as described by Makkar (2000), with a slight modification. Briefly, diluted PJ extracts (500 µl), a blank containing aqueous methanol instead of plant extracts, and concentrations of a standard (gallic acid) were mixed with 1 N Folin-C reagent (0.5 ml) followed by the addition of 2% sodium carbonate (2.5 ml). The absorbance was measured at 725 nm using a Helios Omega UV–vis spectrophotometer (Thermo Scientific technologies, Madison, USA) after 40 min incubation at room temperature. Total soluble phenolic concentrations were calculated from the standard curve and expressed as the mean ± S.E (mg) of gallic acid equivalents (GAE) per 100 ml PJ.

Total flavonoids concentration

The total flavonoids content in PJ extracts was determined as described by Yang et al. (2009). Diluted PJ extract (250 µl) was mixed with distilled water (1.25 ml) followed by the addition of 5% sodium nitrite solution (75 µl), and the mixture was thoroughly vortexed and left for 5 min. Subsequently, aluminium chloride (10%, 150 µl) was added to the mixture and allowed for further reactions for 6 min before adding sodium hydroxide (1 M, 500 µl) and distilled water (775 µl). A standard (catechin, 0 – 125 µg/ml) was prepared and assayed following the same procedure. The absorbance of the mixture was measured at 510 nm using a Helios Omega UV–vis spectrophotometer (Thermo Scientific technologies, Madison, USA). The absorbance values of PJ extracts were compared with those of standard concentrations and expressed as the mean ± S.E (mg) of gallic acid equivalents (GAE) per 100 ml PJ.

Rhodanine assay for total gallotannins

Determination of total gallotannins concentration (TGC) in PJ was carried out as described by Makkar (2000). In triplicate, diluted extracts (50 µL) were mixed with 150 µL of 0.4 N sulphuric acid followed by 600 µL rhodanine. After 10 min, 200 µL of 0.5 N KOH were added and subsequently distilled water (4 mL) after 2.5 min. The absorbance was read at 520 nm against a blank that contained aqueous methanol instead of sample after 15 min incubation at room temperature. Gallic acid was used for the standard curve. TGC was calculated from the standard curve and expressed as gallic acid equivalent (GAE) per 100 mL PJ.

Total anthocyanin concentration

Total anthocyanin concentration (TAC) was quantified using the pH differential method (Wrolstad, 1993). In triplicate, PJ extracts (1 mL) were mixed with 9 mL of pH 1.0 and pH 4.5 buffers, separately. Absorbance was measured at 520 and 700 nm in pH 1.0 and 4.5 buffers and the result was expressed as cyanidin 3-glucoside using the following equations:

$$A = (A_{510} - A_{700})_{pH\ 1.0} - (A_{510} - A_{700})_{pH4.0} \quad (1)$$

$$\text{Total monomeric anthocyanin (mg/mL)} = (A \times MW \times DF) \div (\mathcal{E} \times L) \quad (2)$$

Where, A is the Absorbance; Ɛ is the Cyd-3-glucoside molar absorbance (26,900); MW is the anthocyanin molecular weight (449.2); DF is the dilution factor and L is the cell path length (1 cm). Final results were expressed as cyanidin 3-glucoside equivalents per 100 mL PJ (mg C_3gE/100 mL PJ).

GC-MS determination of volatile constituents

Volatile compounds in PJ samples were profiled by subjecting juice samples to headspace solid phase micro-extraction (HS-SPME). 5 ml of fresh juice sample were dispensed into 22 mL crimp cap headspace vials, followed by the addition of 1 g of table salt (NaCl, Sigma, St. Louis, USA) to enhance the release of the volatile compounds (Lachenmeier et al., 2006). A 50/30 µm DVB/CAR/PDMS (Supelco, Bellefonte, PA, USA) fiber was used for all the analysis. Pre-incubation and extraction times to 50°C were 10 and 20 min, respectively. Volatile organic compounds trapped in the fibre were analysed by an Agilent GC (6890 N, Agilent Technologies, Santa Clara, CA, USA) coupled with a mass spectrometer (5975 N, Agilent Technologies, Santa Clara, CA, USA),

and equipped with an Rxi®-5Sil MS column (30 m length, 0.25 mm i.d., 0.25 µm film thickness). Oven temperature was maintained at 40°C for 2 min and then programmed to maximum temperature of 250°C at 5°C /min, where it was isothermally held for 10 min. Helium was the carrier gas at a constant flow rate of 1.2 mL/min. Tests were run in duplicate per sample, each sample comprising three individual fruit in triplicates. Volatile compounds were identified by comparing their mass spectra with the mass spectra of libraries (NIST, 95) with comparison quality >90%. Semi-quantification of compounds identified was achieved by calculating the relative proportions (%) of each volatile compound as percentage ratio of peak area of each compound to the total peak area of all identified compounds. Measurements were conducted in triplicate on pooled fruit samples.

Antioxidant capacity

DPPH radical-scavenging activity

The DPPH assay was carried out in triplicate, according to the method used by Karioti et al. (2004) with some modifications. Methanolic extract of PJ sample (15 µL) was diluted with methanol (735 µL) in test tubes followed by the addition of methanolic DPPH solution (750 µL, 0.1 mM). The mixtures were incubated at room temperature for 30 min in the dark, and the absorbance was measured at 517 nm using a UV-vis spectrophotometer. Absorbance was compared with the standard curve (ascorbic acid, 0 - 2.0 mM). The free-radical capacity of PJ was expressed as ascorbic acid (mM) equivalents per mL PJ (mM AAE /mL).

Ferric ion reducing antioxidant power (FRAP)

The antioxidant power of PJ was measured colorimetrically according to the method of Benzie and Strain (1996) with a few modifications (Fawole et al., 2012). The FRAP working solution containing mixtures of 300 mM acetate buffer (50 mL), 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) (5 mL) and 20 mM ferric chloride (5 mL) was freshly prepared and incubated in a water bath at 37°C before being used. In triplicates, diluted aqueous methanolic PJ extracts (150 µL) were added to 2850 µL of the FRAP working solution before incubation in the dark for 30 min. Trolox (100 - 1000 µM) was used for the calibration curve, and the results were expressed as trolox (mM) equivalents per mL PJ (mM TE/mL PJ).

Statistical analysis

The results of all studied variables are presented as mean (± S.E) values. Analysis of variance (ANOVA) was carried out using Statistica software (Statistica 11.0, StatSoft Inc., Tulsa, OK, USA) according to Duncan's multiple range test. Where appropriate, 2-way ANOVA was also carried out. Correlation coefficients (r) were determined by the Pearson correlation matrix method using SPSS for Windows. Principal component analysis (PCA) was carried out using the statistical software XLSTAT Version 2012.4.01 (Addinsoft, France).

RESULTS AND DISCUSSION

Juice absorbance, pH, titratable acidity (TA) and total soluble solids (TSS)

Changes in juice absorbance, pH, TA and TSS during cultivars are reported in Table 2. For both cultivars, a gradual increase in juice absorbance value with advancing maturation occurred with cultivars in each season. The absorbance values were notably higher at more advanced stages (S4 and S5) when the fruit aril colour had changed from pink to the desirable red colouration. For both cultivars, no significant interactions were found between fruit maturity and growing season for juice absorbance ('Bhagwa': $p = 0.1552$; 'Ruby': $p = 0.3736$). However, juice absorbance was significantly influenced by fruit maturity for 'Bhagwa' ($p < 0.0001$) while the main differences for 'Ruby' were evidently due to fruit maturity and growing season (maturity; $p < 0.0001$, season; $p = 0.0254$) (Table 2). These results confirm the findings of Gil et al. (1995) on Spanish cultivars. The observed changes in juice absorbance could be explained by increase in biosynthesis and accumulation of red anthocyanins in fruit. Indeed, according to the study of Shulman et al. (1984), pomegranate juice absorbance is a reflection of anthocyanins which are light-absorbing plant-based pigments.

The pH of pomegranate juice characterizes its acidic taste (Zarei et al., 2011). Changes in juice pH during fruit development was significantly ($p < 0.05$) influenced by the interaction between maturity stage and growing season. In the 2011 season, significant ($p < 0.05$) increase in pH value occurred with advancing maturity in 'Bhagwa', whereas the changes in pH value for 'Ruby' did not follow a significant ($p < 0.05$) pattern. In 2012 season, however, for both cultivars, immature fruit (S1 - S2) were characterized by low pH values (more acidic) while at later maturity stages (S3 - S5) had higher pH values (less acidic) (Table 2). The fluctuations observed in juice pH could be a reflection of within-season changes such as orchard management practices which could affect juice acidity during fruit development.

Considerable variation was observed in total soluble solid (TSS) content at all the maturity stages for both cultivars (Table 2). TSS content increased more rapidly during early stages of fruit development (S1 - S3) for both cultivars in each season, a phenomenon associated with active hydrolysis of starch to sugars in maturing fruit (Kulkarni and Aradhya, 2005). High TSS content is highly desirable in pomegranate fruit juice as it enhances sweetness and flavour especially if accompanied by a decrease in juice acidity and tannin concentration (Shwartz et al., 2009; Zarei et al., 2011). TSS content in both cultivars at fully-ripe stage (S5) reached at least 15°Brix in each season. TSS contents increased in 'Bhagwa' cultivar from 10.33 to 16.18°Brix in 2011 season and from 9.28 to 15.56°Brix in 2012 season. The differences in TSS accumulation may be attributed to the significant ($p < 0.0001$) interaction between maturity stage and growing season, although seasonality effect alone was slightly noticeable but not significant ($p = 0.0516$). In contrast, the significant differences observed

Table 2. Changes in chemical composition in pomegranate juice at major maturity stages over two seasons.

Maturity stage	Juice abs.	pH	TSS (°Brix)	TA (% tartaric)	TSS:TA	BrimA (TSS- k*TA)
'Bhagwa'						
Stages (A)						
2011_S1	0.03±0.001^{c}	3.18±0.03^{e}	10.33±0.35^{f}	0.62±0.02^{a}	16.68±0.35^{e}	9.10±0.20^{f}
2011_S2	0.36±0.001^{bc}	3.22±0.05^{de}	11.97±0.19^{e}	0.57±0.03^{a}	21.23±0.58^{e}	10.83±0.14^{e}
2011_S3	0.64±0.12^{bc}	3.24±0.23^{de}	13.83±0.29^{d}	0.45±0.01^{bc}	30.89±0.59^{d}	12.94±0.17^{d}
2011_S4	2.24±0.36^{a}	3.35±0.04^{cde}	15.12±0.25^{bc}	0.39±0.02^{c}	39.19±1.31^{c}	14.42±0.06^{bc}
2011_S5	3.00±0.12^{a}	3.57±0.13^{abc}	16.18±0.21^{a}	0.38±0.02^{c}	41.83±2.21^{c}	15.38±0.06^{a}
2012_S1	0.08±0.01^{bc}	3.44±0.02^{abcd}	9.28±0.13^{g}	0.34±0.01^{d}	18.96±0.26^{e}	8.30±0.13^{f}
2012_S2	0.23±0.02^{bc}	3.37±0.01^{bcd}	14.52±0.10^{cd}	0.48±0.00^{b}	30.11±0.31^{d}	13.55±0.1^{cd}
2012_S3	0.78±0.05^{bc}	3.58±0.02^{ab}	14.90±0.07^{bc}	0.31±0.00^{d}	47.69±0.41^{b}	14.27±0.06^{bc}
2012_S4	1.11±0.09^{b}	3.61±0.03^{a}	15.31±0.06^{abc}	0.31±0.01^{d}	49.68±0.78^{b}	14.69±0.06^{ab}
2012_S5	2.91±0.12^{a}	3.54±0.02^{abc}	15.56±0.03^{ab}	0.28±0.00^{d}	56.70±0.66^{a}	15.00±0.03^{ab}
Season (B)						
2011	1.22 $^{n.s}$	3.30^{b}	13.59 $^{n.s}$	0.50^{a}	28.87^{b}	12.60 ^{b}
2012	1.02 $^{n.s}$	3.56^{a}	13.76 $^{n.s}$	0.37^{b}	41.27^{a}	13.02 ^{a}
Prob. > F						
A	< 0.0001	0.0563	< 0.0001	< 0.0001	< 0.0001	< 0.0001
B	0.2747	0.0061	0.0516	< 0.0001	< 0.0001	0.0017
A * B	0.1552	0.0067	< 0.0001	0.4707	0.0082	< 0.0001
'Ruby'						
Stages (A)						
2011_S1	0.02±0.00^{c}	3.30±0.03^{b}	11.00±0.20^{d}	0.39±0.03^{b}	28.22±2.12^{bc}	10.21±0.25^{e}
2011_S2	0.32±0.13^{bc}	3.11±0.04^{b}	12.63±0.64^{c}	0.37±0.01^{bc}	33.91±2.27^{b}	11.89±0.65^{d}
2011_S3	0.36±0.06^{bc}	3.25±0.09^{b}	14.60±0.06^{ab}	0.33±0.04^{cd}	45.50±5.52^{a}	13.94±0.08^{ab}
2011_S4	0.98±0.02^{b}	3.18±0.05^{b}	14.87±0.26^{ab}	0.33±0.01^{cd}	45.56±2.40^{a}	14.20±0.28^{ab}
2011_S5	2.25±0.29^{a}	3.28±0.05^{b}	15.21±0.48^{a}	0.31±0.01^{d}	49.49±2.61^{a}	14.59±0.49^{a}
2012_S1	0.26±0.01^{bc}	3.25±0.02^{b}	10.90±0.09^{d}	0.49±0.01^{a}	22.28±0.32^{c}	9.91±0.09^{e}
2012_S2	0.37±0.11^{bc}	3.27±0.02^{b}	13.81±0.07^{b}	0.50±0.01^{a}	27.93±0.25^{bc}	12.81±0.07^{cd}
2012_S3	0.83±0.08^{bc}	3.58±0.02^{a}	13.86±0.09^{b}	0.32±0.004^{d}	43.52±0.42^{a}	13.22±0.09^{bc}
2012_S4	2.07±0.26^{a}	3.61±0.02^{a}	14.84±0.07^{ab}	0.32±0.02^{d}	47.87±0.79^{a}	14.24±0.06^{ab}
2012_S5	2.55±0.19^{a}	3.62±0.01^{a}	15.06±0.04^{a}	0.31±0.03^{d}	48.02±0.21^{a}	14.43±0.04^{a}
Season (B)						
2011	0.86^{b}	3.23^{b}	13.41 $^{n.s}$	0.33^{a}	39.34 $^{n.s}$	12.71 $^{n.s}$
2012	1.22^{a}	3.47^{a}	13.37 $^{n.s}$	0.38^{b}	37.39 $^{n.s}$	12.62 $^{n.s}$
Prob. > F						
A	<0.0001	0.0015	<0.0001	<0.0001	<0.0001	<0.0001
B	0.0254	<0.0001	0.8760	0.0003	0.0996	0.8634
A * B	0.3736	0.0046	0.1523	<0.0001	0.3152	0.2489

Factorial ANOVA was performed for Factor A (maturity stage) and Factor B (season). Different letter(s) on column indicate statistically significant differences (p<0.05) according to Duncan's multiple range test.

in TSS contents in 'Ruby' were particularly due to the influence of fruit maturity ($p < 0.0001$). These results show the significant influence of fruit maturity stage in TSS content, which suggests that it might be possible to define TSS content in fruit at a certain maturity stage regardless of growing season. These findings are in agreement with previous studies for other pomegranate cultivars grown under different agro-climatic regions and in different growing seasons (Ben-Arie et al., 1984; Shulman et al., 1984).

Titratable acidity (TA, expressed as % tartaric acid) in both cultivars decreased during fruit development and ripening, especially at S3 stage where a significant decline occurred, and then followed by gradual decrease until the fully-ripe maturity stage (S5) (Table 2). During maturity stages investigated, TA levels in the 'Bhagwa' cultivar decreased primarily due to fruit maturity and season ($p < 0.0001$). The interaction between fruit maturity

and season was evident on TA levels for ‘Ruby’ (Table 2). The TA level decreased from 0.39% to 0.31% in 2011 season and from 0.49% to 0.31% in the subsequent season (Table 2). The observed trends in TA levels in our study are in agreement with those reported by several researchers during fruit development of ‘Ganesh’, ‘Taifi’ and ‘Wonderful’ pomegranates (Ben-Arie et al., 1984; Gil et al., 1995; Kulkarni and Aradhya, 2005; Shwartz et al., 2009).

As a result of changes in TSS and TA contents, the ratio of TSS/TA increased considerably in both cultivars during the two seasons. TSS/TA ratio increased significantly at the first three maturity stages (S1 - S3) for both cultivars. Further increase between the last two maturity stages (S4 - S5) was not significant except for ‘Bhagwa’ in 2012. TSS/TA has been reported as one of the most reliable indicators of fruit maturity in some pomegranates although it is largely dependent on cultivar (as fruit juice ranges from sweet to sweet-sour or sour) and agro-climatic conditions (Ben-Arie et al., 1984; Al-Maiman and Ahmad, 2002; Kulkarni and Aradhya, 2005; Shwartz et al., 2009). Fruit of ‘Wonderful’ (sour cultivar) which is regarded as ‘tasty’ had a TSS/TA ratio range of 11 to 16 (Ben-Arie et al., 1984). According to the study of Chace et al. (1981), a TSS value of 17% and a TA value of 1.8% (TSS/TA = 9.44) was recommended as having minimum maturity for commercial harvesting of pomegranates grown in California. Various TSS/TA values have been successfully used to classify pomegranate cultivars of Spanish and Italian origins (Hernandez et al., 1999; Martinez et al., 2006). Furthermore, TSS/TA value is an important criterion also for evaluating pomegranate juice quality in the processing industry for formulation of food and beverage products (Al-Said et al., 2009). Results obtained in the present study confirmed cultivar differences in TSS/TA ratio as evidenced by the significant interaction effects between fruit maturity and growing season for ‘Bhagwa’ ($p = 0.0082$), whereas changes in TSS/TA values for ‘Ruby’ were mainly due to fruit maturity ($p < 0.0001$) (Table 2).

To further explore the relationship between TSS and TA as a potential maturity indicator, BrimA index was calculated. This index allows smaller levels of acidity in juice than sugar content to make the same numerical change to BrimA (Jordan et al., 2001; Jaya and Das, 2002). This observation was also valid in the present study (Table 2). Specifically, the main differences in BrimA were due to significant ($p < 0.0001$) interactions between fruit maturity and season for ‘Bhagwa’, whereas only fruit maturity had significant ($p < 0.0001$) influence on BrimA for ‘Ruby’ (Table 2).

Phytochemical compounds during fruit development and ripening

Seasonal changes in total phenolics (TPC), total flavonoid concentration (TFC), gallotannin concentration (GTC) and total anthocyanin concentration (TAC) in the investigated cultivars are presented in Table 3. Generally, total phenolic concentration (TPC) has been reported to occur at high concentrations at early stages of fruit development and decline with advancing maturation (Al-Maiman and Ahmad, 2002; Kulkarni and Aradhya, 2005; Shwartz et al., 2009). During 2011 for ‘Bhagwa’ the highest TPC was detected at S1 (2027.46 mg GAE/100 mL) and declined by 71% at late maturity stage (S5). Similarly, for ‘Ruby’, TPC declined by 54% between S1 (1051.60 mg GAE/100 mL) and S5 (483.31 mg GAE /100 mL).

In the following season (2012), the TPC in both cultivars were lower than those observed in the previous season, with a reduction of about 82% (‘Bhagwa’) and 49% (‘Ruby’) between S1 and S5. The significant ($p < 0.05$) interaction between maturity stage and growing season revealed that total phenolic concentration in both cultivars was influenced by fruit maturity (Table 4). Decrease in phenolic concentration during fruit development might result from the oxidation of polyphenols (Amiot et al., 1995; Kulkarni and Aradhya, 2005; Shwartz et al., 2009).

The reduction of phenolic concentration during fruit development has also been attributed to the decline and eventual end of polyphenols biosynthesis during fruit maturation (Kulkarni and Aradhya, 2005; Shwartz et al., 2009).

The decline in TPC corresponded with a significant ($p < 0.05$) decrease in total flavonoids and gallotannin concentrations in fruit of both cultivars. Since flavonoids and gallotannins are phenolic compounds, it was hypothesized that TFC and GTC would contribute to the total phenolic concentration of fruit juice (Gil et al., 2000; Fischer et al., 2011).

A considerable decline in total flavonoids, including condensed and hydrolysable tannins, is desirable in pomegranate fruit. Moderate concentration of these compounds contribute to the typical pomegranate juice flavour, whereas too much of flavonoids concentrations like those measured in immature and unripe fruit stage, give pomegranate juice an unpleasant and astringent taste (Al-Said et al., 2009; Zarei et al., 2011). Comparable values of TFC were observed between the two harvest seasons for both cultivars.

For ‘Bhagwa’ at S5 stage, the TFC value was 201.57 mg CAE/100 mL in 2011 and 165.99 mg CAE/100 mL in 2012. TFC concentrations in ‘Ruby’ were significantly higher at 395.27 and 309.93 mg CAE/100 mL in 2011 and 2012 seasons, respectively.

The significant ($p < 0.05$) interactions between fruit maturity and growing season was limited to significant ($p < 0.05$) influence of maturity for both cultivars. In contrast, for GTC, both main factors contributed to the significant ($p < 0.05$) interactions observed between the factors (Table 3).

Table 3. Changes in phenolic concentration (mg/100 mL) in pomegranate juice at major maturity stages over two seasons.

Stage	'Bhagwa'				'Ruby'			
	Total phenolics	**Total flavonoids**	**Gallotannins**	**Anthocyanis**	**Total phenolics**	**Total flavonoids**	**Gallotannins**	**Anthocyanins**
Stage (A)								
2011_S1	2027.46±17.60^{a}	1459.94±106.91^{a}	125.33±5.78^{a}	0.01±0.00^{d}	1051.60±36.35^{a}	752.18±38.49^{a}	64.80±4.81^{a}	0.20±0.12^{d}
2011_S2	1335.57±10.14^{c}	749.89±109.60^{c}	61.07±6.37^{cde}	6.47±0.80^{cd}	772.59±14.94^{b}	621.11±47.12^{ab}	35.20±1.22^{bcd}	3.25±0.05^{cd}
2011_S3	675.83±76.46^{ef}	568.16±119.86^{cd}	53.60±6.84^{e}	13.11±0.63^{bc}	409.75±11.25^{de}	253.19±18.69^{ef}	38.13±0.41^{bc}	9.44±0.28^{bc}
2011_S4	550.25±7.47^{g}	462.25±23.68^{d}	58.30±3.61^{cde}	40.84±0.31^{a}	429.63±29.99^{d}	356.35±20.51^{de}	21.07±0.82^{e}	11.24±0.16^{b}
2011_S5	583.72±43.2^{gf}	201.57±29.32^{e}	56.53±2.97^{de}	48.61±0.01^{a}	483.31±4.31^{cd}	397.27±26.48^{cde}	29.07±0.62^{cd}	24.23±1.67^{a}
2012_S1	1499.35±2.56^{b}	1045.62±1.96^{b}	91.46±0.10^{b}	0.89±0.48^{d}	771.00±22.55^{b}	529.19±9.39^{bc}	41.23±0.64^{b}	0.81±0.19^{d}
2012_S2	1076.36±39.60^{d}	1002.34±4.91^{b}	89.34±0.24^{b}	4.59±0.27^{d}	707.07±13.73^{b}	531.911±15.43^{bc}	41.41±1.05^{b}	5.63±1.12^{bcd}
2012_S3	753.36±3.56^{e}	697.60±23.55^{c}	80.96±0.75^{b}	13.99±0.84^{bc}	624.07±14.89^{bc}	472.41±10.19^{bcd}	37.38±0.69^{bc}	9.93±0.74^{bc}
2012_S4	257.83±5.53^{g}	465.21±31.56^{d}	76.11±1.29^{bc}	19.42±1.44^{b}	268.79±39.85^{e}	229.34±27.27^{f}	20.89±1.85^{e}	21.67±2.48^{a}
2012_S5	265.54±13.46^{h}	150.99±24.35^{e}	52.59±3.98^{e}	45.79±4.46^{a}	386.59±50.24^{de}	309.93±34.38^{ef}	26.36±2.33^{ed}	27.50±1.97^{a}
Season (B)								
2011	1034.57^{a}	688.36$^{n.s}$	21.81^{a}	74.03$^{n.s}$	629.38^{a}	476.02$^{n.s}$	9.67^{b}	37.65$^{n.s}$
2012	830.49^{b}	672.35$^{n.s}$	16.94^{b}	78.09$^{n.s}$	539.52^{b}	414.56$^{n.s}$	13.11^{a}	33.45$^{n.s}$
Prob. > F								
A	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
B	< 0.0001	**0.7121**	0.0085	**0.3028**	0.0075	**0.0542**	0.0179	**0.0589**
A * B	< 0.0001	0.0011	0.0018	0.0026	0.0003	0.0022	0.0028	**0.1535**

Factorial ANOVA was performed for Factor A (maturity stage) and Factor B (season). Different letter(s) on column indicate statistically significant differences ($p<0.05$) according to Duncan's multiple range test. Total phenolics - mg GAE/100 mL juice; Total flavonoids - mg CAE/100 mL juice, Gallotannins - mg GAE/100 mL juice and Anthocyanins - mg Cy3dE/100 mL juice. GAE - gallic acid equivalent; CAE - catechin equivalent; Cy3dE - cyanidin-3-glucoside equivalent.

Anthocyanins are phenolic compounds that give the characteristic red colouration to pomegranate fruit tissues.

Total anthocyanin concentration (TAC) increased with advancing fruit maturity in each season (Table 3). In both cultivars, TAC was very low concentration at S1 stage but steadily increased until S3 stage, and then the rate of accumulation increased rapidly thereafter. However, there was no significant ($p < 0.05$) between the last two maturity stages of 'Ruby' in 2012, a possible indication of fruit readiness for harvest between S4 and S5 maturity stages. Fruit maturity stage interacted significantly ($p < 0.05$) with growing season for 'Bhagwa' but overall, significant variability was noted for fruit maturity status ($p < 0.0001$).

Furthermore, this study showed that anthocyanin concentration in 'Ruby' was primarily influenced by fruit maturity ($p < 0.0001$) (Table 3). For both cultivars, the increase in anthocyanin concentration was more marked at advanced maturity stages (S4 and S5). These results confirmed previous literature evidence.

Hernández et al. (1999) reported that anthocyanin concentration in ripe fruit of Spanish cultivars was eightfolds higher than the early stages of fruit development.

The accumulation of anthocyanins in plant tissue is directly linked to the contribution of phenolic compounds to the biosynthesis of the flavylium ring of anthocyanins (Kulkarni and Aradhya, 2005). Deep colour formation is one of the important parameters used in assessing pomegranate fruit aril quality. In fact, high anthocyanin concentration results in high fruit red

Table 4. Aroma volatile compounds (peak area %) composition analysed in 'Bhagwa' and 'Ruby' pomegranate fruit cultivars at five maturity stages in 2011/12 season.

Compound	Code	Bhagwa					Ruby				
		S1	S2	S3	S4	S5	S1	S2	S3	S4	S5
Alcohols											
3-Hexen-1-ol	3hOH	n.d	17.4^{c}	29.3^{b}	16.2^{c}	44.5^{a}	n.d	n.d	n.d	n.d	n.d
Hexanol	hexOH	52.4^{a}	53.7^{b}	n.d	n.d	n.d	95.2^{a}	68.3^{b}	8.5^{d}	18.4^{c}	5.7^{d}
3-methyl-1-butanol	3mebOH	n.d	n.d	n.d	n.d	n.d	n.d	n.d	11.2^{b}	19.4^{a}	6.7^{c}
Phenyl ethanol	phenOH	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	1.3
Total		52.4	71.1	29.3	16.2	44.5	95.2	68.3	19.7	37.8	12.4
Ketones											
2-Heptanone	2heONE	n.d	12.0^{ab}	7.0^{b}	7.8^{b}	16.4^{a}	n.d	16.9^{a}	6.4^{b}	n.d	n.d
2-Octanone	2octONE	n.d	4.1^{b}	27.7^{a}	2.9^{c}	5.5^{b}	n.d	8.2^{a}	5.6^{b}	11.2^{a}	2.5^{c}
Total		n.d	16.1	34.7	10.7	21.9	n.d	25.1	12.0	11.2	2.5
Esters											
Hexyl acetate	hexATE	n.d	n.d	0.9^{c}	34.4^{a}	27.8^{b}	n.d	n.d	22.7^{c}	46.0^{a}	32.8^{b}
2-Ethyl acetate	2eATE	n.d	n.d	n.d	n.d	n.d	n.d	5.1^{a}	0.7^{b}	0.1^{b}	n.d
Butyl acetate	butATE	n.d	2.2^{b}	4.4^{a}	2.0^{b}	1.3^{c}	n.d	n.d	0.3^{b}	2.9^{a}	0.6^{b}
Total		n.d	2.2	5.3	36.4	29.1	n.d	5.1	23.7	49.0	33.4
Terpene											
Limonene	lim	47.6^{a}	10.6^{b}	10.3^{b}	3.8^{b}	1.7^{c}	4.8^{a}	1.5^{b}	1.1^{b}	1.9^{b}	0.4^{c}
Total		47.6	10.6	10.3	3.8	1.7	4.8	1.5	1.1	1.9	0.4

Mean values with different letter(s) in the same row indicate statistically significant differences ($p<0.05$) according to Duncan's multiple range test; n.d- not detected.

colouration (Fawole and Opara, 2013). This attribute is, in particular, desirable in pomegranate arils and juice. The results from the present study demonstrate that anthocyanin concentration in fruit juice offers a potential tool to assess fruit maturation pomegranate grown under the South African agro-climate since its accumulation continued throughout fruit development and did not decrease when physiological maturity was reached during the two seasons studied.

Volatile constituents

The relative contents (%) of volatile compounds in juice samples of 'Bhagwa' and 'Ruby' are presented in Table 4. A total of only 10 aroma compounds were detected in the headspace of the juices from both cultivars. This was not surprising because generally pomegranates have very low odour and aroma intensity (Carbonell-Barrachina et al., 2012) which makes it difficult to study the aroma composition. Factors such as sample preparation and method of determination could influence the values obtained. Raisi et al. (2008) used pervaporation to recover more aroma compounds from juice of Iranian pomegranate juice cultivars but were able to identify only nine compounds. However, in a recent study by Calín-Sánchez et al. (2011), a total of 18 compounds were identified in Spanish pomegranate juices using the hydrodistillation technique.

In this study, the identified compounds belong to 4 chemical groups including alcohols, esters, ketones and terpene (Table 4). In general, the composition and relative proportions of the aroma volatiles were different among the fruit maturity stages for both cultivars. Only two aroma volatile compounds (hexanol and limonene) were detected at the first maturity stage (S1) in both cultivars. Hexanol belongs to the alcohol group constituting 52.4% in 'Bhagwa' and 95.2% in 'Ruby', while limonene belongs to the terpenes group and constituted 47.6% and 4.8% of the total aroma in 'Bhagwa' and 'Ruby', respectively. In each of the subsequent maturity stages (S2 - S5), 'Bhagwa' had six compounds while 'Ruby' had seven compounds. At maturity stage S2, the alcohol group had the highest proportion and increased in 'Bhagwa' but decreased in 'Ruby' when compared to the previous stage. As the fruit advanced to maturity stage S3, the ketone group became prominent in 'Bhagwa' accounting for 34.7% of the total volatile compounds. The identified ketones comprised of 2-heptanone (7.8%) and 2-octanone (27.7%). In 'Ruby'

however, the esters group contributed to 23.7% of the total volatiles at S3, comprised of hexylacetate, 2-ethyl acetate and buty acetate, and the group dominated the rest of the maturity stages (S4 = 49%; S5 = 33.4%). In 'Bhagwa' maturity stage S4 was characterized by the dominance of esters with total proportion of 36.4%, while in S5 stage the ethanol group (44.5%) was dominant.

The odour threshold of each volatile compound present in fruit could be used to characterize the aroma intensity (Visai and Vanoli, 1997; Melgarejo et al., 2011). Quantitatively, the alcohol group and limonene were identified in all the maturity stages of both cultivars (Table 4). The alcohol group was mainly represented by hexanol and 3-hexen-1-ol in 'Bhagwa' and hexanol, 3-methyl butanol and phenyl ethanol in 'Ruby'. Limonene has previously been identified in pomegranate juice (Melgarejo et al., 2011), and describes the mild, citrus, sweet, orange and lemon sensory attributes in fruits (Melgarejo et al., 2011). In the present study, limonene content decreased between S1 and S5 stages. Further studies on this compound could possibly be linked to fruit maturity. The esters group was another important group of aroma volatile compounds identified in large proportion in fruit juice at advanced maturity stages (S4 and S5). The hexyl acetate is a major representative in this group (esters) and is known to be responsible for fruity and pineapple odours in fruit (Visai and Vanoli, 1997). Megarejo et al. (2011) reported the aldehydes group as predominant in Spanish cultivars. This group was not identified in the present study on 'Baghwa' and 'Ruby' grown in South Africa.

Antioxidant capacity

The antioxidant capacity of pomegranate juice at different stages of fruit maturation is shown in Figure 1. Antioxidants may act in various ways in different antioxidant assays (Çam et al., 2009). Antioxidant capacity has been determined by several methods based on both the free radical scavenging and the oxidation-reduction mechanisms, although the mechanism of action set in motion by the antioxidant activity of these compounds is still not clearly understood (Viuda-Martos et al., 2010). In this study, antioxidant capacity was measured using DPPH radical scavenging activity (DPPH) and ferric reducing antioxidant power (FRAP). There was a significant ($p < 0.05$) decrease in the antioxidant capacity (DPPH and FRAP assays) of both cultivars with advancing maturity stages in both seasons. For 'Bhagwa', ferric reducing power (FRAP) decreased from 1.67 to 0.74 mM TE/mL (2011 season) and from 1.48 to 0.76 mM TE/mL (2012 season), while the radical scavenging activity (DPPH) decreased from 1.57 to 0.39 mM AAE/mL and from 1.16 to 0.33 mM AAE/mL in 2011 and 2012 seasons, respectively. For 'Ruby' cultivar, FRAP decreased in 2011 and 2012 seasons from 1.43 to 0.71 mM TE/mL and from 0.97 to 0.52 mM TE/mL, respectively, while DPPH decreased from 0.89 to 0.34 AAE/mL in 2011 season and from 0.52 to 0.27 AAE/mL in 2012 season. Fruit maturity stage and growing season had significant ($p < 0.0001$) interaction effects on antioxidant capacity in both FRAP and DPPH assays for both cultivars. It appeared that the variation in antioxidant capacity was brought about by the significant ($p < 0.05$) effects of fruit maturity and growing seasons. The reduction in antioxidant capacity during pomegranate fruit development may be associated with the decrease in quantity of polyphenols in the fruit as shown in Table 3. This contribution of phenolic compound to total anti-oxidant capacity in pomegranate fruit has been reported in previous studies (Gil et al., 2000; Fischer et al., 2011). In particular, fruit harvested during early maturity stages (S1 and S2) showed markedly higher antioxidant values. This further supports the higher polyphenol concentrations such as flavonoid concentrations found in fruit juice (Table 3). Although anthocyanins are known to be antioxidant compounds, their increase during fruit development constituted only a small proportion of total flavonoid concentration of fruit juice; hence the change in flavonoid has a much greater influence than anthocyanins on juice antioxidant capacity. Fully ripe 'Ruby' fruit (S5) had higher (but not significant in some cases) antioxidant capacity than fruit at the preceding maturity stage (S4) for both seasons, although this was not the case in 'Bhagwa' (Figure 1). Higher values of antioxidant capacity at the last maturity stage could, in part, be due to a relatively higher accumulation of anthocyanin compounds, highlighting the important contribution of anthocyanins in total antioxidant capacity of fruit at harvest maturity of the investigated cultivars.

Correlation between maturity indices

Pearson correlation was used to investigate the interrelationships between selected chemical indices of fruit maturity including phenolic components and the antioxidant capacity over the two seasons for both cultivars (Tables 5 and 6). Significantly ($p < 0.05$) strong relationships were revealed among some of the parameters assessed. TSS and TA showed strong negative correlation in both cultivars ($r = -0.98$).

This relationship clearly showed that decrease in fruit titratable acidity may also bring about an increase in TSS during fruit development regardless of fruit maturity stage or season (Tables 5 and 6). Another interesting relationship was the strong positive correlation between juice absorbance and anthocyanin concentration in both cultivars. This suggests that high anthocyanin concentration would contribute to more red colouration of arils, which is a desirable attribute in pomegranate marketing. Considering the reported health benefits of consuming fruit high in phenolic compounds (Gil et al., 2000;

Cultivar	Source	Significance level	
		DPPH	FRAP
'Bhagwa'	Maturity stage (A)	< 0.0001	< 0.0001
	Season (B)	0.0286	0.0004
	*A**B	0.0001	< 0.0001
'Ruby'	Maturity stage (A)	< 0.0001	< 0.0001
	Season (B)	0.0002	0.0031
	*A**B	< 0.0001	< 0.0001

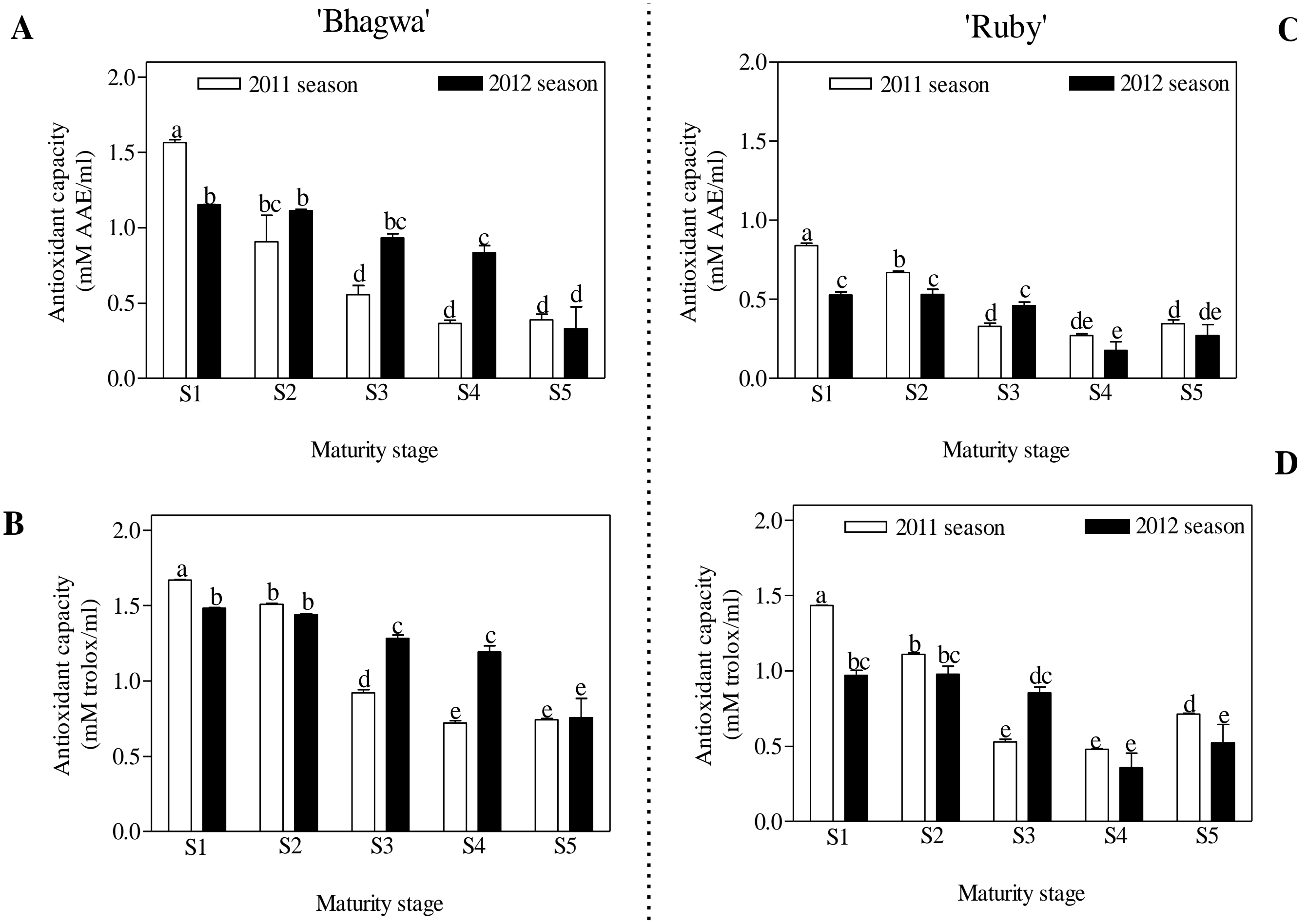

Figure 1. Antioxidant capacity (DPPH and FRAP) of pomegranate juice at major maturity stages over two seasons (2011 and 2012). 'Bhagwa' cultivar: DPPH assay (**A**) and FRAP assay (**B**); 'Ruby' cultivar: DPPH assay (**C**) and FRAP assay (**D**). Different letters on bars mean statistically significant differences (p<0.05) according to Duncan's multiple range test. AAE- Ascorbic acid equivalent.

Tzulker et al., 2007), its therefore not surprising that antioxidant capacity (both DPPH and FRAP) showed a positive correlation with total phenolic concentration but not with the concentration of anthocyanins. Therefore, it is plausible that the changes in the antioxidant capacity of fruit during maturity are largely dependent on the total phenolic concentration. This is supported by evidence from results of the study on 'Wonderful' cultivar grown in Israel (Shwartz et al., 2009).

Principal component analysis

To elucidate the metabolic changes that occur during pomegranate fruit maturation, key maturity indices measured in the two seasons were subjected to principal component analysis (PCA). An Eigen value gives a measure of the significance of the factor; thus the factors with the highest eigenvalues are the most significant and eigenvalues ≥1 are considered significant (Shrestha and

Table 5. Pearson correlation coefficient matrix between chemical indices measured in 'Bhagwa' cultivar during 2011 and 2012 seasons.

S/N	Variable	1	2	3	4	5	6	7	8	9	10	11	12
1	juice abs	**1**											
2	pH	**0.95**	**1**										
3	TSS	**0.93**	0.87	**1**									
4	TA	**-0.89**	-0.79	**-0.98**	**1**								
5	TSS:TA	**0.94**	0.86	**0.99**	**-0.99**	**1**							
6	BrimA	**0.92**	0.86	**1.00**	**-0.99**	**0.99**	**1**						
7	TPC	-0.76	-0.66	**-0.94**	**0.96**	**-0.93**	**-0.95**	**1**					
8	TFC	-0.81	-0.78	**-0.95**	**0.91**	**-0.90**	**-0.94**	**0.95**	**1**				
9	Anthocyanins	**1.00**	**0.92**	**0.94**	**-0.92**	**0.96**	**0.94**	-0.80	-0.83	**1**			
10	GTC	-0.54	-0.48	-0.77	0.75	-0.70	-0.77	**0.89**	**0.92**	-0.57	**1**		
11	FRAP	-0.85	-0.74	**-0.96**	**0.98**	**-0.98**	**-0.97**	**0.96**	**0.88**	**-0.88**	0.74	**1**	
12	DPPH	-0.78	-0.68	**-0.95**	**0.95**	**-0.92**	**-0.95**	**0.99**	**0.97**	-0.82	**0.92**	**0.94**	**1**

Correlation values in bold are significant at $p<0.05$.

Table 6. Pearson correlation coefficient matrix between chemical indices measured in 'Ruby' cultivar during 2011 and 2012 seasons.

S/N	Variable	1	2	3	4	5	6	7	8	9	10	11	12
1	Juice abs	**1**											
2	pH	0.19	**1**										
3	TSS	0.72	-0.05	**1**									
4	TA	-0.78	-0.12	**-0.98**	**1**								
5	TSS:TA	0.76	0.08	**0.99**	**-0.99**	**1**							
6	BrimA	0.72	-0.04	**1.00**	**-0.98**	**0.99**	**1**						
7	TPC	-0.55	0.12	**-0.97**	**0.93**	**-0.95**	**-0.98**	**1**					
8	TFC	-0.46	0.09	**-0.94**	**0.90**	**-0.92**	**-0.94**	**0.99**	**1**				
9	Anthocyanins	**0.97**	0.25	0.84	**-0.90**	**0.88**	0.84	-0.71	-0.65	**1**			
10	GTC	-0.60	0.51	-0.87	0.77	-0.79	-0.86	0.85	0.78	-0.64	**1**		
11	DPPH	-0.60	0.05	**-0.97**	**0.94**	**-0.96**	**-0.97**	**0.98**	**0.96**	-0.74	0.84	**1**	
12	FRAP	-0.48	0.11	**-0.94**	**0.89**	**-0.92**	**-0.95**	**0.99**	**0.98**	-0.64	0.83	**0.99**	**1**

Correlation values in bold are significant at $p<0.05$

Kazama, 2007; Garizi et al., 2011). PCA of the data sets yielded two principal factors (F1 and F2) with Eigen values > 1, explaining more than 80% of the total variance. Acceptable explanations can be drawn from the first factor (F1) which accounted for over 75% of the total variance in both cultivars (Figure 2 A and B). The relationships between the indices were evidenced by short distances between juice absorbance and anthocyanin concentration and between TSS and BrimA. Also, short distances between phenolic groups and antioxidant capacity suggest significant contribution of phenolics to the antioxidant capacity measured by both DPPH and FRAP assays. According to the study of Shwartz et al. (2009), during the early fruit maturity stage, when high acidity and total phenolics prevailed, fruit antioxidant activity was high. However, both concentration of phenolics and antioxidant capacity decreased with advancing fruit maturation. In the present study, the decrease in total phenolics and acidity in immature and unripe fruits, respectively, was also characterized by a shift from right to left, reflecting the beginning of the ripening process in the cultivars between S2 and S3 (Figure 2 A and B).

Close examination of Eigen values, loadings and significant maturity parameters in each cultivar showed that major compositional changes in fruit are developmentally regulated (Table 7). The factor loadings obtained corresponded with the strength of correlation between the original variables and the factors. According to the study of Liu et al. (2003), classification of factor loading is considered 'strong', 'moderate' and 'weak' corresponding to absolute loading values of > 0.75, 0.75 - 0.50 and 0.50 - 0.30, respectively. In both seasons, positive scores of F1 corresponded to immature and unripe fruit (S1 - S2). Ripe and fully ripe fruit at maturity stages S4 and S5 had high negative scores along F1, while fruit at S3 that had low negative scores were semi-ripe for both cultivars (Figure 2 and Table 7). The scores

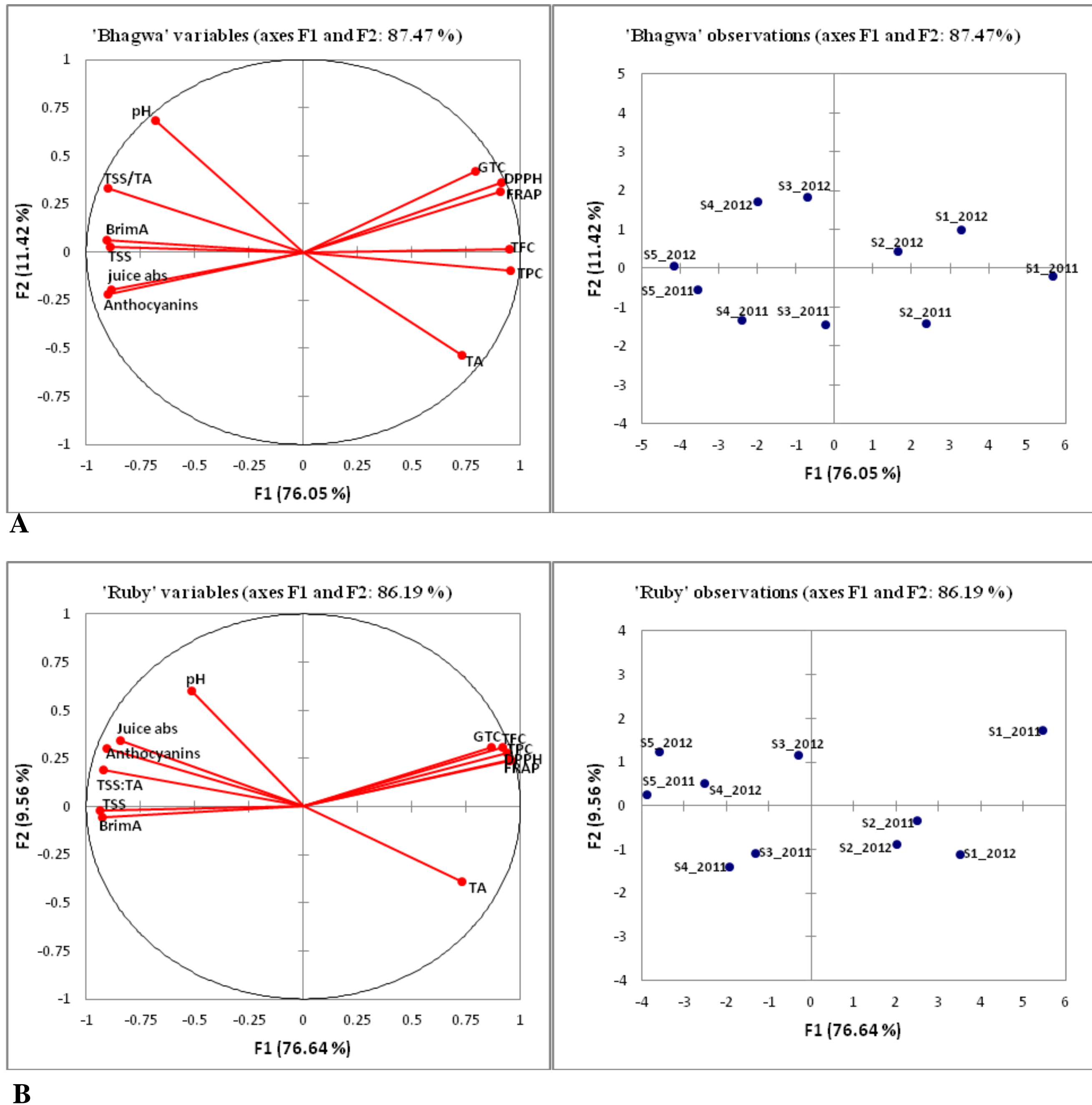

Figure 2. Variables and observations chart using key maturity indices and antioxidant capacity in 2011 and 2012 seasons. 'Bhagwa' cultivar (**A**); 'Ruby' cultivar (**B**).

can be interpreted by the factor loadings (Table 7), with F1 showing strong positive correlations for TA, TPC, TFC, GTC, FRAP and DPPH, and negative loadings for juice absorbance, pH, TSS, TSS, TA, BrimA and anthocyanin concentration. In general, this study showed that young (immature) fruit had higher acidity and phenolic concentrations, while mature but semi- mature to full-ripe fruit had higher anthocyanin concentration and TSS.

Conclusion

Seasonal changes in chemical composition and aroma volatiles as well as antioxidant properties of pomegranate fruit at different maturity stages were investigated during the time course of fruit development and ripening. Results obtained showed that major compositional changes in the fruit are developmentally regulated. Fruit acidity and phenolics declined with advancing maturity,

Table 7. Factor scores, loadings, Eigen values and variance (%) for the first two factors (F1 and F2) based on selected indices and antioxidant capacity in 2011 and 2012 seasons for both cultivars.

Observation	Factor scores			
	'Bhagwa'		'Ruby'	
	F1	F2	F1	F2
S1_2011	5.663	-0.218	5.468	1.717
S2_2011	2.385	-1.436	2.497	-0.340
S3_2011	-0.229	-1.465	-1.316	-1.102
S4_2011	-2.382	-1.325	-1.936	-1.399
S5_2011	-3.543	-0.559	-2.498	0.511
S1_2012	3.299	0.994	3.499	-1.125
S2_2012	1.638	0.446	2.026	-0.893
S3_2012	-0.690	1.814	-0.306	1.161
S4_2012	-1.988	1.704	-3.564	0.244
S5_2012	-4.152	0.044	-3.869	1.227
Loadings				
juice abs	-0.885	-0.194	-0.844	0.344
pH	-0.682	0.684	-0.518	0.604
TSS	-0.892	0.028	-0.928	-0.057
TA	0.730	-0.537	0.731	-0.392
TSS/TA	-0.902	0.333	-0.921	0.189
BrimA	-0.908	0.060	-0.936	-0.021
TPC	0.956	-0.097	0.955	0.241
TFC	0.949	0.015	0.915	0.307
GTC	0.794	0.417	0.904	0.301
Anthocyanins	-0.900	-0.222	0.868	0.307
FRAP	0.906	0.315	-0.904	0.237
DPPH	0.915	0.359	0.938	0.278
Eigenvalue	9.126	1.371	9.196	1.147
Total variance (%)	76.052	11.421	76.636	9.558
Cumulative (%)	76.052	87.473	76.636	86.195

suggesting a decrease in juice sourness, while sugar concentration increased and red colour intensity (anthocyanin) increased in both cultivars. Principal component analysis (PCA) was used to characterize the relationships between the major maturity indicators of fruit. Fruit at advanced maturity stages (S4 and S5) were characterized by higher TSS values as well as intense fruit and aril pigmentation, which coincided with the highest accumulation of anthocyanins.

The interactions between fruit maturity stage and growing season did not influence juice colouration (absorbance) and TA for 'Bhagwa', and juice colouration, TSS, TSS:TA and BrimA for 'Ruby'. However, the effects of fruit maturity and or season were clearly evident. For 'Bhagwa', juice colouration and TSS were not influenced by growing season but rather by fruit maturity stage. Similarly, TSS, TSS:TA and BrimA level in 'Ruby' did not show significant seasonality during fruit maturation.

In combination, the identified maturity indices (absorbance, TSS, TSS:TA and BrimA level) would account for the evolution of juice colour, flavour and taste. The identified maturity indices for each cultivar could aid the search for reliable maturity markers to determine fruit readiness for harvest.

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