Chemical properties, bioactive compounds, organic sugars and acids of harvested pomegranate cv. Wonderful in the Western Cape Province, South Africa

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A pomegranate cultivar (Wonderful) from all three localities in Western Cape province of South Africa during the 2013 season was evaluated for chemical properties. Fruits were analyzed for total soluble solids (TSS) or °Brix, titratable acidity (TA) and juice pH. Chemical properties evaluated were; total phenols, anthocyanin, antioxidant, organic sugars and acids. Results of the study revealed significant variations in all measured parameters attributed to location. Total soluble solids content varied from 16.0 – 17.3 (°Brix), pH values from 2.7 – 3.0, titratable acid content varied from 1.3 – 1.7 and maturity index from 9.7 – 13.4. Anthocyanins, total phenols and antioxidants were 772 – 1134 mg/100ml; 1611 – 1834 mg/100ml and 12.57 – 14.84 mg/100ml, respectively. Organic acids (citric and malic) and organic sugar (fructose, glucose and sucrose) had significant differences, while no differences were shown in Acetic acid across the production areas. Location and cultivar therefore play an important role when assessing chemical properties (TSS, pH, TA, anthocyanin, phenolics and antioxidants) and organic sugar/acids of pomegranate.

Keywords: *Punica granatum*, chemical properties, organic sugar/acid.

Punica granatum (pomegranate) is indigenous to an area of Asia from Himalayas in northern India to Iran (Meerts et al. 2009, 1085-1092) whilst Stover and Mercure (2007, 1088-1092) stated that it is a traditional fruit of the central Iranian plateau where it is thought to have originated. It is also one of the oldest fruit trees to be domesticated (Kumar 1990, 328-347). The ability of pomegranate to adapt to a wide range of temperature regimes has made it possible for it to be grown in the Mediterranean regions of Asia, Africa, America and Europe (Kumar 1990, 328-347; Holland and Bar-Ya´akov 2008, 12-15; Mashavhathakha et al. 2014, 157-164).

Pomegranate fruit quality can be assessed based on external properties such as shape, size and colour (Kader 2006; Holland et al. 2009, 127-191). However, fruit maturity for harvest cannot be assessed by skin colour only (Ben-Arie et al. 1984, 898-902), hence other factors are considered such as aril colour; total soluble solids and acidity to meet market standards (Ben-Arie et al. 1984, 898-902; Kader 2006; Holland et al. 2009, 127-191; Fawole and Opara (2013, 23-31).

The chemical composition of the fruit differs, depending on the cultivar, growing region, climate, maturity, cultural practice and storage conditions (Melgarejo et al. 2000, 185-190; Fadavi et al. 2005, 113-119). The nature and level of the organic acids present in pomegranate may provide information concerning the origin of the fruit, microbiological growth and even processing techniques (Aarabi et al. 2008, 45-55). Significant variations in organic acids, phenolic compounds, sugars, water-soluble vitamins, and minerals of pomegranate have been reported over the years by various authors

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(Aviram et al. 2004, 423-433; Mirdehghan and Rahemi 2007, 120-127; Tezcan et al. 2009, 873-877).

A range of phenolics has been found in pomegranate juice. Galloylglucose, punicalagin, punicalin, ellagic acid and gallic acid are the main active compounds in the juice and husk (Gil et al. 1996, 481-485; Seeram et al. 2006). Phenolics play an important role in the health benefits of moderate juice consumption. They are also responsible for colour, flavour, astringency and bitterness, the levels of which are related to juice quality. Pomegranate juice contains plenty of anthocyanins such as delphinidin 3-glucoside, cyaniding 3-glucoside, pelargonidin 3, 5-diglucoside (Hernandez et al. 1999, 39-42). The main aim of the study was to evaluate chemical properties, bioactive compounds as well as organic sugars and acids of cultivar Wonderful from three locations of the Western Cape Province, South Africa.

Material and methods

Study site

This study was conducted during the harvest season of 2013, with fruits of pomegranate cultivar Wonderful, randomly selected from trees located in Bonnievalle (33°55′39″S 20°6′2″E, 175 m.a.s.l), Ladismith (33°29′S 21°16′E, 544 m.a.s.l), and Calitzdorp (33°32′14.59″S 21°41′6.59″E, 279 m.a.s.l), farms of the Western Cape Province, South Africa (Table 1). Healthy plants with uniformity in truck size were randomly selected for the study. One hundred fruit samples were harvested from each selected growing location. Juice was extracted from the arils using a 600W Mellaware® Model 26300B Liquafresh juice extractor.

Chemical analysis

Total soluble solids (TSS, ºBrix %) were measured using a refractometer (Atago, Tokyo, Japan) at room temperature. Juice pH was measured using a pH meter, while titratable acidity (TA), expressed as milligrams of malic acid per millimetre was measured by titration to an end point of pH 8.2 using CRISON TiCom V1.7 from Spain. Results were expressed as means ± S.E (n= 30).

The method of Fuleki and Francis (1968, 72-77) was followed with minor modifications for the total anthocyanin. The juice samples (50 µL) were pipetted in triplicate into the wells of a 96-well polystyrene microplate, followed by 150 µL of a 0.55 mol L^{-1} HCl solution. The total polyphenol content of the juice sample was determined using the method described by Singleton and Rossi (1965, 144-158) with minor modifications in accordance with Arthur et al. (2011, 581-588). Free radical scavenging activity of the infusions was determined in triplicate using the method of Rangkadilok et al. (2007, 328-336) with slight modifications. Organic sugar and organic acid concentrations in pomegranate juice was analysed using high performance liquid chromatography (HPLC) (Agilent 1100 Series, Waldron, Germany) equipped with a diode array detector (DAD).

Table 1: Climatic conditions at three locations in the Western Cape Province, South Africa

2012/2013	Minimum Temperature (^{0}C)	Maximum Temperature (^{0}C)	Average Rainfall (mm)	Average Heat units
Bonnievalle	12.93	27.33	18.99	273.10
Calitzdorp	13.36	31.08	16.29	347.15
Ladismith	10.99	27.70	26.26	268.15

Statistical Analysis

Analysis of variance (ANOVA) was performed using PROC General Linear Model (GLM) of SAS 9.2. (SAS 2012). Significant differences between means were determined by the Student-t LSD (least significant difference) test. Differences at p<0.05 were considered statistically significant. Results were presented as mean values with the standard deviations in parenthesis.

Results and discussion

Chemical properties

There were significant differences in total soluble solids (TSS) among the three locations, varying from 17.3 (Bonnievalle) compared to 17.2 (Calitzdorp) and 16.0 (Ladismith) (Table 2). This is reinforced by reports of pomegranate TSS, which consists mostly of sugar but varied across regions and cultivars (Al-Said et al. 2009, 129-143; Zaouay and Mars 2011, 151-166; Ferrara et al. 2011, 599-606; Martinez et al. 2012, 100-106). Previous research has shown that rainfall impacts fruit TSS (Mditshwa et al. 2013, 81-90). In this study low rainfall at Bonnievalle and Calitzdorp may be responsible for the high TSS value (Table 1). The low TSS for Ladismith fruit might be attributed to the higher rainfall. Total soluble solids for all the study locations ranged higher than the minimum threshold of 12% generally required for the commercial use for Spanish cultivars (Martinez et al. 2006, 241-246).

The highest pH of fruit was found in Bonnievalle, with a significant value of 3.0 (Table 1). However, there was no significant difference in pH values between Calitzdorp (2.8) and Ladismith (2.7). Legua et al. (2012, 115-119) reported higher pH of 3.9 to 4.1 on six Mollar group pomegranate cultivars in Spain. In Saudi Arabia, Al-Maiman and Ahmad (2002, 437-447) obtained a pH of 3.6 in pomegranate juice for Taifi cultivar while Fadavi et al. (2005, 113-119) in Iran reported pH values ranging between 2.9 to 4.2. The results from this study thus still fall within the similar range from these two previous studies.

There was a significant difference between the titratable acid of the fruit among the three locations from 1.7% (Ladismith) compared to 1.6% (Calitzdorp) and 1.3% (Bonnievalle). Our results are similar to those reported by Shulman et al. (1984, 265-274) for cultivar Wonderful with values not less than 1.5% in coastal plain Bet Shean valley. Chace et al. (1981, 15) also reported a value of 1.8% for cultivar Wonderful. Titratable acidity (TA) is an important quality attribute of pomegranate juice (Shwartz et al. 2009, 965-973). High acidity content in the juice of Wonderful during fruit development was used to classify the fruit as a late cultivar (Shulman et al. 1984, 265-274).

Table 2: Total soluble solids, pH, titratable acidity and maturity index from the three study location

Area	TSS	pH	TA	MI
Bonnievalle	17.33±0.30	3.04±0.20	**1.32±0.12**	13.43±1.21[a]
Calitzdorp	17.22±0.45	**2.75±0.88**	**1.56±0.16**	**10.86±1.83**
Ladismith	**16.01±0.26**	**2.71±0.02**	**1.70±0.14**	**9.69±1.09**

* Means in bold show significant differences at $P < 0.05$

The maturity index (1T1) of fruit from Bonnieville was significantly higher (13.43) than from the other localities. However, there was no significant difference between Calitzdorp (10.9) and Ladismith (9.7). Our results concur with work done in some production regions (5.04 to 46.31), Tehranifar et al. 2010, 180-185; (6.6 to 29.1), Ferrara et al. 2011, 599-606). In contrast, Legua et al. (2012, 115-119) reported higher value of 59.1 to 88.0 in Alicante Province in Spain. Pomegranates are appropriate for the fresh market when their acidity content is lower than 1.8%, with the MI ranging 7 – 12. The fruit becomes more acceptable as MI level goes beyond 12 (Chace et al. 1981, 15).

Anthocyanins are phenolic compounds that contribute to the red, blue, or purple coloration of many fruits and are well known for their antioxidant activity (Tehranifar et al. 2010, 180-185). Anthocyanin showed in significant differences among the three localities (Table 3) with 1134 for Bonnievalle compared to 1009 for Calitzdorp and 772 for Ladismith. Zaouay et al. (2012,81-89) reported total content anthocyanin of 50.5 mg L^{-1} (CH8-2) to 490.4 mg L^{-1} (JR1) while sour cultivars did not reveal high anthocyanin amounts compared to sweet ones.

Phenolics were high in both Calitzdorp (1834.6) and Ladismith (1814.8), while Bonnievalle had the lowest value of 1611.3 (Table 3). The results are lower than those reported by Gil et al. (1996) in Spain; Özgen et al. (2008, 703-706) in Turkey.

Antioxidants in Bonnievalle were significantly lower, with a value of 12.6, than Ladismith and Calitzdorp where no significant differences with values of 14.84 and 13.58, respectively, was observed. Borochov-Neori et al. (2009, 189-195) indicated that antioxidant and quality characteristics of pomegranate fruit are more dependent on cultivar and ripening date, while fruit ripening later in the harvest season contained more soluble phenolics and exhibited higher antioxidant activity.

Table 3: Anthocyanin, phenolics and antioxidant of cultivar Wonderful from the three study location

	Anthocyanins	Phenolics	Antioxidants
Bonnievalle	**1134**	**1611.3**	**12.57**
Calitzdorp	**1009**	1834.6	13.58
Ladismith	**772**	1814.8	14.84

† Means in bold, in the same column, indicate significant differences at $P < 0.05$

Table 4: Organic acids and sugars of cultivar Wonderful from the three study location

	Organic Acids			Organic Sugars		
	Acetic acid	Citric acid	Malic acid	Fructose	Glucose	Sucrose
Bonnievalle	0.048	**16.293**	**0.620**	77.087	68.160	7.607
Calitzdorp	0.030	**23.718**	0.388	77.465	68.403	8.262
Ladismith	0.051	**19.952**	0.468	**63.448**	**55.143**	**6.188**

‡ Means in bold, in the same column, indicate significant differences at $P<0.05$

Organic sugars and acids

The build-up of simple sugars is one of the processes occurring during the final developmental stages of fruit, resulting in increased sweetness as the fruit approaches ripeness (Shwartz et al. 2009, 965-973; Zarei et al. 2011, 121-129). Fructose, glucose and sucrose were individually analysed, as they play an important role in pomegranate quality. Glucose and fructose were the major soluble sugars found in all growing regions (Table 4).

This result is in agreement with previous studies on other pomegranate cultivars (Melgarejo et al. 2000, 185-190; Al-Maiman and Ahmad 2002, 437-447; Shwartz et al. 2009, 965-973; Tezcan et al 2009, 873-877; Mena et al. 2011, 1893-1906). Fructose values in Bonnievalle and Calitzdorp were not significantly different albeit higher than Ladismith with values of 77.1 mg/100 ml and 77.5 mg/100 ml respectively. Ladismith had a significantly lower fructose value of 63.5 mg/100 ml. Glucose revealed the same trend with Bonnievalle, having 68.2 mg/100 ml and Calitzdorp 68.4 mg/100 ml while Ladismith was significantly lower at 55.14 mg/100 ml. Fructose and glucose in particular serve as energy sources and contribute to fruit sweetness. It has been reported that fructose is double as sweet as glucose (Nookaraju et al. 2010, 1-15), and could be used as a measure of juice sweetness during fruit ripening (Al-Maiman and Ahmad 2002, 437-447). Sucrose which was detected in small amounts ranged from 8.3 mg/100 ml in Calitzdorp, 7.6 mg/100 ml in Bonnievalle and 6.2 mg/ ml in Ladismith. Our results concur with those obtained by Al-Maiman and Ahmad (2002, 437-447) in Saudi Arabia where fructose (~53% of total sugars) and glucose (~47% of total sugars) were found to be the dominant sugars among the pomegranate accessions, while sucrose contents were lower. In contrast, the work done by Özgen et al. (2008, 703-706) and Legua et al. (2012, 115-119) reported glucose levels were higher than fructose levels in pomegranate fruits. This difference can be attributed to climatic conditions (Table 1), ripening times and cultivars. The low sucrose concentration in our results may be due to its conversion to reverse sugars such as fructose and glucose during ripening (Hasnaoui et al. 2011, 374-381).

Previous studies suggested that although several organic acids were found in pomegranate aril juices, the major acid accounting for titratable acidity is citric acid (Melgarejo et al. 2000, 185-190). In the present study, the results obtained show that this might also be the case since citric acid was found to be the major organic acid in juice. Organic acid distribution of pomegranate was dominated by citric acid in Calitzdorp (23.7 mg/100 ml), Ladismith (19.3 mg/100 ml) and Bonnievalle (16.3 mg/100 ml). Citric acid contents of between 0.22 g/100 ml and 2.16 g/100 ml have been reported in pomegranates from Turkey and the United States of America, where it was found to be the predominant acid. Different amounts of citric acid levels were reported in Spain (Melgarejo et al. 2000, 185-190), and in Turkey (Poyrazog˘lu et al. 2002; and Özgen et al. 2008, 703-706). Malic acid was found to be the second most dominant acid for the three localities in this study with Bonnievalle at 0.62 mg/100 ml, Ladismith 0.47 mg/100 ml) and Calitzdorp at 0.39 mg/100 ml. While the reported levels of malic acid in literature are 0.135–0.176 mg/100 ml by Melgarejo et al. (2000, 185-190); 0.056 to 0.686 mg/100 mL by Poyrazog˘lu et al. (2002), and 0.09 to 0.15 mg/100 mL by Özgen et al. (2008, 703-706). Acetic acid was also found in small amounts and was not significantly different in all the areas.

Table 5: Pearson (n) correlation matrix

Variables	TSS	pH	TA	Anthocyanin	Phenol	Antioxidants	AceticAcid	CitricAcid	L_MalicAcid	D_Fructose	D_Glucose	Sucrose
TSS	**1**											
pH	**0.458**	**1**										
TA	**-0.678**	**-0.599**	**1**									
Anthocyanin	**0.668**	**0.760**	**-0.515**	**1**								
Phenol	**-0.497**	**-0.506**	**0.680**	**-0.403**	**1**							
Antioxidants	**-0.765**	**-0.579**	**0.642**	**-0.702**	**0.649**	**1**						
AceticAcid	0.065	-0.121	0.207	-0.154	0.092	0.050	**1**					
CitricAcid	-0.246	**-0.406**	0.191	-0.211	**0.417**	0.303	**-0.565**	**1**				
L_MalicAcid	0.244	0.239	-0.319	0.003	**-0.635**	-0.181	0.138	**-0.496**	**1**			
D_Fructose	**0.815**	0.261	**-0.579**	**0.623**	-0.359	**-0.574**	0.031	-0.062	0.067	**1**		
D_Glucose	**0.815**	0.235	**-0.574**	**0.605**	**-0.379**	**-0.578**	0.060	-0.074	0.085	**0.997**	**1**	
Sucrose	**0.603**	**0.505**	**-0.464**	**0.808**	-0.216	**-0.370**	-0.193	0.067	-0.022	**0.679**	**0.654**	**1**

§Values in bold are significantly different from 0 with a significant level of = 0.05

Table 6: Confusion matrix for the estimation sample from cultivar Wonderful in three locations in the Western Cape Province

Area	G1	G2	G3	Total	% correct
G1	8	0	0	8	100.00%
G2	0	12	0	12	100.00%
G3	0	0	10	10	100.00%
Total	8	12	10	30	100.00%

Correlation tests of all measured variables

Pearson correlation tests were used to determine the relationship of all variables measured (Table 5). There was a highly positive correlation between TSS with all the sugars (TSS: Fructose = 0.815; TSS: Glucose = 0.815; TSS: Sucrose = 0.603), while TSS: pH = 0.458 has a weak positive correlation. A positive correlation was also found in TSS: Anthocyanin = 0.668 but TSS: Phenols = -0.497 and TSS: Antioxidant = -0.765 all had negative correlations.

Interestingly, anthocyanin also had a highly positive correlation with measured sugars (Anthocyanin: Fructose = 0.623; Anthocyanin: Sucrose = 0.808). Anthocyanin had a strong negative correlation with antioxidant = -0.702. Antioxidant had a moderate negative correlation with all sugars (Antioxidant: Fructose = -0.574; Antioxidant: Glucose = -0.578 and Antioxidant: Sucrose = -0.370).

Conclusion

The results showed significant differences in TSS, pH, TA and MI attributed to location. Anthocyanins, phenolics and antioxidants were also significantly affected by growing location. These parameters are important for health benefits through juice consumption and are used by food industries for the expansion of juices and various other processed products.

Fructose and glucose in this study were the major soluble sugars in all growing locations, and have an important role in pomegranate quality. In conclusion this study showed that when assessing chemical properties (TSS, pH, TA); bioactive compounds (anthocyanins, phenolics and antioxidants) and organic sugar/acids of pomegranate, it is important to consider location and cultivar as these attributes are directly dependent on the latter.

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