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FULL LENGTH ARTICLE

The physico-chemical characteristics of Morrocan pomegranate and evaluation of the antioxidant activity for their juices☆

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Abstract The characteristics of pomegranate cultivars is important not only to put in evidence the diversity of varieties, but also to meet current market demand for quality fruits. The study was performed on a pomegranate collection from the National Institute for Agricultural Research experimental station, in a semi-arid climate. The aim of the current work was to study the morphological and biochemical characteristics and antioxidant capacity of eighteen pomegranate cultivars grown in Morocco. The results of fruit weight, aril percentage, seed percentage, and skin percentage were between 206.6–506.67 g, 56.45–69.41%, 9.36–18.56%, 22.09–42.60% respectively. This study showed that there were significant variations among the investigated cultivars for all measured parameters except aril length and seed weight. However, the results displayed that the values of total phenolic content varied from 1384.85 to 9476.32 mg GAE/L for local cultivars and from 1284.42 to 8295 mg GAE/L for foreign cultivars. These cultivars showed a high antioxidant capacity as high as 4577.12 ± 29.73 mg L^{-1} juice and correlated with high phenol content. The result of morphological characteristics and chemical properties implies the great potential of pomegranate grown in Morocco for both fresh consumption and fruit processing. Additionally, the 'Grenade

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☆ Novelty statement: In Morocco, pomegranate fruits have taken notable attention for their antioxidant compositions in the last years. Because no previous research achieved in regard to pomological and Biochemical Traits for Moroccan genotypes. These descriptors based on morphological characteristic and biochemical, using polyphenol content, anthocyanin, condensed tannins, hydrolysable tannins and antioxidant activity (DPPH) showed that there were significant disparities among the studied cultivars in all measured parameters except aril length and seed weight. As described in this study, the morphological characteristics, antioxidant capacity and composition of phenolic compounds of pomegranate juices were influenced by the type of cultivar.

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jaune', 'Chioukh' and 'Gordo de Jativa' cultivars are suitable for the production of juices because of its high phenolic compositions.

1. Introduction

The pomegranate tree has been grown in different regions such as the North Africa, Asia, the Middle East and the Mediterranean areas (Sarkhosh et al., 2006). The medicinal properties of this fruit, especially in traditional use, are known from time immemorial (Roy and Waskar, 1997). Morocco is among the main pomegranate producer countries. However, the pomegranate cultivation occupies an area of 5000 ha and provides an average yearly harvest of 58,000 tons fruit. Pomegranate arils are consumed as fresh fruit, but there are great efforts by industrial companies to convert a part of this production to juice. The consumption of processed pomegranate in Morocco has been increasing in the last ten years. However, there is not much work on the study of the antioxidant activity of pomegranate juice of Morocco. The major antioxidant activity of pomegranate juice was attributed to punicalagin, contained in the skin of fruit (Fuhrman et al., 2005). The commercial was prepared by pressing the whole fruit, which allows extraction of a large amount of bioactive compounds; therefore, commercial juices are reputed to have high antioxidant capacity.

Pomegranate has become more popular for the attribution of essential to human health such as anticancer (Afaq et al., 2005), HIV-I entry inhibitory, cardioprotective (Sumner et al., 2005), and improve quality sperm (Türk et al., 2008). The edible parts of pomegranate fruit contain large amounts of organic acids, sugars, minerals, vitamins and polyphénols (Gil et al., 2000; Tehranifar et al., 2010).

In this context, the purpose of this study was to determine the physico-chemical characteristics and evaluate the antioxidant activity for 18 cultivars grown in Morocco in order to gain more knowledge about the pomegranate.

2. Materials and methods

2.1. Sample preparation

The study was performed in a pomegranate (*Punica granatum* L.) collection with 18 cultivars planted in 1996 (Table 1) at the INRA (National Institute for Agricultural Research) Experimental Station, Meknes-Morocco (altitude 500 m), which has a semi-arid climate. There is about 400 mm of rainfall per year. The soil is calcareous with a high percentage of clay. Trees are planted at 5 × 3 m spacing and irrigated at 3500 m^3 per year supplied from May to October. The cultivars are cultivated under the same geographic conditions. However, no treatment is applied except removing old branches.

Twenty fruits of each cultivars were harvested at maturity in the beginning of October. All the cultivars studied had a similar maturity date, starting from the end of September to the beginning of October, this period induced by the staggered flowering of the tree.

Five fruits were harvested randomly from each of the four geographic orientations of the tree. The fruit was picked when all greenness had disappeared from fruit peel surface. Then, they were immediately taken to the laboratory for analysis. The fruits were peeled and the skins covering arils were eliminated manually. Then, the juice was obtained from pomegranate arils by mechanical press, and was kept at (−20 °C) until achievement of assay. Three repeats were maintained for each analysis.

2.2. Chemicals and reagents

Potassium iodate, Folin–Ciocalteu reagent, sodium carbonate, Folin Denis reagent, potassium chloride, sodium acetate, and methanol were purchased from R&M Chemicals (Essex, UK). 1,1-Diphenyl-2-picrylhydrazyl (DPPH) was from the Fluka company (Switzerland). Trolox (6-hydroxy-2,5,7,8-tetra methylchroman-2-carboxylic acid), which is a hydrophilic analogue of vitamin E, Gallic and tannic acid were purchased from Sigma–Aldrich (St. Louis, MO, USA).

2.3. Physical characteristics

For each cultivar, twenty fruits were analyzed for physical characteristics. Fruits were weighted by using a balance of exactitude of 0.001 g. The length and diameter of the fruit and calyx were determined with a digital vernier caliper. The fruit length was performed as the polar axis, between the apex and the end of stem. The fruit diameter is defined as the maximum width of the fruit, and it was measured in the direction perpendicular to the polar axis. Then, the arils were extracted

Table 1 Origins geographic of eighteen pomegranate cultivars collected at the experimental station (National Institute for Agricultural Research) of Meknes-Morocco.

	Code	Name of variety	Origins geographic
Local	L1	Grenade jaune	Morocco
	L2	Grenade rouge	
	L3	Chioukhi	
	L4	Ounk Hmam	
	L5	Gjebali	
	L6	Djeibi	
	L7	Chelfi	
	L8	Bzou	
	L9	Sefri	
	L10	Sefri2	
Foreign	F1	Gordo de Jativa	Spain
	F2	Negro Monstrioso	Spain
	F3	Wonderful	USA
	F4	Ruby	USA
	F5	Dwarf semi Evergreen	USA
	F6	Mollar Osin Hueso	China
	F7	Zherie précoce	Tunisia
	F8	Zherie d'Automne	Tunisia

from the fruits manually, and total arils, seed and skin per fruit were measured separately. The determination of the skin thickness, the size of the 25 arils and seed were evaluated employing the digital vernier caliper. Finally, a panel of 6 expert tasters evaluated the hardness of the seeds, quality valuation, taste and visual color. Seed hardness was determined on a scale from 1 to 3 in increasing order of hardness, and the quality was evaluated according to the scale: poor, acceptable, good and excellent.

2.4. Juice yield, Titrable acidity, pH and total soluble solids

The titrable acidity (TA) was determined by titration to pH 8.1 with 0.1 M NaOH solution and expressed as g of citric acid per 100 ml of juice. The pH measurements were performed using a digital pH meter (Thermo Orion 3 star) at 21 °C. The total soluble solids (TSS) were determined with a digital refractometer (Mettler-Toledo Gmbh, 30 PX, Switzerland, calibrated using distilled water). Results were reported as °Brix at 21 °C. The yield of juice, was obtained from extraction of juice from the five fruits of each cultivar taken random and expressed as volume of juice per 100 g de fruits.

2.5. Total phenolic content (TPC)

The determination of total phenolic was based on Folin–Ciocalteu method (Singleton et al., 1965). Briefly, 300 μL of juice diluted at 1:20 with methanol:water (6:4) was mixed with 1.2 mL of 7.5% sodium carbonate and 1.5 mL of Folin–Ciocalteu reagent diluted in the ratio of 1:10. The absorbance was measured at 760 nm, by a UV–Visible spectrophotometer (SAFAS UV mc1, Monaco), after 90 min of the reaction at room temperature. The results were presented as mg gallic acid equivalent in liters of juice.

2.6. Total anthocyanin content (TAC)

The total anthocyanin content (TAC) was evaluated by applying by the method described by Ozgen et al. (2008) using two buffer systems: sodium acetate buffer pH 4.5 (0.4 M) and potassium chloride buffer pH 1.0 (25 mM). Briefly, 0.4 mL of pomegranate juice was mixed with 3.6 mL of corresponding buffers and the absorbance was determinated at 510 and 700 nm. The water was used as a blank. The Absorbance (A) was expressed as follows:

$$A = (A_{520\ nm} - A_{700\ nm})pH_{1.0} - (A_{520\ nm} - A_{700\ nm})pH_{4.5}$$

TAC of juice was presented as mg cyanidin-3-glucoside L^{-1} of PJ and was determinated using equation below:

$$TAC = [A \times MW \times DF \times 100] \times 1/MA$$

with: A: absorbance; MW: molecular weight (449.2 g moL^{-1}); DF: dilution factor (10); MA: molar absorptivity of cyanidin-3-glucoside (26.900).

2.7. Hydrolyzable tannins (HT)

The estimation of hydrolyzable tannins was assessed according to method of Willis and Allen (1998). Briefly, 1 mL of diluted juice in the ratio of 1:10 and 5 mL of 2.5% KIO_3 were introduced into a vial and vortexed for 10 s. After 2 min for diluted juice and 4 min for standard solutions, then the absorbance was measured at 550 nm. The water was used as a blank. Then, the standard solutions were prepared from diverse concentrations of tannic acid solutions (500–2000 mg/L). The results were presented as mg tannic acid equivalent (TAE) per liter of juice.

2.8. Condensed tannins (CT)

The condensed tannins were evaluated by the colorimetric assay Folin Denis mentioned by Joslyn (1970). Reducing phosphomolybdic acid and tungstic in alkaline medium with the presence of tannins gives a blue color whose intensity is measured at 760 nm. Briefly, 10 mL of saturated solution of CO_3-Na_2, 75 mL of water, 1 mL of 1/20 diluted juice, and 5 mL of Folin Denis reagent were mixed in the flask of 100 mL.

This saturated solution is prepared from 43.75 g of sodium carbonate dissolved in 100 mL of hot water (70–80 °C). After cooling, the solution was filtered and then adjusted to 125 mL. After 30 min, the density of the mixture was at 760 nm. However, for calibrations the diverse concentrations of tannic acid solutions (0–0.1 g/L) were used. The results were presented as mg tannic acid equivalent (TAE) per liter of juice.

2.9. Antioxidant activity by DPPH assay

The antioxidant activity of the pomegranate juice was achieved by method of Brand-Williams et al. (1995). Briefly, 100 μL of juice diluted at 1:100 with methanol:water (6:4) and 2 mL of 0.1 mM DPPH in methanol were mixed. After 30 min of reaction, the absorbance was determined at 517 nm. For the background correction, the mixture was prepared without DPPH. The results were expressed as efficient concentration (EC50) and antioxidant activity (AA). The antioxidant activity was determined according the equation mentioned below:

$$\text{Antioxidant activity\%} = [1 - (\text{Abs sample 517 nm}/\text{Abs control 517 nm})] \times 100$$

2.10. Statistical analysis

The data were treated by Statistical Analysis System (SAS) software 917 SAS Institute Cay N.C. (USA) using analysis of variance (ANOVA) and differences between means were resolved for significance at $P < 0.05$ using the PROC GLM procedure.

3. Results

3.1. Physical characteristics

The physical aspect of eighteen pomegranate fruits investigated is presented in Table 2. Significant variations ($P < 0.05$) were showed for the all analyses.

The average fruit weight ranged between 206.6 g 'Chelfi' (L7) and 506.67 g 'Sefri 2' (L10) (Table 2). Thus, in this study the fruit length values were 58.93 'Chelfi' (L7) and 86.07 mm 'Sefri 2' (L10), fruit diameter 72.13 'Chelfi' (L7) and 96.33 mm 'Sefri 2' (L10), calyx length 12.40 'Wonderful' (F3) and 21.93 mm 'Ounk Hmam' (L4), calyx diameter 16.81

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Table 2 Morphological characteristics of eighteen pomegranate fruits.

Cultivars	FW (g)	FD (mm)	FL (mm)	CD (mm)	CL (mm)	SkT (mm)	SkP (%)	AP (%)	SP (%)
L1	466.00 ± 86.12^{ab}	93.33 ± 7.740^{a}	80.73 ± 6.23^{de}	20.80 ± 2.11 ^{bcd}	13.20 ± 3.65^{de}	2.33 ± 0.56^{ab}	27.89 ± 6.48^{bcde}	59.72 ± 4.30^{bc}	18.56 ± 2.73^{a}
L2	317.66 ± 65.46^{de}	83.60 ± 5.91^{c}	73.46 ± 5.71^{fg}	19.66 ± 2.92^{cde}	16.53 ± 2.42^{cde}	1.83 ± 0.59^{bc}	33.57 ± 9.96^{bcde}	56.45 ± 9.60^{cd}	13.15 ± 1.93^{bcd}
L3	359.00 ± 76.98^{cd}	85.41 ± 6.62^{bc}	74.26 ± 4.91^{ef}	21.73 ± 1.29^{bc}	17.26 ± 3.97^{bcde}	2.16 ± 0.51^{ab}	33.37 ± 5.37^{bcde}	68.65 ± 9.41^{a}	13.76 ± 5.33^{bc}
L4	392.66 ± 52.12^{c}	88.80 ± 6.09^{b}	75.86 ± 5.39^{fg}	26.33 ± 4.11^{a}	21.93 ± 5.45^{bcde}	2.51 ± 0.93^{ab}	29.79 ± 8.26^{bcde}	59.71 ± 6.43^{bcd}	12.68 ± 3.95^{bcd}
L5	237.00 ± 29.02^{g}	75.86 ± 3.96^{ef}	61.93 ± 3.13^{fg}	19.46 ± 1.77^{cdef}	14.8 ± 2.43^{cde}	2.00 ± 0.6^{abc}	27.20 ± 4.70^{f}	64.16 ± 3.77^{cd}	16.61 ± 1.56^{ab}
L6	300.66 ± 52.91^{e}	80.80 ± 4.93^{cd}	71.80 ± 5.02^{i}	17.93 ± 2.91^{efg}	13.40 ± 2.06^{de}	1.91 ± 0.20^{bc}	22.09 ± 3.42^{f}	63.46 ± 8.73^{d}	15.21 ± 3.45^{bc}
L7	206.60 ± 54.02^{g}	72.13 ± 6.73^{f}	58.93 ± 5.48^{i}	18.73 ± 2.02^{defg}	14.80 ± 2.78^{cde}	2.70 ± 0.86^{bc}	36.30 ± 7.15^{f}	61.15 ± 3.47^{d}	16.17 ± 1.91^{ab}
L8	394.33 ± 83.04^{c}	88.53 ± 6.28^{b}	78.53 ± 5.21^{ef}	18.61 ± 2.67^{defg}	15.13 ± 2.72^{cde}	2.05 ± 0.42^{ab}	30.12 ± 6.05^{ef}	69.41 ± 1.60^{bcd}	16.48 ± 4.42^{bc}
L9	446.01 ± 77.67^{b}	95.21 ± 7.78^{a}	82.82 ± 4.97^{cd}	25.66 ± 5.26^{a}	18.62 ± 6.02^{cde}	2.65 ± 0.36^{abc}	42.60 ± 7.86^{bcde}	61.52 ± 2.62^{bc}	11.26 ± 1.37^{bcd}
L10	506.66 ± 91.45^{a}	96.33 ± 4.61^{a}	86.06 ± 4.01^{bc}	20.33 ± 2.41^{bcde}	14.00 ± 3.30^{cde}	2.05 ± 0.51^{abc}	32.40 ± 5.14^{bcde}	67.57 ± 7.51^{d}	9.45 ± 2.16^{cd}
F1	222.66 ± 42.59^{g}	74.53 ± 4.64^{ef}	62.8 ± 5.43^{h}	18.00 ± 3.98^{efg}	14.80 ± 3.23^{cde}	1.24 ± 0.24^{c}	31.43 ± 5.27^{f}	65.51 ± 7.80^{cd}	10.45 ± 5.61^{cd}
F2	309.02 ± 62.85^{de}	80.81 ± 5.70^{cd}	70.66 ± 4.85^{ef}	16.81 ± 2.24^{g}	14.66 ± 3.75^{cde}	2.79 ± 1.20^{a}	30.75 ± 6.91^{bcdef}	64.92 ± 6.28^{bcd}	15.19 ± 3.37^{bc}
F3	314.00 ± 80.65^{de}	83.86 ± 6.81^{c}	74.26 ± 6.94^{ef}	17.06 ± 2.81^{fg}	12.40 ± 7.60^{e}	2.25 ± 0.20^{ab}	28.13 ± 3.85^{cdef}	63.73 ± 2.89^{d}	14.92 ± 1.73^{bc}
F4	331.66 ± 41.09^{de}	84.86 ± 4.43^{bc}	76 ± 4.46^{ih}	20.86 ± 3.42^{bcd}	15.60 ± 3.62^{bcde}	2.79 ± 0.71^{a}	35.50 ± 7.05^{bcde}	66.30 ± 12.44^{cd}	11.97 ± 0.42^{bcd}
F5	311.33 ± 30.85^{de}	81.66 ± 3.29^{cd}	73.6 ± 4.19^{g}	22.40 ± 3.87^{b}	16.53 ± 3.16^{bcde}	2.29 ± 0.41^{ab}	26.84 ± 5.54^{def}	58.56 ± 4.27^{d}	9.36 ± 1.48^{cd}
F6	308.66 ± 49.84^{de}	83.86 ± 5.21^{c}	71.80 ± 3.55^{bc}	18.02 ± 2.86^{efg}	13.86 ± 3.29^{de}	1.96 ± 0.46^{abc}	32.07 ± 8.81^{bcdef}	67.04 ± 9.43^{bcd}	15.78 ± 2.57^{ab}
F7	289.33 ± 28.15^{ef}	83.80 ± 2.57^{c}	68.93 ± 3.35^{bc}	20.73 ± 2.19^{bcd}	13.40 ± 2.67^{de}	2.08 ± 0.69^{abc}	22.21 ± 2.71^{f}	61.74 ± 4.04^{d}	13.23 ± 2.35^{bc}
F8	243.66 ± 22.79^{fg}	78.46 ± 3.51^{ed}	64.80 ± 3.17^{ef}	18.93 ± 1.94^{defg}	15.53 ± 3.34^{bcde}	2.49 ± 0.73^{ab}	28.26 ± 7.49^{f}	62.06 ± 9.81^{bcd}	13.97 ± 2.72^{bc}

All data were expressed as means ± SD. The same letter (a–h) indicates no significant difference at the 95% confidence level. FW: Fruit weight; FD: Fruit diameter; FL: Fruit length; CD: Calyx diameter; CL: Calyx length; SkT: Skin thickness; SkT: Skin percentage; AP: Aril percentage; SP: Seed percentage; L1: Grenade jaune; L2: Grenade rouge; L3: Chioukhi; L4: Ounk Hmam; L5: Gjebali; L6: Djeibi; L7: Chelfi; L8: Bzou; L9: Sefri; L10: Sefri 2; F1: Gordo de Jativa; F2: Negro Monstrioso; F3: Wonderful; F4: Ruby; F5: Dwarf semi Evergreen; F6: Mollar Osin Hueso; F7: Zherie précoce; F8: Zherie d'Automne.

'Negro Monstrioso' (F2) and 26.33 mm 'Ounk Hmam' (L4) (Table 2).

The skin thickness varies between 1.24 for the 'Gordo de Jativa' cultivars (F1) and 2.79 mm for 'Ruby'(F4). Martinez et al. (2012) found the values between 3.13 and 5.36 mm. Similarly, our values were lower than values (2.4 and 6.9 mm) observed by Tehranifar et al. (2010).

As presented in Table 2, the skin percentage, aril percentage and seed percentage have important variation, ranged between 22.09%'Djeibi' (L6) – 42.60%'Sefri' (L9), 56.45% 'Grenade rouge'(L2) – 69.41% 'Bzou'(L8) and 9.36 'Dwarf semi Evergreen'(F5) – 18.56% 'Grenade jaune' (L1) respectively.

The arils are composed of pulp, juice, and seed, with high composition of crud fiber and other compounds such as sugars and polyphenols. The parameters produced on the arils can be interesting for farmers and industrial. The physical aspect of arils and seeds is described in Table 3. However, the weight, length, and diameter of arils were within the range of 0.508 'Sefri' (L9) – 0.209 'Zherie précoce' (F7); 12.42 'Zherie précoce' (F7) – 9.46 'Gjebali' (L5); 8.25 'Sefri' (L9) – 5.91 'Ounk Hmam' (L4), respectively.

While for the seed, the weight, length, and diameter ranged from 0.027 'Gjebali'(L5) – 0.055 g 'Sefri'(L9); 5.983 'Gjebali'(L5) – 7.772 mm 'Ruby'(F4); 2.183 'Chelfi'(L7) – 2.903 mm 'Mollar Osin Hueso' (F6), respectively.

Table 4 lists the evaluation of the following sensory analysis: hardness, taste and color; however, the flavor and hardness of the seeds have a strong influence on the eating quality of the fruit, and the following cultivars (Granade jaune (L1), Granade rouge (L2), Gjebali (L5), Djeibi (L6), Chelfi (L7), Bzou (L8), Sefri (L9), Sefri2 (L10), Gordo Jativa (F1), Zherie précoce (F7) and Zherie d'automne (F8)) have a good or excellent quality and must be intended for consumption fresh, because they have a sweet or slightly sweet taste, and their seeds are

Table 3 Mean values of morphological parameters of arils and seeds.

Cultivars	AW (g)	AL (mm)	AD (mm)	SW (g)	SL (mm)	SD (mm)
L1	0.344 ± 0.045[gh]	10.876 ± 0.776[b]	6.746 ± 0.834[fg]	0.045 ± 0.008[b]	7.540 ± 0.738[a]	2.345 ± 0.296[hi]
L2	0.383 ± 0.041[ef]	10.492 ± 0.711[b]	7.274 ± 0.628[de]	0.038 ± 0.013[b]	6.491 ± 0.491[de]	2.612 ± 0.317[defg]
L3	0.477 ± 0.048[c]	11.499 ± 0.793[b]	7.535 ± 0.755[cd]	0.045 ± 0.056[b]	7.014 ± 0.701[bc]	2.543 ± 0.350[efgh]
L4	0.261 ± 0.025[m]	9.649 ± 0.907[a]	5.913 ± 0.745[i]	0.035 ± 0.006[b]	6.918 ± 0.806[cd]	2.501 ± 0.415[bcde]
L5	0.284 ± 0.033[kl]	9.461 ± 0.629[b]	6.553 ± 0.492[gh]	0.027 ± 0.003[b]	5.983 ± 0.586[f]	2.193 ± 0.378[i]
L6	0.385 ± 0.088[e]	10.708 ± 2.043[b]	6.676 ± 1.321[fgh]	0.039 ± 0.009[b]	6.784 ± 1.351[cd]	2.445 ± 0.509[hi]
L7	0.267 ± 0.034[lm]	9.566 ± 0.594[b]	6.306 ± 0.567[hi]	0.031 ± 0.005[b]	6.151 ± 0.299[ef]	2.183 ± 0.282[efg]
L8	0.363 ± 0.027[efg]	10.613 ± 0.497[b]	7.033 ± 0.683[ef]	0.043 ± 0.007[b]	7.016 ± 0.404[bc]	2.673 ± 0.336[bcde]
L9	0.508 ± 0.037[a]	11.953 ± 0.671[b]	8.256 ± 0.777[a]	0.055 ± 0.011[a]	7.313 ± 0.544[ab]	2.871 ± 0.493[a]
L10	0.401 ± 0.043[b]	11.201 ± 0.659[b]	7.006 ± 0.875[b]	0.039 ± 0.004[b]	7.269 ± 0.706[ab]	2.581 ± 0.293[bc]
F1	0.356 ± 0.068[fgh]	11.304 ± 0.409[b]	7.082 ± 0.979[ef]	0.035 ± 0.006[b]	6.937 ± 0.737[bc]	2.400 ± 0.249[fghi]
F2	0.313 ± 0.035[ij]	10.599 ± 0.743[b]	6.649 ± 0.646[fgh]	0.049 ± 0.073[b]	7.061 ± 0.422[cd]	2.701 ± 0.265[efg]
F3	0.305 ± 0.031[jk]	10.101 ± 0.646[b]	6.661 ± 0.592[fgh]	0.035 ± 0.004[b]	6.726 ± 0.638[cd]	2.551 ± 0.388[efgh]
F4	0.368 ± 0.039[efg]	10.913 ± 0.691[b]	7.601 ± 0.561[cd]	0.048 ± 0.057[b]	6.772 ± 0.681[cd]	2.831 ± 0.424[bcd]
F5	0.335 ± 0.027[hi]	10.381 ± 0.663[b]	7.085 ± 0.877[ef]	0.042 ± 0.011[b]	6.832 ± 0.622[cd]	2.705 ± 0.475[hi]
F6	0.451 ± 0.045[d]	11.691 ± 0.824[b]	7.932 ± 0.811[bc]	0.037 ± 0.006[b]	7.434 ± 0.643[a]	2.903 ± 0.491[b]
F7	0.209 ± 0.117[n]	12.42 ± 0.767[b]	8.102 ± 0.983[b]	0.032 ± 0.006[b]	7.312 ± 0.602[ab]	2.380 ± 0.369[ghi]
F8	0.464 ± 0.064[cd]	11.912 ± 0.987[b]	7.661 ± 0.785[cd]	0.028 ± 0.006[b]	6.676 ± 0.951[cd]	2.286 ± 0.349[i]

All data were expressed as means ± SD. The same letter (a–h) indicates no significant difference at the 95% confidence level. AW: Aril weight; AL: Aril length; AD: Aril diameter; SW: Seed weight; SL: Seed length; SD: Seed diameter; L1: Grenade jaune; L2: Grenade rouge; L3: Chioukhi; L4: Ounk Hmam; L5: Gjebali; L6: Djeibi; L7: Chelfi; L8: Bzou; L9: Sefri; L10: Sefri 2; F1: Gordo de Jativa; F2: Negro Monstrioso; F3: Wonderful; F4: Ruby; F5: Dwarf semi Evergreen; F6: Mollar Osin Hueso; F7: Zherie précoce; F8: Zherie d'automne.

Table 4 Evaluation of characteristics by sensory panel.

Cultivars	Seed hardness	Aril color	Taste	Sensorial test
L1	1	Pink-Red	Sweet	Tendre
L2	1	Pink-Red	Sweet	Tendre
L3	2	Pink	Sour-Sweet	Semi dure
L4	1	Red	Sour-Sweet	Tendre
L5	2	Red	Sweet	Semi dure
L6	1	Pink	Sweet	Tendre
L7	3	Pink	Sweet	Dure
L8	1	Red	Sweet	Tendre
L9	1	Pink-Red	Sweet	Tendre
L10	1	Red	Sour-Sweet	Tendre
F1	3	Red	Sweet	Dure
F2	3	Dark red	Sour	Dure
F3	3	Dark red	Sour	Dure
F4	3	Red	Sour	Dure
F5	3	Dark red	Sour	Dure
F6	2	Red	Sour-Sweet	Semi dure
F7	1	Pink	Sweet	Tendre
F8	2	Pink	Sweet	Semi dure

tender or semi-hard. The fruits of other cultivars should be destined for processing.

As regards seed color, it varies considerably between all cultivars year ranging from pink to dark red. However cultivars (Ounk Hmam (L4), Gjebali (L5), Bzou (L8) Sefri2 (L10), Gordo Jativa (F1), Negro Monstrioso (F2), Wonderful (F3), Ruby (F4), Dwarf semi Evergreen (F5) and Mollar Osin Hueso (F6)) having red or dark red seeds can be used as a potential source of dietary or cosmetic colorants. This red coloring is due to the composition in anthocyanin pigments, which in addition to their coloring properties, there is significant antioxidant activity (Gil et al., 2000).

3.2. Juice yield, Titrable acidity, pH and Ytotal soluble solids

The results for Juice yield, Titrable acidity (TA), pH and total soluble solids (TSS) from the different cultivars are presented in Table 5. Significant differences ($P < 0.05$) were revealed among the pomegranate cultivars for all parameters.

The juice percentage varied from 29.73% L1 to 54.42% F1, which agree with the results 26.95–46.55% reported by Tehranifar et al. (2010). The greatest volume of juice per 100 g of fruits was observed for the cultivars F1, F2, F6, L6 and L9. These cultivars can be interesting for production of industry juice. The highest total soluble solid content was in L10 17.07 °Brix and the lowest was in L2 12.33 °Brix. Our results were lower than values observed 16–19 °Brix by Poyrazoglu et al. (2002), while our results were in agreement with values 10–16.5 °Brix reported by Fadavi et al. (2005). The pH values ranged between 2.85 L4 and 4.22 L8. The pH values obtained in the current study are greater than those reported by Tehranifar et al. (2010) on pomegranate cultivars grown in Iran (3.16–4.09). The titrable acidity content varied from 0.19 L4 to 2.31 g/100 ml F2. Similar results were also reported by Fadavi et al. (2005), whereas the values reported by Legua et al. (2012) for ten cultivars grown in different regions of Morocco are relatively higher than the results obtained in this work (0.24–3.7 g/L). According to the results, cultivar type plays an important role in terms of their total soluble solids, pH and titrable acidity of the pomegranate juice.

3.3. Total phenolic (TP) and total anthocyanin content (TAC)

The results for TP and TAC of eighteen PJ are displayed in Fig. 1. A significant diversity in TP composition was showed between the 18 PJ and the data were included among 1385

Table 5 Chemical analysis of the juice from the pomegranate cultivars grown in Morocco.

Cultivars	JP (%)	TA (g/100ml)	pH	TSS (°Brix)
L1	29.73 ± 6.31^{cd}	0.36 ± 0.03^{ef}	3.65 ± 0.17^{abc}	17.00 ± 0.45^{a}
L2	30.08 ± 2.87^{d}	0.48 ± 0.02^{ef}	3.54 ± 0.15^{de}	12.33 ± 0.12^{i}
L3	39.64 ± 6.11^{ab}	0.27 ± 0.02^{ef}	3.18 ± 0.08^{de}	16.00 ± 0.21^{b}
L4	38.73 ± 4.84^{bcd}	0.21 ± 0.02^{f}	2.85 ± 0.09^{h}	15.40 ± 0.22^{cd}
L5	44.28 ± 2.71^{ab}	0.42 ± 0.10^{ef}	4.17 ± 0.32^{bc}	16.13 ± 0.12^{fg}
L6	46.07 ± 3.16^{b}	0.35 ± 0.31^{b}	3.66 ± 0.12^{fgh}	15.00 ± 0.34^{e}
L7	38.09 ± 3.11^{bcd}	0.38 ± 0.26^{c}	4.01 ± 0.51^{h}	16.20 ± 0.21^{b}
L8	43.71 ± 7.74^{ab}	0.67 ± 0.06^{c}	4.22 ± 0.30^{a}	15.26 ± 0.31^{cde}
L9	32.85 ± 3.35^{bcd}	0.22 ± 0.03^{e}	3.38 ± 0.14^{cd}	16.80 ± 0.12^{g}
L10	45.97 ± 9.29^{ab}	0.26 ± 0.08^{a}	3.12 ± 0.12^{de}	17.07 ± 0.31^{fg}
F1	54.42 ± 8.45^{a}	0.19 ± 0.02^{f}	3.83 ± 0.06^{ab}	14.40 ± 0.20^{f}
F2	45.87 ± 8.23^{b}	2.31 ± 0.28^{d}	3.18 ± 0.15^{de}	15.50 ± 0.52^{c}
F3	39.98 ± 2.07^{bc}	0.46 ± 0.02^{ef}	3.04 ± 0.17^{ef}	16.06 ± 0.12^{b}
F4	42.51 ± 5.51^{b}	0.32 ± 0.02^{ef}	3.71 ± 0.07^{ab}	15.06 ± 0.31^{de}
F5	42.41 ± 4.73^{b}	0.24 ± 0.04^{ef}	3.18 ± 0.20^{efg}	15.06 ± 0.12^{de}
F6	47.51 ± 8.73^{ab}	0.34 ± 0.02^{ef}	3.73 ± 0.16^{de}	16.06 ± 0.12^{b}
F7	39.79 ± 4.08^{bc}	0.25 ± 0.05^{ef}	3.48 ± 0.10^{h}	14.60 ± 0.50^{h}
F8	43.44 ± 2.88^{ab}	0.46 ± 0.13^{b}	3.69 ± 0.21^{gh}	14.26 ± 0.31^{fg}

JP: juice percentage (Volume of juice/Weight of fruit), TA: titrable acidity, TSS: total soluble solids. Values within rows uncommon superscripts (a–h) were significantly different ($P < 0.05$). All values are reported as ± standard deviation.

and 9476 mg GAE/L of local cultivars and foreign cultivars were between 1284 and 8295 mg GAE/L. The hierarchy for the values observed was L1 > L3 > F1 > F5 > L 4 > F4 > F2 > L8 > L7 > F3 > L10 > L2 > L9 > L6 > F6 > F7 > L5 > F8. The highest composition of TP was observed for the cultivars L1 (9476 ± 102 mg/L), L3 (8805 ± 65 mg/L), and F1 (8295 ± 127 mg/L).

As shown in Fig. 1B a high diversity of TAC was found among the cultivars. TAC of local clones varied from 64.16 (L3) to 188.7 mg/L (L4) and of foreign cultivars ranged between 56.58 (F1) and 178.79 mg/L (F3). The hierarchy for the values observed was L4 > F3 > L6 > L1 > L1 0 > L9 > L8 > L2 > F6 > F5 > L5 > F2 > F7 > L 3 > L7 > F8 > F4 > F1.

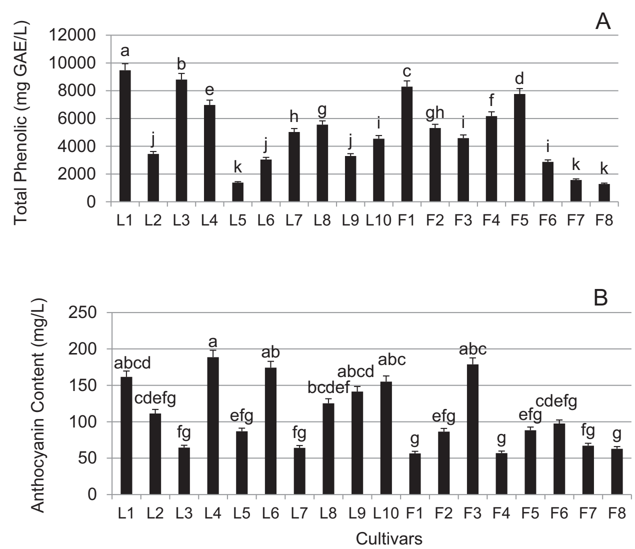

Figure 1 Total Phenolic content (A) and total anthocyanin content (B) of eighteen pomegranate juices. The data presented represent the mean ± standard error of three replicates from accession. ANOVA was used to determine statistically significant difference at ($P < 0.05$) as identified by different letters.

3.4. Hydrolyzable tannins and condensed tannins

The results for hydrolyzable and condensed tannins of the pomegranate juices are presented in Fig. 2. As displayed in Fig. 2, a high diversity of hydrolyzable tannins content was showed between the local cultivars (216.3 and 730.8 mg/L) and for Foreign cultivars among 216.3 and 730.8 mg/L, while the condensed tannin content is almost the same between local cultivars (251.9–644.7 mg/L) and foreign (296.1 and 648.5 mg/L). The variabilities were statistically significant at ($P < 0.05$). Indirectly, these data are very interesting, because Filippich et al. (1991) reported that the intestine can absorb the pomegranate tannins. In fact it has been showed that the ellagitannins of pomegranate are hydrolyzed in mice, and the ellagic acid was excreted in the urine and feces.

3.5. Antioxidant activity

The results for antioxidant activities measured by DPPH method of the cultivars studied are presented in Fig. 3. The DPPH radical scavenging assay is usually applied to determine the capacity of antioxidant to scavenge free radicals and the scavenging potentials of the antioxidant extract were measured according to the degree of discoloration of mixture. In this current work, the variation of antioxidant capacity between the juices was statistically significant and the data were among 31.16% and 66.82% for local cultivars and for foreign cultivars among 45.65% and 76.3%. When the value is high, the total antioxidant capacity is high (Hmid et al., 2013). However, for measuring the EC50, reaction between DPPH free

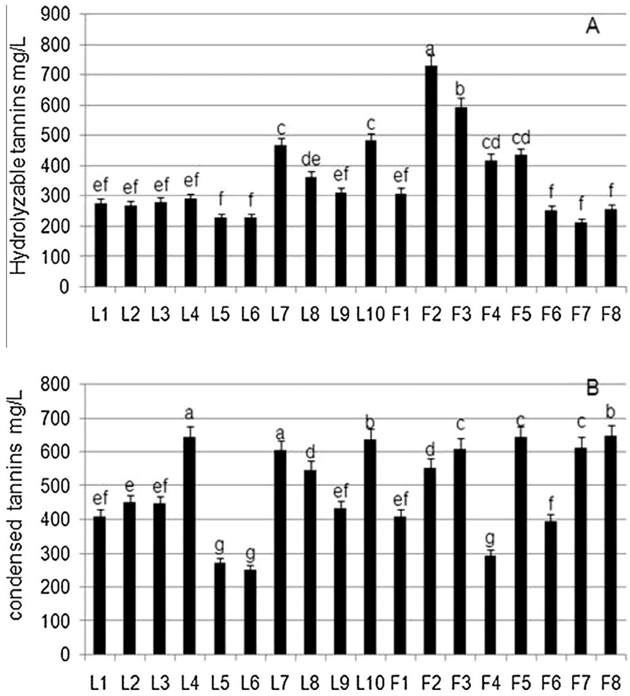

Figure 2 Hydrolyzable tannins content (A) and condensed tannins content (B) of eighteen pomegranate juices. The data presented represent the mean ± standard error of three replicates from accession. ANOVA was used the determined statistically significant difference at ($P < 0.05$) as identified by different letters.

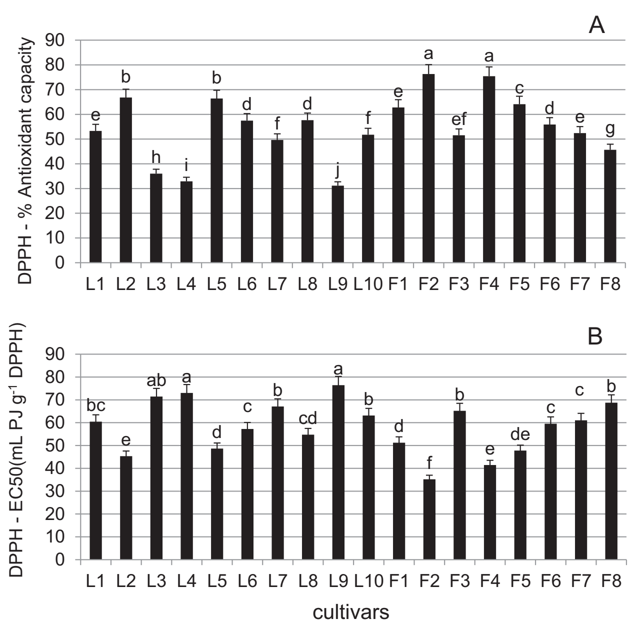

Figure 3 Antioxidant capacity of 18 pomegranate juices measured by DPPH method and expressed as antioxidant activity (AA) (A) and efficient concentration (EC50). The data presented represent the mean ± standard error of three replicates from accession. ANOVA was used to determine statistically significant difference at ($P < 0.05$) as identified by different letters.

radical and antioxidant of juices achieved the steady state in 1 h. Final results were expressed using the term EC50. The lower the EC50 is the higher antioxidant capacity (Brand-Williams et al., 1995). All cultivars showed scavenging effects against DPPH radical ranging from 35.21 to 76.45 ml PJ/g DPPH. Çam et al. (2009) showed that the values of EC50 for eight cultivars grown in Turkey were ranged between 29.8 and 72.3 ml PJ/g DPPH.

4. Discussion

It was already displayed that the physical characteristics of pomegranate fruits grown in Iran found that the fruit weight, fruit length, fruit diameter, calyx length and calyx diameter were between 196.89 g and 315.28 g; 69.49 mm and 81.56 mm, 64.98 mm and 86.88 mm, 13.45 mm and 24 mm, 12.52 mm and 23.96 mm (Tehranifar et al., 2010). Our results in general were higher than results reported by this research. Similarly, the slight difference observed between our work and that of Oukabli et al. (2004), who worked on the same collection, can be explained by variability in climatic conditions. Melgarejo (1993) showed that the fruit weight varies according to the cultivar. The values observed (196.89–315.28 g) by Tehranifar et al. (2010) were lower than our results, while our results were in agreement with values found in another study concerning five cultivars grown in Morocco (Rouge Marrakech, Ounk Hmam, Bouaâdime, Jaune Marrakech and Sefri) and one foreign cultivar (Ruby) grown in different regions of Morocco present fruit weights (430–535.1 g) (Martinez et al., 2012).

The data of the physical aspect of the fruits in this study showed that eighteen cultivars are different in all investigated analysis except two parameters : seed weight and aril length. However, the local cultivars 'Chioukhi' (L3), 'Bzou' (L8), and 'Sefri 2' (L10) seem the most interesting, combined more aril percentage, least skin percentage, and least seed percentage that was a highly desirable characteristic by consumer. The foreign cultivars are also very promising such as 'Gordo de Jativa' (F1), 'Ruby' (F4), 'Mollar Osin Hueso' (F6) and Wonderful cultivars (F3) which having a high percentage of Arils.

All cultivars are evaluated under the same climatic conditions and agricultural practices; therefore, the observed variability in terms of TP was due to genetic variability. Gil et al. (2000) found that TP of pomegranate juice was extracted from fresh arils as 2117 ± 95 mg/L and for a commercial juice as 2566 ± 131 mg/L, while Çam et al. (2009) showed that the TP of eight pomegranate arils extensively cultivated in Turkey varied among 2083–3436 mg/L (Çam et al., 2009), and our data were their results were in similarity with their results. Also this study showed that the cultivar 'Grenade jaune' (F1) has the highest polyphenol composition and a high °Brix (17 °Brix), while the lowest value for the TP observed cultivar Zherie d'automne (F8) corresponding to a relatively low value of ° Brix (14.26 °Brix). As regards the Wonderful cultivar, our results (4587.71 mg GAE/L), are higher than those reported by Gil et al. (2000) (2117 ± 95 mg/L), which may be explained by the arid climate in Morocco. So the effect of water stress causes this increase in total polyphenols. Our results for anthocyanin content were lower than the results presented for eight pomegranate gynotypes widely cultivated in Turkey, with

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anthocyanin composition was among 81–369 mg/L of juice extracted from seeds by press (Çam et al., 2009).

However, the Ounk Hmam, Wonderful, Djeibi and Sefri 2 cultivars contain the high total polyphenol content and the high anthocyanin composition; therefore, they may be of interest to pharmaceutical and food industries to extract these bioactive compounds. The polyphenol and anthocyanin contents are known to be influenced by genotype, growing region, climate and cultural practices. Our results give interesting data on the physico-chemical characteristics of pomegranate juice extracted from various cultivars. The most relevant characterization method can now be used in fruit juice industry for sorting out the best cultivars, agronomic and processing and practices, based on the health benefits of this juice for the consumers.

The Hydrolyzable tannins are various combinations of gallic acid, glucose, tetragallic acid, and hexahydroxydiphenic acid (after hydrolysis, this acid gives rise to ellagic acid). In previous work, the composition of hydrolyzable tannins in pomegranate juice was 539.20 mg/L (Gil et al.,2000).

Previous studies have shown that pomegranate juice (PJ) was found to be a powerful antioxidant drink more than other fruit juice such as red grape and apple juice (Gil et al., 2000), which can be explained by the higher composition of pomegranate in polyphenols. In the present study, the values of antioxidant activity were in agreement with the values (10.37–67.46%) reported by Tezcan et al. (2009) on seven commercial pomegranate juices from Turkey, all marks contain 100% pomegranate juices and without added ingredients, while our results were higher than values reported by Tehranifar et al. (2010) on twenty pomegranate juices extracted from arils in Iran (15.59–40.72%).

5. Conclusion

This paper presents the analysis of eighteen pomegranate cultivars based on the morphological and biochemical diversity. Statistically significant variabilities were showed among pomegranate cultivars studied in all analyses performed except seed weight and aril length. This confirms that the physico-chemical properties and antioxidant activity of pomegranates were strongly related to the factor cultivar.

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