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Characterization and evaluation of Tunisian pomegranate quality during storage as ready to eat arils

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ABSTRACT

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Purpose: Ready to eat arils can be a value-added product as an alternative use for the whole pomegranate fruit by offering more convenience to the consumer. Recently, the diffusion of local cultivars with typical and unique quality characteristics will offer new opportunities for the fresh international market. Research method: This study aimed to evaluate the quality of arils from five cultivars, namely Gabsi (GB), Jebali (JB), Khalledi (KH), Tounsi (TN), and Zehri (ZH) to be used for ready to eat market, as well as to provide a form of valorization for these cultivars. Findings: Significant differences found between cultivars for most of the evaluated quality parameters. KH, GB, and JB were the cultivars with the best initial quality. PCA separated the investigated cultivars based on the storage period. Among the studied cultivars, the main changes in color and sensory quality attributes during storage have been registered for the cultivars JB and KH. TN showed slight color difference during storage. GB had right color intensity, maintained high content of anthocyanins, and the best sensory evaluation at the end of storage. Limitations: Based on their nutritional quality, cultivars GB and KH were the best cultivars for ready to eat arils processing. However, a proper selection of initial quality should be considered. Originality/Value: The richness of local Tunisian pomegranate cultivars with its typical and unique quality traits. They could be used as a ready to eat form to valorize the whole fruit thereby, will enhance marketing demand.



INTRODUCTION

Mediterranean countries are full of vast local cultivars of pomegranate. Only a few cultivars are commercially cultivated, and most are just locally presented as whole fruit. The introduction of different cultivars has been spread recently in many countries, including Tunisia.

In Tunisia, pomegranate is being one of the leading fruit trees with great interest. The production is based on a few cultivars, with exciting market characteristics, despite the relatively large number of local cultivars reported in the Tunisian germplasm. Tunisia is considered as a micro-gene center of this species with more than 60 local ecotypes already collected (Mars & Marrekchi, 1998). Nevertheless, some cultivars are extinct despite their high potential for valorization (Zaouay et al., 2012).

Recently, arils consumption showed huge demand either for fresh market or industrial processing. Thus, it is crucial to characterize different cultivars to meet the current market demand for high quality. Aril quality depends on cultivar and other preharvest and postharvest factors. Zaouay and Mars (2011) studied the diversity of Tunisian pomegranate cultivars as assessed by further criteria and highlighted some particular varieties for different uses. Alcaraz-Mármol et al. (2017) characterized twenty Spanish pomegranate cultivars. They found arils with soft seeds increased sweetness, and considered for fresh consumption.

Ready to eat arils (REA) were peeled and prepared to attract consumer attention and enhance consumption. REA has become attractive due to their convenience and health benefits (Martinez-Romero et al., 2013). Some properties of pomegranate fruit are useful aspects to evaluate fruit processing (Çam et al., 2009), mainly bioactive antioxidant compounds. These attributes change significantly by several factors, such the storage period. Depending on the cultivar, pomegranate arils have a storage period of 1 to 2 weeks when stored under 5°C (Caleb et al., 2012). Postharvest treatments used to keep or enhance aril quality include packaging material, passive or active modified atmosphere packaging, and coatings (Banda et al., 2015; Belay et al., 2017; Moradinezhad et al., 2020).

In the frame of this enormous diversity, local pomegranate cultivars have not yet been valorized as ready to eat arils. Different studies have focused on the evaluation of pomegranate fruit characteristics (Zaouay & Mars, 2011) and on juice or some by-product characterization (Abid et al., 2018). Currently, no studies were carried on the evaluation of local pomegranate cultivars as ready to eat aril, and the change of quality during storage. In the current study, the potential of five local cultivars for processing as fresh ready-to-eat arils were determined. Physico-chemical, nutritional and sensory quality attributes were analyzed initially and after storage at 5° C.

MATERIALS AND METHODS

Fruit samples

In this report five pomegranate (*Punica granatum* L.) cultivars 'Gabsi' (GB), 'Khalledi'(KH), 'Jebali' (JB), 'Tounsi'(TN), and 'Zehri'(ZH) were studied (Fig. 1). Characteristics of whole fruit and arils in the pomegranates are reported in Table 1. Fruit were harvested at optimum maturity stage in October 2017, from the research orchard of the Higher Agronomic Institute of Chott-Mariem, Sousse, Tunisia. The cultivars selected according to their importance in the region. The fruit was cultivated under homogenous conditions, without any fertilization and irrigation except natural rainfall. Fruits were transported to the postharvest laboratory of the Higher Agronomic Institute of Chott-Mariem. Fruit with visible physical defects and unhealthy skins were discarded. For each cultivar, a lot of 25 fruits were prepared.





Fig. 1. Pomegranate fruit cultivars studied from left to right. 'Gabsi' (GB), 'Khalledi'(KH), 'Jebali' (JB), 'Tounsi' (TN), and 'Zehri'(ZH).

 Table 1. Characteristics of the studied pomegranate cultivars

Cultivar	Code	External color	Internal color
Gabsi	GB	Pink yellowish	Red
Khalledi	KH	Dark pink	Dark red
Jebali	JB	Red greenish	Purple red
Tounsi	TN	Green yellowish	Red whitesh
Zehri	ZH	Dark red purple	Red

Fruit handling and processing

Arils were hand-extracted and washed in 100μ L L⁻¹ of sodium hypochlorite (NaOCl), and collected on a sterile plastic box to air dry and manually remove the damaged. Samples of 200 g were uniformly prepared and triplicated per storage period for each cultivar. Arils were filled in rigid polyethylene punnets (10/30) and covered with 15µm PE food grade film (SIPEL) (Ben Amara et al., 2020), and stored at 5 °C and 90–95 % RH for up to 10 days. For each cultivar, a total of 9 packs analyzed on day 0, 5, and 10 days of storage. The quality parameters assessed are the physical, chemical, bioactive, and sensory quality attributes. All analysis was triplicated.

Chemical analysis

Color attributes

The color of arils was measured on the basis of the CIE $L^*a^*b^*$ color system using a digital chromameter (Minolta Chroma Meter, CR-400, Japan). The arils were spread to cover a Petri dish. L^* , a^* and b^* color measurements were taken from 15 different points of the dish. Chroma and color difference were calculated using the following formula (1 and 2):

Chroma (*C*) intensity was calculated as the following equation: $C *= \sqrt{a^{*2} + b^{*2}}$ (1)

Color difference (ΔE) as described by the following equation: $\Delta E = \sqrt{(\Delta L^{*2}) + (\Delta a^{*2}) + (\Delta b^{*2})}$ (2)

 ΔE indicates the degree of the overall color change between the fresh arils and the arils after storage.

Firmness

The firmness of the arils was measured using a texture meter TA-XT2 Texture Analyzer (Stable Micro Systems Ltd., England, UK). The texture analyzer linked to a computer that recorded and analyzed the data via a software program. The test consisting to compress 10 g of the aril sample, and the firmness expressed in (N). The compression used, the platen of 75 mm with a mini-Kramer cell probe (approximately 2×2 cm). The test speed was 0.5 mm s⁻¹. All measurements were triplicated.



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

Arils were juiced using an ULTRA-TURRAX (IKA, T18 Basic; Wilmington, NC, USA) and filtered through cheesecloth. The TSS was recorded using a digital refractometer (ATAGO, Japan), and values are expressed in percentage. The pH and the TA were determined on one gram of aril juice, with an automatic titrator (T50 M Terminal, METTLER TOLEDO, Switzerland) against a volume of 0.1 M NaOH until it reaches the final pH of 8.2. TA expressed, as % of citric acid. Each sample had three replicates.

Total phenol content

The total phenol content (TPC) was measured using the Folin-Ciocalteu (Folin-C) method, as described by Singleton et al. (1999) with some modification. In a cuvette, 100 μ L of diluted pomegranate juice (PJ) mixed with 100 μ L Folin-C reagent, and then 300 μ L sodium carbonate (20 %, NaCO₃) solution after 2 min. After 2 hr incubation, absorbance was read at 725 nm using a UV-vis spectrophotometer (SHIMADZU-1700, Jiangsu, China). The TPC expressed, as milligram of gallic acid equivalent (GAE) per L of PJ (mg.L⁻¹). Each sample had three replicates, and each replicate had two readings.

Total anthocyanin content

Total anthocyanin content (TAC) was quantified using the pH differential method (Cam et al., 2009) with two buffer systems comprising of potassium chloride (pH 1, 0.025 mol.L⁻¹) and sodium acetate (pH 4.5, 0.4 mol.L⁻¹). Briefly, 0.4 mL of juice sample was mixed with 3.6 mL of pH 1.0 and pH 4.5 buffers, separately. The absorbance (A) was determined after 10 min incubation at 510 and 700 nm using a UV–vis spectrophotometer (SHIMADZU-1700, Jiangsu, China) where:

$$A = (A_{510 \text{ nm}} - A_{700 \text{ nm}}) \text{ pH } 1.0 - (A_{510 \text{ nm}} - A_{700 \text{ nm}}) \text{ pH } 4.5$$

The TAC expressed, as mass Cyanidin-3-glucoside per unit volume of PJ using the following equation (3):

TAC (mg L - 1) =
$$\frac{A \times MW \times DF \times 100}{\varepsilon \times L}$$
 (3)

With (ϵ) molar extinction coefficient of 26,900, MW=molecular weight of Cyanidin-3-glucoside (449.2 g.mol⁻¹), DF = dilution factor and L=cell path length (1 cm). Each sample had three replicates.

Antioxidant activity

The antioxidant activity (AA) was conducted on the same extract made for TPC, as described by Brand Williams et al. (1995) with slight modifications. 50 μ L of the extract was adedd to 950 μ L of DPPH (2, 2-Diphenylpicrylhydrazyl) solution, and absorbance was read after 24 h at 515 nm against a blank made with water instead of sample extract. Trolox was used as a standard, and results were expressed in milligrams of Trolox equivalents (TE) per L of juice (mg.L⁻¹). All measurements were in triplicate, and each replicate had two readings.

Vitamin C

A sample of 2 mL PJ homogenized with 2 mL of MeOH plus citric acid (21 g.L⁻¹) with EDTA (0.5 g.L⁻¹) and NaF (0.168 g.L⁻¹). The homogenate filtered through cheesecloth and C18 Bakerbond SPE cartridge (Baker, Deventer, Holland). Ascorbic acid (AA) and dehydroascorbic acid (DHAA) contents were determined as described by Zapata and Dufour



(1992). The HPLC analysis carried out after the derivatization of DHAA with 1, 2phenylenediamine dihydrochloride (OPDA). 750 μ L of sample analyzed by the HPLC (Agilent Technologies 1200 Series, Waldbronn, Germany) equipped with a DAD detector and a Binary pump. The separation of DHAA and AA achieved on a Zorbax Eclipse XDB-C18 column (150 mm × 4.6 mm, 5 μ m particle size; Agilent Technologies, Santa Clara, CA, USA). The mobile phase was MeOH containing 5 mM cetrimide and 50 mM potassium dihydrogen phosphate at pH 4.5. The detector wavelengths were 348 nm for DHAA and 251 nm for AA. Vitamin C calculated, as the sum of AA and DHAA, and expressed in mg L⁻¹. All analyses were done in triplicate.

Difference in sensory evaluation

Sensory evaluation was performed by 20 trained panelists from the research group of the postharvest laboratory of the Higher Agronomic Institute of Chott-Mariem. Panelists were asked to evaluate color, aroma, taste, sweetness and acidity of each sample using a 9-point quantitative descriptive scale (1= extremely weak intensity, 2 = very weak, 3 = weak, 4 = moderately weak, 5 = regular, 6 = moderately strong, 7 = strong, 8 = very strong and 9 = extremely strong) previously defined by the trained panelists. Scores of 5 and above are considered desirable for commercial purposes, and scores below three are unacceptable for consumption (Ben Amara et al., 2020). Plates with 40 g of arils from 5 fruit were served in a randomized order. Distillate water was provided to panelists to clean their palates between samples.

Statistical analysis

The effect of cultivar, storage period, and their interaction on the measured parameters were analyzed by Two-way ANOVA using STATGRAPHICS® Centurion XVI version. Mean differences were separated by the honestly significant difference (HSD) procedure using the Tukey test when F-values were significant at $P \leq 0.05$. Sensory analysis were analyzed by two-way ANOVA and presented with a spider graph using Excel 2007 (Microsoft). A Principal Component Analysis (PCA) was performed. PCA enabled to discriminate the group of cultivars during the storage period. Correlations between instrumental and sensory data were analyzed using Pearson correlations.

RESLUTS AND DISCUSSION

Color attributes

Initially, the color attributes varied between cultivars. Significant differences in color attributes were found on redness a, * yellowness b*, and chroma intensity C* between cultivars and storage periods, as reported in ANOVA (Table 2). Results indicate that lightness varied among different cultivars at the initial date. Arils from cultivars KH and JB presented darker arils corresponding to the lowest L* values of 27.4 and 21.0, respectively. Arils of the cultivar ZH with the highest value, L* = 35.3, followed by TN (33.1) and GB (29.2). Fawole and Opara (2014) reported for the cultivar "Ganesh" that the L* value of arils was the highest among the studied cultivars, which corresponds with its good visual appeal. Based on these criteria, ZH, TN, and GB would have an excellent visible color based on L* values.

With regards to aril redness, JB had the highest a^* value (24.7) compared to GB (20.1), KH (15.1), and TN (14.4), while ZH has shown less red aril color (11.0). For yellowness color (b^*), JB had the lowest b^* value (10.2) followed by KH (13.4), ZH (15.6), and TN (18.0) while GB had a high b^* value (24.7) (Table 2). Regarding the chroma intensity (C^*), some similar chroma values could be seen, as reported in Table 2. The arils from cultivars ZH, and

KH could be grouped with the lowest C* values, with 19.1 and 20.3, respectively. JB and TN arils had mean values of 23.3 and 26.7 respectively. In contrast, GB has shown the highest C^* (31.9). High chroma value indicates red color and corresponds to a high level of anthocyanin content (Belay et al., 2017). According to chroma values obtained, arils from cultivar GB had red color corresponding with a high level of anthocyanins (Table 4).

Based on ANOVA, the storage period had a significant effect on L, b^* and C^* between cultivars while a^* did no varied during the storage (Table 2). Different cultivars behaved differently during storage period, with more important changes observed for arils from cultivar KH, where significant differences were observed for all color parameters. Concerning the cultivar JB, differences between initial and ten days of storage were registered, mainly for L^* and a^* . Thus, an increase of L^* from 21.0 to 26.7 and a decrease of a^* from 24.7 to 14.6 (Table 2). Color changes of GB and ZH during storage were significant for a^* , b^* , and C^* . These changes were more critical for C^* with an increase from 31.9 and 19.0 to 36.7 and 26.2 respectively for both cultivars. Among all cultivars, TN has shown the most stable color attributes after the storage period. Color difference ΔE was low for the cultivar TN during storage as well as ZH. The cultivars GB, JB, and KH showed high color difference, which increased after ten days (Table 2). The color difference is mainly due to cultivars, and a slight change during storage was registered.

The studied cultivars did not exhibit dark red color compared to cultivar 'Mollar de Elche' ($L^* = 12$ to 38), and less dark than 'Wonderful' accession ($L^* = 0.8$ to 17) (Mena et al., 2011). The differences in color coordinates were due to the differences between cultivars. The non-significant effect of the storage period was reported previously for different pomegranate aril cultivars. Sepùlveda et al. (2000) observed no color change in minimally processed 'Wonderful' arils stored for 14 days. Ayhan and Estùrk (2009) reported no effect on a^* and b^* for 'Hicaznar' cultivar throughout the 18 days of storage. However, Palma et al. (2009) showed that the color of minimally processed arils had no significant difference in L^* , b^* , and C^* and a slight rise in a^* . Belay et al. (2017) observed a slight increase in C^* at the end of 12 days.

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

Cultivars and storage periods did not significantly affect on arils firmness, as reported in ANOVA (Table 3). The initial firmness of arils ranged between 10.0 N for the cultivar ZH and 14.1 N for the cultivar GB (Table 3). The cultivars JB and KH had a mean value of 13.9 N, and TN registered 11.6 N. The firmness fluctuated after storage. Statistically, changes of the firmness during the storage period was not significant for each cultivar. These results are in accordance with Caleb et al. (2013); the authors reported no significant change in aril firmness of 'Acco' and 'Herskawitz' cultivars during 14 days of storage. The firmness is crucial quality parameter that determines appearance and influence consumer appreciation.

Total soluble solids, titratable acidity and pH are reported in Table 3. TSS and TA are important traits of pomegranate aril taste and consumer acceptability. Arils of cultivar ZH (TSS = 15.0 %) showed significantly lower TSS than the other cultivars for which TSS ranged between 15.9 % and 16.4 %. TSS values of 'Wonderful' pomegranate arils reduced with storage and ranged from 17.1% to 15.3% by the end of 12 days (Banda et al., 2015). It could be seen from Table 3 that TA differed significantly among cultivars and during storage. Initially, KH had the highest TA (0.65 %) followed by GB (0.57%), TN (0.53 %) and JB (0.51 %). The lowest TA value, 0.30 %, recorded for the arils of the cultivar ZH. Regarding the initial value, arils from all cultivars have shown an increase in titratable acidity after ten days.



pH was cultivar dependent and ranged from a minimum value of 3.7 for the cultivar ZH to a maximum of 4.8 for TN, slight changes registered after storage without showing any clear trend.

TA values registered in the studied cultivars are lower than then cultivar 'Wonderful' TA= 1.11 to 1.58 % (Kader et al., 1984). The increase observed in acidity during storage could be related to water loss or also to the metabolic activity such as the conversion of soluble sugars into other organic acids (Bhatia et al., 2015). Palma et al. (2009) observed an increase in titratable acidity due to the absorption of CO₂, which lowers pH when dissolved in the aqueous phase, and high values of titratable acidity are considered advantage to inhibit off-flavor of pomegranate aril. Generally, TA in pomegranate juice differed depending on the cultivar, growing region, and postharvest handling practices. Our findings on the effect of storage in physical and chemical parameters between various cultivars was determined previously. Caleb et al. (2013) studied the effect of storing of two pomegranate cultivars and found a significant decrease in TA from day 3, while it stayed relatively unchanged until 15 days. Regarding TSS, Ayhan and Estürk (2009) observed that TSS remained unchanged for the nine days and started to decrease for the rest of the storage. The cultivar was one of the main factors influencing changes in pH, TSS, and TA values (Caleb et al., 2011).

cat point grana	te ams non	li live cultivais u	uning storage per			
	Days	GB	JB	KH	ZH	TN
L*	0 5 10	$\begin{array}{c} 29.2 \pm 9.1^{A}{}_{b} \\ 30.0 \pm 6.1^{A}{}_{ab} \\ 30.5 \pm 6.4^{A}{}_{bc} \end{array}$	$\begin{array}{c} 21.0 \pm 1.7^{\text{B}}_{\text{c}} \\ 23.8 \pm 2.5^{\text{AB}}_{\text{b}} \\ 26.7 \pm 3.7^{\text{A}}_{\text{c}} \end{array}$	$\begin{array}{c} 27.4 \pm 6.6^{B}{}_{bc} \\ 30.0 \pm 7.1^{B}{}_{ab} \\ 33.1 \pm 5.0^{A}{}_{a} \end{array}$	$\begin{array}{c} 35.3 \pm 5.5^{A}{}_{a} \\ 32.5 \pm 7.2^{A}{}_{a} \\ 34.2 \pm 5.0^{A}{}_{ab} \end{array}$	$\begin{array}{c} 33.1 \pm 2.2^{A}{}_{b} \\ 29.0 \pm 3.6^{B}{}_{ab} \\ 32.8 \pm 3.4^{A}{}_{ab} \end{array}$
a*	0 5 10	$\begin{array}{c} 20.1 \pm 4.1 ^{B}{}_{b} \\ 26.9 \pm 4.6 ^{A}{}_{a} \\ 24.4 \pm 6.5 ^{AB}{}_{a} \end{array}$	$\begin{array}{c} 24.7 \pm 1.4^{A}{}_{a} \\ 17.9 \pm 2.7^{B}{}_{bc} \\ 14.6 \pm 2.0^{B}{}_{ab} \end{array}$	$\begin{array}{c} 15.1 \pm 4.7^{\rm B}{}_{c} \\ 15.3 \pm 5.5^{\rm B}{}_{c} \\ 20.4 \pm 4.0^{\rm A}{}_{ab} \end{array}$	$\begin{array}{c} 11.0 \pm 2.1^{B}{}_{d} \\ 14.1 \pm 3.1^{A}{}_{c} \\ 14.1 \pm 2.3^{A}{}_{bc} \end{array}$	$\begin{array}{c} 14.4 \pm 3.6^{A}{}_{c} \\ 13.6 \pm 1.6^{A}{}_{b} \\ 12.9 \pm 3.4^{B}{}_{c} \end{array}$
b*	0 5 10	$\begin{array}{c} 24.7 \pm 4.0^{AB}{}_{a} \\ 22.7 \pm 4.0^{B}{}_{a} \\ 27.4 \pm 5.1^{A}{}_{a} \end{array}$	$\begin{array}{c} 10.2 \pm 0.1^{AB}{}_{d} \\ 11.7 \pm 2.0^{A}{}_{c} \\ 12.0 \pm 0.2^{A}{}_{d} \end{array}$	$\begin{array}{c} 13.4 \pm 3.2^{B}{}_{c} \\ 18.5 \pm 3.0^{B}{}_{b} \\ 20.4 \pm 2.0^{A}{}_{b} \end{array}$	$\begin{array}{c} 15.6 \pm 1.9^{B}{}_{c} \\ 17.2 \pm 2.4^{AB}{}_{b} \\ 19.3 \pm 2.7^{A}{}_{c} \end{array}$	$\begin{array}{l} 18.0 \pm 3.0^{A}{}_{b} \\ 19.2 \pm 1.0^{B}{}_{c} \\ 20.0 \pm 2.5^{A}{}_{bc} \end{array}$
C*	0 5 10	$\begin{array}{c} 31.9 \pm 5.6^{A}{}_{a} \\ 35.3 \pm 5.5^{A}{}_{a} \\ 36.7 \pm 8.0^{A}{}_{a} \end{array}$	$\begin{array}{c} 26.7 \pm 1.2^{A}{}_{b} \\ 21.5 \pm 2.0^{B}{}_{b} \\ 23.3 \pm 2.8^{B}{}_{b} \end{array}$	$\begin{array}{c} 20.3 \pm 5.3^{B}{}_{c} \\ 24.2 \pm 5.5^{B}{}_{b} \\ 29.0 \pm 4.0^{A}{}_{a} \end{array}$	$\begin{array}{c} 19.0 \pm 2.0^{B}{}_{c} \\ 22.9 \pm 4.0^{A}{}_{b} \\ 26.2 \pm 5.0^{A}{}_{b} \end{array}$	$\begin{array}{c} 23.3 \pm 3.0^{A}{}_{b} \\ 23.6 \pm 1.5^{A0}{}_{b} \\ 24.1 \pm 1.0^{A}{}_{b} \end{array}$
ΔΕ	5 10	$\begin{array}{c} 15.2 \pm 7.0^{A}{}_{a} \\ 17.3 \pm 8.0^{A}{}_{a} \end{array}$	$\begin{array}{c} 12.0{\pm}~4.4^{\rm B}{}_{a} \\ 17.0{\pm}~3.7^{\rm A}{}_{a} \end{array}$	$\begin{array}{c} 15.8 \pm 6.2^{A}{}_{a} \\ 19.6 \pm 5.3^{A}{}_{a} \end{array}$	$\begin{array}{c} 12.3 \pm 5.4^{A}{}_{a} \\ 9.8 \pm 4.4^{B}{}_{b} \end{array}$	$\begin{array}{l} 7.4 \pm 3.1^{A}{}_{b} \\ 7.1 \pm 3.2^{A}{}_{b} \end{array}$
ANOVA	df	F-ratio				
		L^*	a^*	b^*	\mathbf{C}^*	ΔE
A: cultivar	4	22.8*	79.0*	120.7*	90.0*	10.6*
B: storage	2	15.0*	0.3 ns	14.3*	18.2*	2.3 ns
$\mathbf{A} \times \mathbf{B}$	8	4.4*	6.5*	12.5*	9.2*	1.2 ns

Table 2. Color attributes (Lightness L^* , redness a^* , yellowness b^* , Chroma C^* , color difference ΔE) of ready to eat pomegranate arils from five cultivars during storage period at 5°C

Values are means \pm standard error of fifteen replicates. Different Super script letters in the same column are significant for each cultivar during storage, according to the Tukey HSD test at P \leq 0.05.

Different lower script letters in the same row are significant for each storage period among cultivars, according to the Tukey HSD test at $P \le 0.05$. Significance of F-ratio: * $P \le 0.05$. ns not significant at P > 0.05. df: the degree of freedom.

	Days	GB	JB	KH	ZH	TN		
Firmness (F) (N)	0	$14.1\pm2.4^{\rm A}{}_{\rm a}$	$13.9\pm2.2^{A}_{a}$	$13.9\pm2.2^{A}_{a}$	$10.0 \pm 1.5^{A_{b}}$	$11.6 \pm 0.6^{A_{b}}$		
	5	$13.2 \pm 3.5^{A}_{a}$	$9.6\pm2.3^{\rm B}{}_{\rm c}$	$13.1 \pm 0.7^{A}_{\ a}$	$9.0 \pm 1.0^{A}{}_{c}$	$11.7\pm0.6^{\rm A}{}_{\rm b}$		
	10	$10.5\pm5.2^{\rm B}{}_{ab}$	$12.6\pm5.2^{A}_{a}$	$11.7\pm3.4^{\rm A}{}_a$	$8.6 \pm 1.2^{A_{b}}$	$9.4\pm6.8^{A}{}_{b}$		
Total soluble	0	16.4 ± 0.05^{A}	15.9 ± 0.1^{A}	16.3 ± 0.3^{A}	15.0 ± 0^{A}	16.2 ± 0.2^{A}		
solids (TSS) (%)	5	$10.4 \pm 0.05^{\circ}a$ $15.4 \pm 0.05^{\circ}A$	$15.9 \pm 0.1^{\circ}a$ $15.6 \pm 0.2^{\circ}A$	$10.3 \pm 0.3^{\circ}a$ 15.3 ± 0.0 ^A	15.0 ± 0.6	16.2 ± 0.2 a 16.7 ± 0.0^{A}		
solius (155) (70)	10	15.4 ± 0.05 b 15.1 ± 0.05 A	15.0 ± 0.2 ab 16.7 ± 1.1A	15.5 ± 0.0 b 15.6 ± 0.8 A	13.3 ± 0.0 b 14.2 ± 0.5 A	16.7 ± 0.9 a		
	10	13.1 ± 0.03 ab	10.7 ± 1.1 a	13.0 ± 0.0 ab	14.3 ± 0.3 b	10.7 ± 0.3 a		
pН	0	$4.5 \pm 0.05^{A}{}_{b}$	$4.0 \pm 0.03^{\circ}{}_{c}$	$4.5 \pm 0.03^{\rm C}{}_{\rm b}$	$3.7 \pm 0.02^{A}_{d}$	$4.8 \pm 0.07^{A}_{a}$		
1	5	$3.0 \pm 0.02^{C_{d}}$	$4.6 \pm 0.06^{A_{b}}$	$5.2 \pm 0^{A}_{a}$	3.5 ± 0.1^{B}	$4.8 \pm 0.1^{A_{b}}$		
	10	$3.9\pm0.02^{\rm B}{}_{\rm b}$	$4.3\pm0.05^{B}{}_{a}$	$4.8\pm0.02^{\rm B}{}_a$	$2.7 \pm 0.03^{C}{}_{b}$	4.3 ± 0.6^{A_a}		
Total titrable	0	$0.57 \pm 0.02^{B}{}_{b}$	$0.51\pm0.0^{\rm C}{}_{\rm b}$	$0.65 \pm 0.01^{AB}{}_{a}$	$0.31 \pm 0.0^{B}{}_{c}$	$0.53 \pm 0.04^{A}{}_{b}$		
acidity (TA) (%)	5	$0.48 \pm 0.02^{\circ}{\rm c}$	$0.62\pm0.0^{B}{}_{ab}$	$0.60 \pm 0.00^{\rm B}{}_{\rm b}$	$0.37\pm0.0^{A}{}_{d}$	$0.70 \pm 0.01^{A}_{a}$		
	10	$0.69 \pm 0.02^{A}_{a}$	$0.78\pm0.0^{\rm A}{}_a$	$0.71 \pm 0.04^{\rm A}{}_{a}$	$0.45\pm0.0^{A}{}_{a}$	$0.80 \pm 0.20^{A}{}_{a}$		
ANOVA		F-ratio						
	df	F	TSS	pН	r	ГА		
A: cultivar	4	2.0 ns	14.3*	113.7*	2	29.3*		
B: storage	2	1.1 ns	1.9 ns	12.5*	2	46.5*		
A×B	8	0.4 ns	3.8*	23.5*	13.3*			

Table 3. Physico-chemical properties of ready to eat pomegranate arils from five cultivars during storage period at 5 °C

Values are means \pm standard error of fifteen replicates. Different Super script letters in the same column are significant for each cultivar during storage, according to the Tukey HSD test at P ≤ 0.05 .

Different lower script letters in the same row are significant for each storage period among cultivars, according to the Tukey HSD test at $P \le 0.05$. Significance of F-ratio: * $P \le 0.05$. ns not significant at P > 0.05. df: the degree of freedom.

Table 4. Total phenol content (TPC)	, total anthocyanin content (TAC)	, antioxidant activity (AA)	and vitamin C
(Vit C) of ready to eat pomegranate a	arils from five cultivars during sto	rage period at 5 °C	

	Days	GB	JB	KH	ZH	TN
TPC	0	$1.593 \pm 120^{A}_{a}$	$1.597 \pm 40^{A}{}_{a}$	$1.377 \pm 90^{AB}{}_{a}$	$1.677 \pm 220^{B}_{a}$	$1.407 \pm 121^{A}_{a}$
(mg L ⁻¹)	5	$1.300\pm33^{AB}{}_b$	$1.453\pm56^{\rm B}{}_{\rm b}$	$1.410\pm128^{\rm A}{}_{\rm b}$	$1.903\pm6^{A}{}_{a}$	$1.100 \pm 22^{B}{}_{c}$
	10	$1.201 \pm 220^{B}{}_{c}$	$1.581 \pm 57^{AB}{}_b$	$1.134 \pm 104^{B}{}_{c}$	$2.054 \pm 97^{A}_{a}$	$1.187 \pm 60^{\rm B}{}_{\rm c}$
TAC	0	$55.8\pm9.2^{AB}{}_a$	$45.7\pm9.0^{A}{}_{ab}$	$44.8 \pm 2.2^{A}_{ab}$	23.8 ± 7.2^{A_b}	$35.1 \pm 11.1^{A}{}_{b}$
$(mg L^{-1})$	5	$62.2\pm5.3^{A}{}_{a}$	$34.7 \pm 1.3^{A}_{bc}$	$38.3 \pm 6.5^{B}{}_{b}$	$34.8\pm12.6^{A}{}_{b}$	$11.1 \pm 1.8^{B}{}_{c}$
	10	$46.5 \pm \ 5.7^{B}{}_{a}$	$32.5\pm12.2^{A}{}_{b}$	$44.7\pm1.6^{\rm A}{}_{\rm a}$	$21.5\pm6.3^{A}_{c}$	$17.8\pm6.7^{B}{}_{d}$
AA	0	$1.985\pm37^{A}_{b}$	$2.986 \pm 76^{A_{a}}$	$1.632 \pm 15^{\circ}{\rm C_{c}}$	$1.802 \pm 130^{\rm B}{}_{\rm bc}$	$1.866\pm01^{\rm A}{}_{b}$
(mg L ⁻¹)	5	$2.004\pm93^{A}_{bc}$	$2.467 \pm 17^{B}_{ab}$	$2.674 \pm 337^{A}_{a}$	$1.913 \pm 134^{AB}{}_{c}$	$1.363 \pm 147^{C}_{d}$
-	10	$1.575\pm23^{\rm B}{}_d$	$2.532{\pm}50^{B}{}_{a}$	$1.872 \pm 100^{B}{}_{c}$	$2.090\pm102^{\rm A}{}_{\rm b}$	$1.574\pm90^B{}_d$
Vit C						
(mg L ⁻¹)	0	$71.1\pm4.0^{A}{}_{a}$	$67.6 \pm 4.0^{A}{}_{b}$	$76.0\pm1.1^{\rm A}{}_{a}$	$72.7 \pm 13.4^{A_{a}}$	$71.8\pm5.5^{\rm A}{}_a$
	5	$59.4\pm16.5^{\rm A}{}_{\rm b}$	$67.5\pm5.0^{A}{}_{a}$	$60.0\pm6.4^{B}{}_{b}$	$52.7\pm5.8^B{}_c$	$61.2 \pm 2.0^{A_{b}}$
	10	$61.0 \pm 12.0^{A}_{ab}$	$68.8 \pm 5.7^{A}_{a}$	$48.7 \pm 1.8^{\circ}{}_{c}{}$	$56.9\pm4.2^{\rm B}{}_{b}$	$54.0\pm4.1^{B}{}_{b}$
ANOVA		F-ratio				
	df	TPC	TAC	AA	Vit C	
A: cultivar	4	9.2*	103.6*	96.5*	9.0 *	
B: storage	2	4.0*	3.1 ns	7.3*	9.6*	
A×B	8	7.1*	17.1*	25.1*	5.1*	

Values are means \pm standard error of fifteen replicates. Different Super script letters in the same column are significant for each cultivar during storage, according to the Tukey HSD test at P \leq 0.05.

Different lower script letters in the same row are significant for each storage period among cultivars, according to the Tukey HSD test at $P \le 0.05$. Significance of F-ratio: * $P \le 0.05$. ns not significant at P > 0.05. df: the degree of freedom.



Total phenol content (TPC), total anthocyanins (TAC), vitamin C, and antioxidant activity (AA)

TPC of arils varied significantly among different cultivars ($P \le 0.05$) (Table 4). The initial total phenol content values ranged from 1,377 mg.L⁻¹ for the cultivar KH and 1,407 mg L⁻¹ for the cultivar TN, to 1,677 mg L⁻¹ GAE for the cultivar ZH (Table 4). The storage period showed a slight effect on TPC. Thus, after ten days, a decrease in TPC was registered for GB and TN, while TPC increased significantly for the cultivar ZH. The possible addition could be related to an indication of tissue stress and likely senescence during processing (Ansah et al., 2018). The pattern of the observed decrease in TPC was following with those reported by D'Aquino et al. (2010), they observed a significant reduction in TPC for cv 'Primosole' after storage. TPC concentrations reported in the literature show that the contents in pomegranate fruits are varied. In fact, the TPC depends on factors such as cultivar type, ripeness, fruit part (Alcaraz-Mármol et al., 2017).

TAC greatly varied between all cultivars as summarized in ANOVA (Table 4), the storage period had no significant effect. The lowest TAC, 21.8 mg L⁻¹, recorded at the initial date for the cultivar ZH, which corresponded to L^* and a^* color values, as shown in Table 2. Moreover, arils from cultivars GB, JB and KH exhibited high TAC content, in accordance with, low L^* and high a^* values (Table 2). The highest decrease in TAC was registered for the cultivar TN. The lowest TAC value, 17.8 mg L⁻¹, obtained at the end of 10 days of storage.

Caleb et al. (2012) observed a general trend of decrease in total anthocyanin content as the storage time increased. Meanwhile, Banda et al. (2015) observed fluctuation of total anthocyanin concentration during 12 days. The total anthocyanin content of 'Primosole' packaged pomegranate arils was maintained at the end of ten days of storage (Palma et al., 2009). It was suggested that degradation of anthocyanin is affected by oxidation or cleavage of covalent bonds, which increases during storage or processing (Laleh et al., 2006). Brownmiller and Howard (2008) reported that, total anthocyanin content could be reduced during storage due to the polymerization with other phenolic compounds.

Differences in antioxidant activity (AA) observed among cultivars, and slight differences were due to the storage period (ANOVA, Table 4). The highest AA obtained for the cultivar JB (2.986 mg.L⁻¹) followed by the cultivars GB, TN, and ZH. The lowest value of AA recorded for the cultivar KH (1.632 mg.L⁻¹). Antioxidant activity of KH and ZH increased after storage compared to the value recorded initially at day 0; by contrast, arils from the rest of the cultivars showed a decrease in AA, which could be attributed to the high content in phenols and, or anthocyanins compounds after storage. Cisneros-Zevallos (2003) reported stress-induced phenolic compounds might increase the antioxidant activity of cv. 'Yousef-Khani' arils, despite the reduced levels of total phenolic, relate it to the increased amount of acidity.

It could conclude that the accordance between antioxidant activity and phenolic contents, on the one hand, and anthocyanins, on the other, depends on the type of the particular cultivar under study.

At the initial date, arils from all cultivars did not show high differences on vitamin C (Table 4), and it ranged from 67.6 mg.L⁻¹ (JB) to 76.0 mg.L⁻¹ (KH), a mean value of 72.7, 71.8, and 71.1 mg.L⁻¹ for cultivars ZH, TN and GB respectively. During storage, vitamin C decreased significantly for all cultivars except JB. By the end of 10 days of storage, all arils exhibited a reduction in vitamin C compared to the content registered initially. The lowest content recorded after 10 days for the cultivar KH (48.7 mg.L⁻¹). The decrease in vitamin C could be related to the irreversible oxidation of dehydro-L-ascorbic acid (DHAA) to 2, 3-



diketone-L-gluonic acid (Arendse et al., 2015). O'Grady et al. (2014) observed a decline of ascorbic acid over storage in 'Ruby' arils stored for seven days.

Difference in sensory evaluation

Sensory attributes varied significantly (Table 5) among cultivars and after storage periods. Initially, ready to eat pomegranate arils (REA) from all cultivars revealed differences in sensory quality. Initially, differences in color attributes were registered by panelists with the best score given to KH, followed by GB and JB (Fig. 2). REA from cultivars TN and ZH were the lowest scored for visual color. Concerning the aroma, the cultivars ZH, and TN, has shown the lowest scores compared to the rest of cultivars. Considering all sensory attributes, arils of the cultivar ZH was the least appreciated while, arils of the cultivar KH was the most appreciated.

After ten days, all cultivars showed significant losses for most the sensory attributes with different degrees. Based on visual color, ZH and TN arils remained the colorless cultivars. The score color for KH, JB, and GB ranged around six, which considered regular to vigorous intensity. The cultivar JB has shown the highest decrease in taste and aroma at the end of 10 days, falling to have a similar quality to ZH, while KH, GB, and TN were more appreciated for their smell and taste. Despite the critical changes in quality attributes, mainly in acidity, REA from the cultivar KH showed the best sensory quality after ten days. GB has also demonstrated a good aril sensory quality being the most stable for its quality attributes during storage. Except for whitish arils color, TN maintained good characteristics after storage. Despite its pleasing color, REA from the cultivar JB lost its aroma, taste, acidity, and sweetness after ten days, which led to low appreciation. Like its quality at day 0, REA from cultivar ZH were less appreciated for its sensory attributes after ten days. Generally, all cultivars exhibited REA with scores above the limit of acceptance (> 3). At the end of storage, the best-evaluated cultivars with a score of 5 and above (Fig. 2) were GB, KH, and TN. They considered as desirable for commercial purposes.

Principle Component Analysis (PCA) of arils during storage

The principal component analysis resulted in seven principal components, out of which only PCs 1-4 showed good correlations and accounted for 86.8% of the total variability. The cumulative variance showed that almost all the variations in bioactive content, the physico-chemical and sensory evaluation of fresh arils and after ten days from different cultivars could be explained either strongly or moderately by these four principal components. Acceptable explanations drawn from the first two PC plots, which accounted for 57.8% of the total variance (Fig. 3).

Considering factor loadings, principal component 1 (PC1) showed high positive scores for TAC, AA, vitamin C, color, aroma, taste, and sweetness associated with fresh arils from cultivars GB, JB, and KH. Moreover, high negative scores along PC1 (Fig. 3) corresponded to arils of the cultivars TN and ZH after ten days of storage. These factors were associated with TA, L*and b*. Meanwhile, along PC2, we can distinguish arils of cultivars GB, JB, and KH after ten days with high positive scores corresponded to TSS, pH, TA, and a*. Negative numbers along PC2 grouped L*, and TPC were for arils from the cultivar ZH initially and after ten days of storage.



	F-ratio					
ANOVA	df	Color	Aroma	Taste	Sweetness	Acidity
A: cultivar	4	6.9^{*}	9.9^{*}	9.8^{*}	3.6*	10.3*
B: storage	2	27.7^{*}	18.3^{*}	54.5^{*}	36.0^{*}	21.0^{*}
A×B	8	0.9 ^{ns}	10.2^{*}	9.3*	6.2^{*}	11.5^{*}

Fable 5. Analysis of variance	for the evaluated sensory	attributes of ready	to eat pomegranate arils
2	2	2	

*Significance of F-ratio at $P \le 0.05$. ns not significant at P > 0.05, df: degree of freedom.





Fig. 2. Radar plots of sensory attributes of ready to eat pomegranate arils from five cultivars at initial day (A) and after ten days (B) of storage at 5 °C. Cultivars: GB: Gabsi; JB: Jebali; KH: Khalledi; ZH: Zehri; TN: Tounsi.



Correlations between instrumental and sensory attributes

Cultivars of high nutritional content and sensory quality and stability during storage are key indices for ready to eat pomegranate arils. Pearson's correlation analysis showed positive and negative correlations among several traits were summarized in Table 6. TAC was positively correlated with redness a^* (r = 0.88; P ≤ 0.05) and negatively with sweetness (-0.89; P ≤ 0.05).

Table 6. Correlation between instrumental and sensory analysis of ready to eat pomegranate arils after ten days of storage

	TAC	TPC	AA	VIT C	F	TSS	pН	TA	L*	a*	b*	C*	Color	Aroma	Acidity	Sweetness
TAC	1															
TPC	-0.53	1														
AA	-0.02	0.6	1													
VIT C	-0.36	0.4	0.59	1												
F	0.59	-0.52	-0.12	-0.80	1											
TSS	-0.03	-0.50	0.15	0.21	0.19	1										
pH	0.57	-0.85*	-0.12	-0.28	0.64	0.76										
TA	0.18	-0.74	-0.09	0.07	0.26	0.94^{**}	0.88^{*}	1								
L*	0.11	-0.11	-0.48	-0.92*	0.62	-0.46	-0.10	-0.40	1							
a*	0.88^*	-0.21	0.11	-0.01	0.17	-0.29	0.21	-0.09	-0.13	1						
b*	0.32	-0.44	-0.87^{*}	-0.66	0.19	-0.52	-0.04	-0.25	0.60	0.28	1					
C*	0.70	-0.46	-0.58	-0.46	0.22	-0.49	0.12	-0.18	0.33	0.73	0.86^{*}	1				
Color	0.36	0.21	-0.22	-0,54	0.25	-0.89^{*}	-0.43	-0.79	0.69	0.44	0.63	0.64	1			
Aroma	0.24	-0.23	0.17	0,70	-0.47	0.38	0.29	0.48	-0.89^{*}	0.44	-0.22	0.12	-0.51	1		
Acidity	0.55	0.13	0.42	0.52	-0.30	-0.20	-0.03	-0.12	-0.57	0.84^{*}	-0.10	0.38	0.14	0.70	1	
Sweetness	0.89^{*}	-0.18	0.40	0.03	0.35	-0.03	0.39	0.08	-0.20	0.91^{*}	-0.05	0.44	0.25	0.39	0.79	1

*: correlations significant at $P \le 0.05$ according to Pearson correlation.

**: correlations significant at $P \le 0.01$ according to Pearson correlation



Fig. 3. Plot of the first two components of physico-chemicals, bioactive, and sensory attributes of different pomegranate cultivars at the initial day (0) and after ten days of storage. GB0: fresh arils of Gabsi. GB10: arils from Gabsi after ten days. JB0: fresh arils of Jebali. JB10: arils from Jebali after ten days. KH0: fresh arils of Khalledi. KH10: arils from Khalledi after ten days. ZH0: fresh arils of Zehri. ZH10: arils from Zheri after ten days. TN0: arils of Tounsi. TN10: arils from Tounsi after ten days. F: Firmness, TA: Titratable acidity, TSS: Total soluble solids, TPC: Total phenol content, TAC: Total anthocyanin content, AA: Antioxidant activity, and Vit C: Vitamin C.



The TPC was negatively correlated to pH (r = -0.85; P ≤ 0.05). The AA correlated negatively to yellowness b^{*} (-0.87; P ≤ 0.05), which means arils with high b^{*} values are poor in antioxidants. Vitamin C was negatively correlated (-0.92; P ≤ 0.05) to lightness L^{*}. Titratable acidity, pH, and TSS were positively correlated and negatively to visual color evaluation. Sensory acidity and sweetness were positively correlated with redness a^{*} (r = 0.84; P ≤ 0.05 ; 0.91; P ≤ 0.05). The yellowness b^{*} correlated positively to chroma C^{*} (r = 0.86; P ≤ 0.05). The aroma correlated negatively to lightness L^{*}. Based on these correlations, we found that the evaluation of ready to eat pomegranate arils was strongly dependent on L^{*}, a^{*}, and b^{*}color attributes and sensory traits.

CONCLUSION

The studied cultivars have shown different quality traits based on the fresh arils, where arils from the cultivar KH distinguished for the best nutritional and sensory quality compared to cultivars GB and JB. The less appreciated ready to eat arils were from the cultivar ZH, for its lightness and low sensory quality. Even though the chemical analysis showed great changes between cultivars rather than changes during the storage periods, principle component analysis enabled us to discriminate groups of the studied cultivars based on the storage period. Among the studied cultivars, the main changes during storage resulting for the cultivars JB and KH; and these changes registered mainly in their color attributes and sensory traits. Although, GB has shown minimal changes on its quality, giving better aril quality after storage. Despite their low initial rate, ready to eat arils from cultivars ZH and TN showed slight changes. From the obtained results, cultivars GB and KH were considered as the best cultivar for ready to eat arils marketing.

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Conflict of interest

There is no conflict of interest to declare.

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