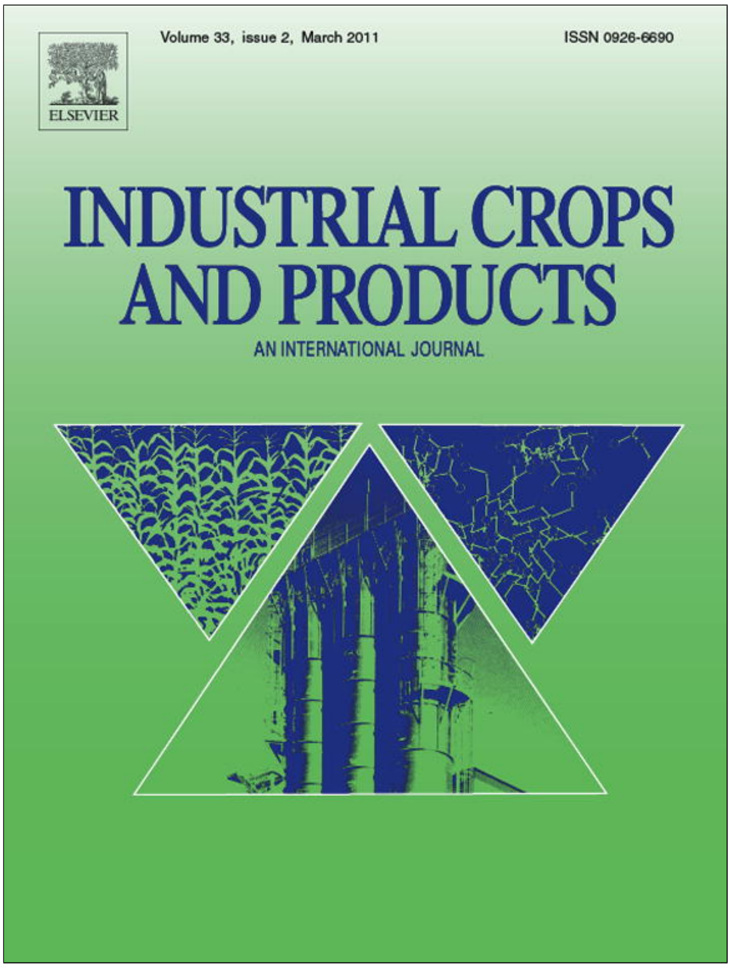
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Seed and juice characterization of pomegranate fruits grown in Tunisia: Comparison between sour and sweet cultivars revealed interesting properties for prospective industrial applications

Nejib Hasnaoui[a,c,*], Messaoud Mars[a], Sana Ghaffari[b], Mokhtar Trifi[c], Pablo Melgarejo[d], Francisca Hernandez[d]

[a] U.R. Agrobiodiversity, Higher Agronomic Institute, 4042 Chott Mariem, Sousse, Tunisia
[b] Laboratory of Aridland and Oases Cropping, Institute of Arid regions, Medenine, Tunisia
[c] Laboratory of Molecular Genetics, Immunology & Biotechnology, Faculty of Sciences of Tunis, 2092 El-Manar, Tunis, Tunisia
[d] Department of Plant Production and Microbiology, University of Miguel Hernández, Alicante, Spain

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Dedicated to the memory of Professor Mohamed Marrakchi, who passed away in April 2008.

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ABSTRACT

Tunisian pomegranate genetic resources consist of sweet and sour cultivars, showing large morphometric variability. In the present work we characterized seeds and juice contents of sugars and organic acids of 5 sour and 7 sweet pomegranate cultivars. Results showed that citric acid was predominant in sour pomegranates, while malic acid was the most prevalent in sweet ones. Paradoxically, sour cultivars have higher sugar content than the sweet ones. A strong correlation was found between sourness and citric acid content, which is assumed to be the major factor that determines sour taste in pomegranate fruits. Besides, some of the seed parameters showed a significant positive correlation with acidity. Sweet cultivars were appropriate for fresh consumption and juice production due to several attributes in addition to their sweetness. Equally, sour pomegranate showed several characteristics that could be of great interest for food and nutraceutical industries.

1. Introduction

In recent years, there has been an explosion of interest in pomegranate fruit, based on its high antioxidant activity as well as its several medical benefits (Caligiani et al., 2010; Gonzalez-Molina et al., 2009; Kasimsetty et al., 2010; Lansky and Newman, 2007; Panichayupakaranant et al., 2010; Ryan and Prescott, 2010; Tehranifar et al., 2010). It follows an increasing demand for industrial processing to make juice, jams, syrup, sauce, nutraceuticals, etc., in addition to the growing demand for fresh consumption. The edible part of pomegranate fruits (the arils) contains large amounts of organic acids, sugars, minerals, vitamins and polyphenols (Al-Maiman and Dilshad, 2002; Poyrazoglu et al., 2002; Tehranifar et al., 2010).

Mediterranean and Middle-East countries are the main regions of pomegranate cultivation and production (Jbir et al., 2008; Melgarejo et al., 2009). In Tunisia, pomegranate is one of the most important fruit trees. The total production exceeded 70,000 t in 2008 (Bchir et al., 2009). Almost all these productions are based on few cultivars, with interesting market characteristics, despite the quite large number of local ecotypes listed (Mars, 2001). In fact, in new plantations that are meant for exportation, many cultivars are becoming abandoned, despite their high potential of valorization. For instance, acidity (or sourness) could be an interesting trait for several purposes (blending juice of other fruits for example).

In the frame of this growing interest in this fruit species, it is highly important to knowledge fruit characteristics, particularly the edible part. This is a necessary step to get essential and useful information for fresh market and processing industry, as well as for cultivars classification. This will help for the best germplasm management and further utilization in breeding program and cultivar selection using cultivar with desirable traits.

Pomegranate preference characteristics are determined through the taste of juicy seed coat and the unpalatability due

* Corresponding author at: Higher Institute of Applied Biology, Rte El-Jorf, km 22.5, 4119, Medenine, Tunisia. Tel.: +216 95819947.
E-mail address: nejibhasnaoui@yahoo.fr (N. Hasnaoui).

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to the woody portion. These traits are of interest not only for consumer, but also for cultivators, breeders and industrials. Taste is determined mainly by organic acid–sugar content balance of the fruit, and these compounds serve as unequivocal markers for sensory attributes assessment and genotype characterization (Melgarejo et al., 2000; Poyrazoglu et al., 2002). As for the unpalatability of pomegranate arils, it is due to the seed hardness, which can be measured by the woody portion index (WPI) (Martínez et al., 2006). Obviously, these characteristics depend on many factors and the interaction between them: genotypes, climate, cultural practice, etc. (Borochov-Neori et al., 2009; Schwartz et al., 2009a,b).

Several reports have shown high variation in morphometric traits and chemical characteristics in different pomegranate accessions from several geographic regions (Martínez et al., 2006; Melgarejo et al., 2000; Poyrazoglu et al., 2002; Sarkhosh et al., 2009; Tehranifar et al., 2010; Zamani et al., 2010). However, in Tunisia there is scarcity in seed morphological and chemical characterization of local cultivars of pomegranate. In the current study we assess the variation of some seed and juice characteristics of Tunisian pomegranate cultivars, and we perform a comparison between sweet and sour pomegranate accessions originated from different locations.

2. Materials and methods

2.1. Fruit samples

Pomegranate fruits were sampled at ripe stage, *i.e.* ready for fresh consumption, from one, two or three trees depending on the tree's number per cultivar available. Twelve autochthonous cultivars, namely: 'Gabsi 1', 'Mezzi 1', 'Mezzi 2', 'Mezzi 3', 'Garoussi 2', 'Gabsi 5', 'Gabsi 9', 'Chelfi 1', 'Chelfi 3', 'Zehri 6', 'Garoussi 1' and 'Tounsi 4' are included in the present study. The five formers produce sour fruits and the seven latters have sweet fruits. Although pomegranate accessions are classified by several authors as sour, sour–sweet and sweet accessions (Poyrazoglu et al., 2002; Melgarejo et al., 2000), the cultivars included in the present study were carefully chosen to be either sour or sweet, to avoid unambiguous clear-cut grouping that may affect results and comparisons. All trees are maintained in the Tunisian national germplasm collection of pomegranate located at Zerkin (33°45′N, 10°16′E) (Mars, 2001), and cultivated under homogenous conditions, without any special management (no fertilizers, no irrigation except natural rainfall).

2.2. Seed characters

Five characteristics were measured on a homogenized sample of 75 seeds (woody part of the arils) extracted from 3 fruits representing each cultivar (25 seeds per fruit): SL (seed length), ST1 and ST2 (seed thicknesses, which correspond to the two maximum thicks in the transverse section), SW (seed weight), and SS (seed shape). For seed shape, we identified 3 kinds of seeds: oblongs, ovate and globose, for which we gave the digit: 1, 2 or 3, respectively. In addition, the aril weight (AW) was also measured on 75 arils extracted from the same 3 pomegranate fruits, as well as the woody portion index (WPI). The latter was determined as: $[SW/AW] \times 100$ (Martínez et al., 2006). The measurements were carried out using digital calliper (Furiya, Japan) and the weights using an electronic balance (Mettler AJ50).

2.3. Juice extraction and HPLC analyses

In the laboratory, 3 ripe fruits representing each cultivar were hand-peeled and the arils were extracted. Subsequently they were juiced using a commercial blender. The crude juices were sieved to eliminate solid particles of pips. The pre-filtered juices were centrifuged at 15,000 rpm for 20 min. One millilitre of the centrifuged liquid was passed through a 0.45 μm Millipore filter and then injected into a Hewlett-Packard HPLC series 1100. The elution system consisted of 0.1% phosphoric acid with a flow rate of 0.5 mL/min. Organic acids were separated on a Supelcogel™ C-610H column (30 cm × 7.8 mm i.d., Supelco, Bellefonte, PA, USA) and detected by absorbance at 210 nm. Sugar analysis was performed on a μBondapak-NH2 column (30 cm × 3.9 mm i.d., Waters, Milford, MA, USA) using acetonitrile/water (85:15, v/v) as mobile phase. Sugars were detected with a refractive index detector. Standard curves for pure organic acids (oxalic, citric, tartaric, malic, acetic, fumaric, succinic and ascorbic acids) as well as for pure sugars (glucose, maltose, fructose, sucrose and sorbitol) purchased from Sigma (Poole, Dorset, UK), were used for quantification. Results were expressed as concentrations (g/100 g).

2.4. Statistical analysis

XLSTAT 2010 (http://www.xlstat.com) software is used to carry out descriptive statistics, as well as the correlation between similarity matrices (based on seed characters and juice parameters) using Mantel's test (Mantel, 1967) based on 10,000 random permutations. Histograms were built using Excel 2003 (Microsoft).

3. Results and discussions

3.1. Seed characteristics

The parameters measured on edible portion of pomegranate fruits are of economic interest and therefore they are of the most important targeted traits by growers, breeder and industrials. Fig. 1 shows obtained data concerning seed parameters measured on sour and sweet pomegranate cultivars. The woody portion index (WPI), the seed weight (SW) and the aril weight (AW) are the characteristics showing the highest variability. Their coefficients of variation were 42%, 29% and 25%, respectively (data not shown). Average WPI ranged between 2.16% ('Chelfi 3') and 7.33% ('Mezzi 1') with an overall mean of 4.43%. A significant difference was observed between sour and sweet accessions. The WPI mean in sour cultivars is twice higher than in sweet cultivars. Thus, the relatively high WPI, in addition to their sourness, increases the unpalatability of sour cultivars. Martínez et al. (2006) consider WPI as good parameter that reflects the wood quantity in edible part of pomegranate fruit. The WPI is significantly correlated with all other seed characters, except the seed length (SL). The remaining parameters presented less variation, and therefore were less discriminatory between cultivars, and between sour and sweet groups.

In comparison with similar previous studies, the Tunisian sweet pomegranate cultivars, for which the highest WPI was 3.35%, showed a very interesting trait dealing with fresh consuming preferences, since much higher WPI values were obtained from pomegranate cultivars from Spain (mean value ∼8%) (Martínez et al., 2006) and from Iran (mean value ∼7.5%) (Sarkhosh et al., 2009). It is also worth to report that this character had also the highest variation among all the other fruit traits in Iranian soft-seed cultivars (Sarkhosh et al., 2009). Thus, this character should be considered for any prospective selection (in addition to crude fibre and seed hardness). For instance, the cultivar 'Chelfi 3', which presents the lowest WPI and the highest AW, offers very promising genotypes for breeders to generate cultivars with greater agronomic potential.

3.2. Organic acids

HPLC was used to quantifying 8 organic acids: acetic, ascorbic, citric, fumaric, malic, oxalic, tartaric and succinic acids. Among

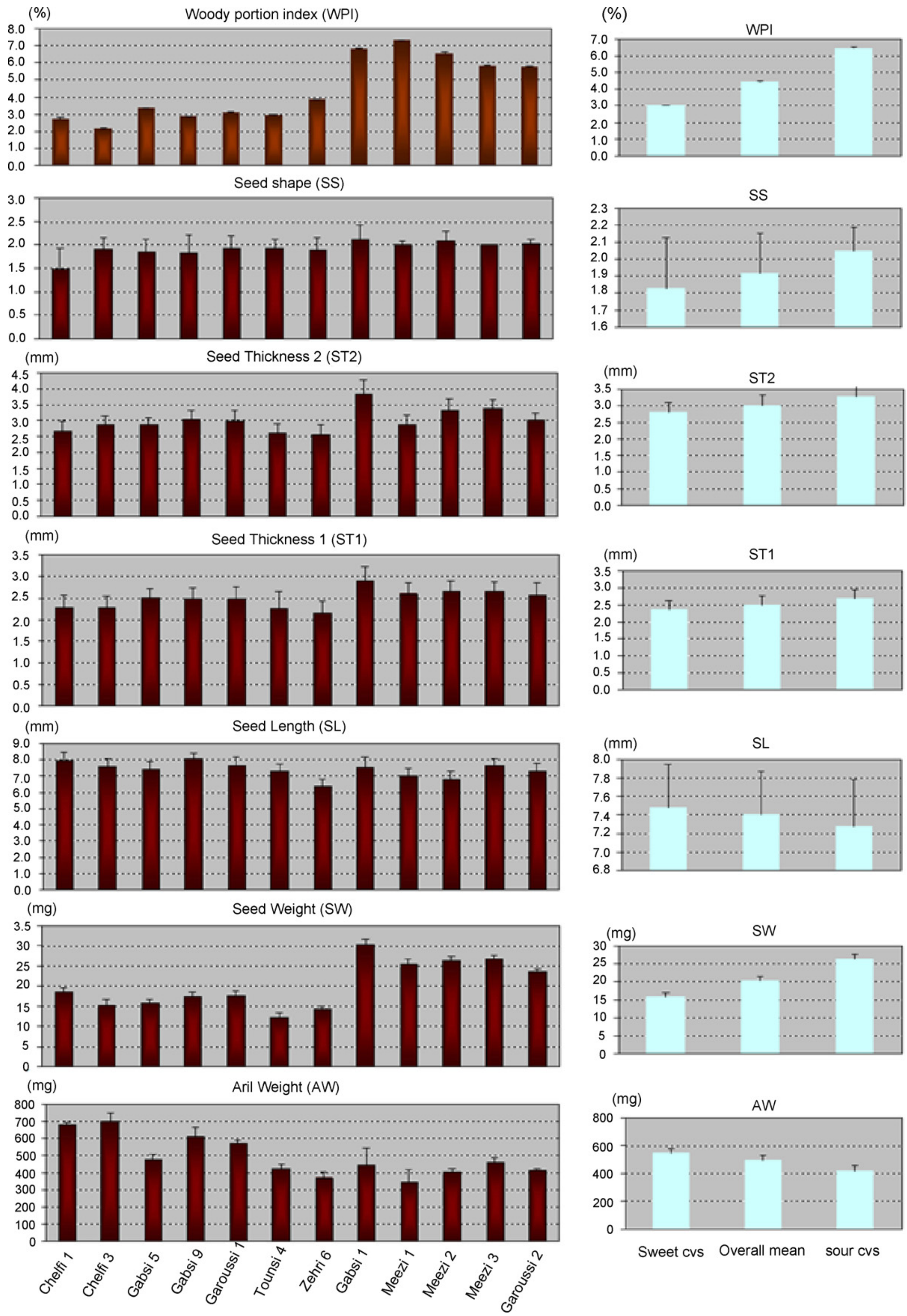

Fig. 1. Seed characteristics of pomegranate cultivars, and mean comparison between sweet and sour cultivars. The data are the mean of 75 replicates of each cultivar.

them, neither fumaric acid nor acetic acid, which have been previously identified in Spanish and Iranian pomegranate samples (Melgarejo et al., 2000; Aarabi et al., 2008), could be detected in the present study. The contents of the six remaining organic acids are displayed in Fig. 2. Few amounts of tartaric and ascorbic acids were found; only traces of tartaric acid were detected in all the sour cultivars, whereas in sweet ones its contents ranged between 10 mg/100 g ('Tounsi 4') and 151 mg/100 g ('Gabsi 5'). Lesser quantities of ascorbic acid have been detected, but with significant difference between sour and sweet cultivars, since it was possible to quantify the ascorbic acid only in one sweet cultivar: 'Gabsi 9' (9 mg/100 g), while higher quantities were detected in all sour cultivars, where it reached 37 mg/100 g in 'Mezzi 2'.

Relatively higher contents of oxalic and succinic acids were registered, with respective general means of 0.219 and 0.513 g/100 g. In detail, registered values of the oxalic acid are much higher in sweet than in sour cultivars, whereas the situation is reversed regarding the distribution of succinic acid (Fig. 2).

Besides, the major organic acids in pomegranate fruit are: citric and malic acids. As for these two acids, sour and sweet cultivars have not the same profile. Citric acid was the major organic acid in sour cultivars, while in sweet cultivars the malic acid was the predominant. In average the sour cultivars contain ~15 times more of citric acid than sweet ones (Fig. 2). Similar findings regarding major acids and their distribution in sweet and sour accessions originated from diverse countries have been previously reported by Dafny-Yalin et al. (2010), Melgarejo et al. (2000), Ozgen et al. (2008), Pande and Akoh (2009), Poyrazoglu et al. (2002) and Schwartz et al. (2009a,b), though some authors did not give specifications as for classification of the studied accessions. Globally, a large variation was showed within Tunisian pomegranate accessions, and the values' ranges were consistent with previous studies.

From the present results, we can infer that sourness (or acidity) of pomegranate fruits and their citric acid content are well correlated. Such correlation was clearly established for sour and sweet pomegranate cultivars from Turkey (Ozgen et al., 2008) and Spain (Melgarejo et al., 2000). Thus, the cultivar 'Mezzi 2', with very strong-acid taste, has the highest citric acid content, and also the highest total organic acid content. In a previous study, Mars (2001) reported the highest titratable acidity and the lowest pH values for 'Mezzi 2' in comparison with 30 cultivars studied.

3.3. Sugars

Among the targeted seven sugars, neither maltose nor sorbitol could be detected in any of the studied cultivars. Also, galactose could not be detected in the majority of studied cultivars (data not shown). Globally, these results are consistent with the literature dealing with sugars identification and quantification in pomegranate fruit. Indeed, only Dafny-Yalin et al. (2010) and Melgarejo et al. (2000) report the existence of traces of maltose in pomegranate, while sorbitol and galactose have never been reported. Thus, the total sugar content of pomegranate fruit, which varied between 17.77 and 19.98 g/100 g for the sour cultivars and between 13.13 and 16.55 g/100 g for the sweet ones (data not shown), is made up essentially of fructose and glucose (Fig. 3), in addition to fewer quantities of arabinose. Sucrose was less prevalent, since it could be quantified only in two cultivars: 'Gabsi 1' (208 mg/100 g) and 'Chelfi 3' (32 mg/100 g). The predominance of fructose and glucose found in the present work was in agreement with all previous works. However, results differ regarding the major sugar (fructose or glucose). For example, Al-Maiman and Dilshad (2002), Cam et al. (2009) and Ozgen et al. (2008) reported that glucose concentrations were higher than those of fructose. Schwartz et al. (2009a,b) found equal amounts of these two sugars in 10 pomegranate varieties, while Melgarejo et al. (2000), Poyrazoglu et al. (2002) and Tezcan et al. (2009) results were similar to our findings, since in all cultivars fructose was the predominant. In 'Gabsi 9' and 'Garoussi 1' there is 1.3 times more of fructose than glucose. These two sweet cultivars have a major advantage with respect to fruit and juice qualities by accumulating more fructose than glucose, given that fructose is approximately twice sweeter than glucose (Levin et al., 2000).

It is important to note that fructose as well as glucose levels were higher in the sour cultivars than that in the sweet ones. For sour pomegranates, fructose concentrations ranged between 9.46 g/100 g ('Garoussi 2') and 10.61 g/100 g ('Mezzi 2'), and for sweet ones from 7.21 g/100 g ('Zehri 6') to 9.02 g/100 g ('Gabsi 9'). Subsequently, a statistical correlation between sourness and sugars content of pomegranate arils could be inferred. The present results are in agreement with sugars profile of sour and sweet pomegranate accessions from Turkey (Ozgen et al., 2008), but in contrast with results reported by Melgarejo et al. (2000) and Schwartz et al. (2009b).

Obviously, variations in these parameters could be assigned to the diversity of agro-climatic conditions, but we believe that cultivar (genotype) effect has the major effect on the accumulation of sugar as well as of organic acids in fruits, in this case pomegranate.

3.4. Sugars to acids ratios and relation between measured attributes

Fruits' organic acid profiles play an important role in their organoleptic properties by enhancing or reducing their palatability. Given that sourness is generally attributed to proton release from acids (Sweetman et al., 2009), and based on our results, it ensues that citric acid content of arils appears as the main determinant of sourness in pomegranate fruits, given that paradoxically sour cultivars contained more sugars than the sweet. Thus, both sourness and sweetness in pomegranate are closely linked to the citric acid concentration. Similar conclusion was advanced for orange (Karadeniz, 2004) and melon (Albuquerque et al., 2006). This hypothesis is strongly supported genetically. By studying the acidity inheritance in pomegranate, Jalikop (2007) concluded that sourness is dominant over sweetness and that this trait is controlled by one major dominant gene. He used "low acid variety" as synonym of "sweet variety", *i.e.* sweetness and sourness are the "heads and tails" of the same trait, at least genetically.

Both sugars and organic acids play a major role in determination of fruit quality and ripeness, and the sugars to organic acids ratio is commonly considered as good index of fruit quality. As shown in Table 1, this ratio varied from 3.19 to 4.92 for sour cultivars and from 3.84 to 11.73 for the sweet ones. If we omit the cultivar 'Zehri 6', the difference between sour and sweet pomegranate accessions is clear. For 'Zehri 6', though it has low sugars/organic acids ratio (3.84), which is situated in the range value of the sour cultivar, this cultivars has sweet taste. Its sweet taste is due to the low quantity of citric acid.

In the aim to get more reliable classification criterion, we calculated the total sugars to citric acid ratio (Table 1). We will pass this ratio as "sourness index: SI" for pomegranate classification. Practically all the sour cultivars have an SI < 10, and the SI of sweet ones are all above 60, except the cultivar 'Zehri 6'. For this latter, which has an SI of ~40, now the situation is more clear, since its SI is four times higher than the highest value of SI in sour group. Its relatively low SI is due, in fact, to it its low sugars' content. Thus, the SI appears as good and reliable classification criterion of pomegranate cultivars/accessions. It is more efficient than the sugars to organic acids ratio or total soluble solids/acidity (TSS/A) known as maturity index (MI). This is well illustrated by the case of 'Tounsi 4' also. Fruits from this cultivar have a very-strong sweet taste and have shown the highest SI (~ 354), but their sugars/organic acids ratio

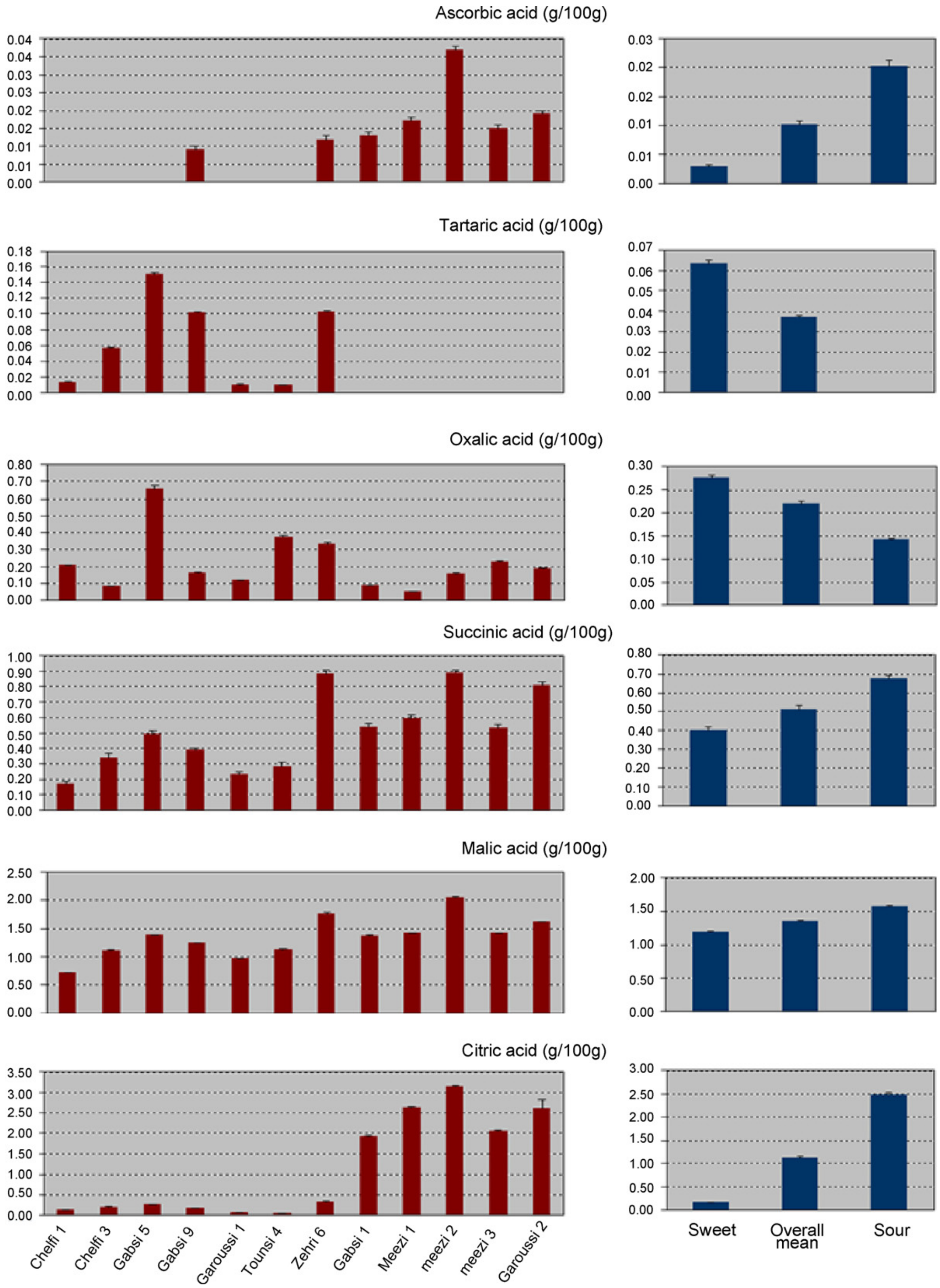

Fig. 2. Organic acid contents of sour and sweet pomegranate and mean comparison among them. Mean values are of 3 fruits for each cultivars.

do not reflect their strong sweetness. As we assume that acidity in pomegranate is governed by the citric acid concentration, the SI takes account directly of this assumption and therefore gives more reliable indication regarding pomegranate sourness and therefore its classification. Surely, that sensometric study, using larger fruit samples and pomegranate cultivars, is compulsory to validate the usefulness of the SI and establish accurate scale of classification (Table 1).

Correlation between all the studied characters was generated using XLSTAT 2010 (Table 2), and several significant correlations

Table 1
Total organic acids (Organic A.), total sugars (Sugars) (value ± standard deviation), sugars to organic acids ratio (Sug/Org A.) and sourness index (SI) of sweet and sour Tunisian pomegranate cultivars.

	Organic A. (g/100 g)	Sugars (g/100 g)	Sug/Org A.	SI
Sweet cultivars				
'Chelfi 1'	1.24 ± 0.01	14.57 ± 0.04	11.73	113.81
'Chelfi 3'	1.79 ± 0.01	16.55 ± 0.05	9.22	80.72
'Gabsi 5'	2.91 ± 0.01	15.21 ± 0.05	5.23	63.37
'Gabsi 9'	2.09 ± 0.01	15.98 ± 0.03	7.65	91.84
'Garoussi 1'	1.40 ± 0.01	14.93 ± 0.07	10.67	229.74
'Tounsi 4'	1.83 ± 0.01	15.96 ± 0.03	8.70	354.56
'Zehri 6'	3.42 ± 0.01	13.13 ± 0.07	3.84	40.15
Sour cultivars				
'Gabsi 1'	3.91 ± 0.01	19.27 ± 0.05	4.92	10.09
'Mezzi 1'	4.71 ± 0.01	19.20 ± 0.05	4.08	7.28
'Mezzi 2'	6.26 ± 0.01	19.98 ± 0.05	3.19	6.37
'Mezzi 3'	4.24 ± 0.01	17.78 ± 0.05	4.19	8.64
'Garoussi 2'	5.26 ± 0.04	18.36 ± 0.04	3.49	7.00

arise from. The highest significant correlation value (+0.975) was registered between citric acid and arabinose and lowest value (−0.582) was the one between glucose and tartaric acid.

Besides, it is worth to bring to the fore the correlation between sourness and some of the studied characters. Such correlations can be inferred from Figs. 1–3 that display comparison between sour and sweet mean values. It is also supported by the highly significant correlation value between citric acid and some morphometric seed attributes (Table 2). Moreover, the Mantel's test (Fig. 4) showed a high positive correlation between the two similarity matrices (based on seed and juice parameters) (r = 0.715, p-value < 0.0001).

One of the main characteristics of sour cultivars is their high woody portion index (WPI) and therefore they have big seeds. Indeed, the citric acid and WPI have a highly significant correlation: 0.935. Obviously, these traits are unacceptable for fresh mar-

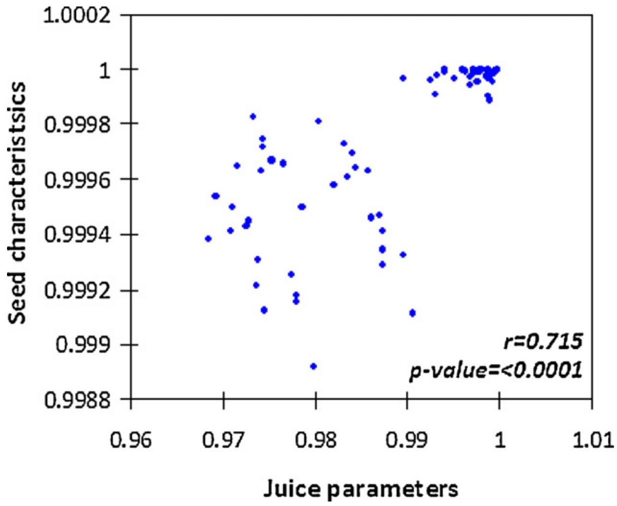

Fig. 4. Correlation of the two proximity matrices (based on seed and juice characters) of 12 pomegranate accessions using Mantel's test.

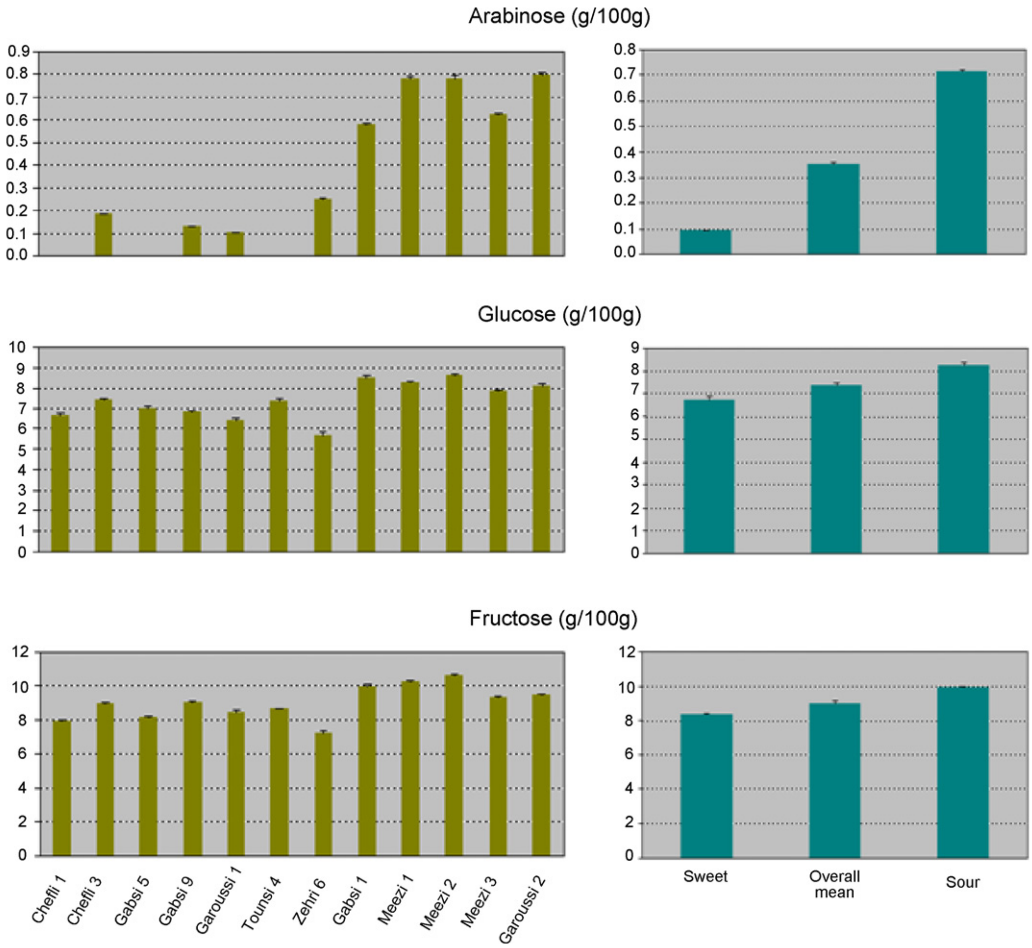

Fig. 3. Sugars contents of sour and sweet pomegranate and mean comparison among them. Mean values are of 3 fruits for each cultivars.

Table 2
Correlation between seed and juice parameters in pomegranate. On diagonal either side are correlation values and their significance.

Variables	AW	SW	SL	ST1	ST2	SS	WPI	Oxalic	Tartaric	Malic	Ascorbic	Citric	Succinic	Arabinose	Fructose	Glucose	Sucrose
AW	1	ns	**	ns	ns	*	**	ns	ns	**	*	*	**	*	ns	ns	ns
SW	−0.361	1	ns	***	***	ns	***	ns	*	ns	*	***	ns	***	**	**	ns
SL	0.757	−0.008	1	ns	ns	ns	ns	ns	ns	**	ns	ns	**	ns	ns	ns	ns
ST1	−0.323	0.894	0.134	1	**	*	**	ns	ns	ns	ns	*	ns	*	**	**	*
ST2	−0.135	0.834	0.209	0.912	1	*	*	ns	ns	ns	ns	ns	ns	ns	*	*	*
SS	−0.628	0.553	−0.405	0.639	0.608	1	*	ns	ns	*	*	*	ns	*	*	*	ns
WPI	−0.716	0.893	−0.394	0.771	0.618	0.687	1	ns	ns	*	**	***	*	***	**	**	ns
Oxalic	−0.176	−0.496	−0.130	−0.312	−0.375	−0.252	−0.348	1	*	ns	ns	ns	ns	ns	ns	ns	ns
Tartaric	0.162	−0.588	−0.034	−0.375	−0.373	−0.303	−0.519	0.653	1	ns	ns	ns	ns	ns	ns	*	ns
Malic	−0.728	0.382	−0.740	0.311	0.272	0.654	0.616	0.059	0.041	1	***	*	**	*	ns	ns	ns
Ascorbic	−0.587	0.688	−0.501	0.515	0.470	0.589	0.791	−0.323	−0.363	0.850	1	***	**	***	*	*	ns
Citric	−0.602	0.861	−0.354	0.695	0.563	0.660	0.935	−0.387	−0.563	0.670	0.883	1	*	***	***	***	ns
Succinic	−0.730	0.395	−0.770	0.263	0.202	0.575	0.634	0.014	0.021	0.963	0.815	0.676	1	*	ns	ns	ns
Arabinose	−0.591	0.842	−0.380	0.659	0.550	0.706	0.922	−0.485	−0.553	0.673	0.860	0.975	0.707	1	**	**	ns
Fructose	−0.313	0.793	−0.032	0.768	0.683	0.684	0.765	−0.537	−0.568	0.412	0.702	0.832	0.320	0.790	1	***	ns
Glucose	−0.338	0.787	−0.021	0.750	0.674	0.647	0.755	−0.362	−0.582	0.369	0.610	0.825	0.302	0.759	0.934	1	ns
Sucrose	−0.038	0.494	0.115	0.586	0.706	0.377	0.344	−0.289	−0.205	−0.018	0.036	0.165	0.001	0.191	0.307	0.397	1

ns, not significant.
* Significant at $\alpha = 0.05$.
** Significant at $\alpha = 0.01$.
*** Significant at $\alpha = 0.001$.

kets by decreasing the palatability of sour cultivars and therefore affect drastically their suitability for fresh consumption. However, acid pomegranate fruits show highly interesting properties. For instance, their acidity, which is due to their richness in citric acid, is a much sought-after property for meat storage (Shuming et al., 2009; Naveena et al., 2008). Also, by having “big” seeds (woody portion index), the sour cultivars can be used as source for nutraceutical substances production, given that pomegranate seeds are among the rare plants having high concentration of sex-steroid hormone (Kho et al., 2010). Furthermore, their lipid fraction showed beneficial biological effects (Caligiani et al., 2010). More commonly, sweet pomegranate cultivars can be used for production of fresh juice with high potential of health benefit. Also, based on some of their attracting characteristics for fresh consumption (low WPI); they can be of interest for food processing industry for commercialization of ready-to-eat arils.

4. Conclusion

Data collected during this work showed a quite large diversity among local pomegranate germplasm. Several parameters allowed clear discrimination between sour and sweet pomegranate accessions, as well as within each group. Citric acid content seems to be the major determinant of sourness in pomegranate. Sweetness is due to a low content of this acid, since sour pomegranate cultivars contained more sugars than sweet ones. We proposed the sourness index (SI); the ratio between total sugars and citric acid contents, as reliable scale of classification of pomegranate fruit taste.

Data issued from the present work dealing with important seed attributes and chemical parameters could help to develop biochemical and ampelographic markers to obtain a cultivar-specific pattern useful for germplasm conservation, exploitation and valorization. If valorization of sweet accessions is more evident, sour pomegranates, which are not meant for fresh consumption, showed very interesting characteristics too, that could be of great interest in food and health sectors.

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