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Geographical Discrimination of Iranian Pomegranate Cultivars Based on Organic Acids Composition and Multivariate Analysis

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ABSTRACT

In this study, geographical discrimination of some Iranian pomegranate cultivars was investigated using chromatographic analyses and multivariate statistical methods. The organic acid content of 24 pomegranate samples of different cultivars (5 sweet, 7 sweet-sour and 12 sour cultivars) from different production sites (Yazd and Markazi Provinces, Iran) were analyzed by HPLC/UV. Ten organic acids including oxalic, tartaric, malic, shikimic, ascorbic, maleic, succinic, citric, acetic and fumaric were identified and quantified in freshly prepared juices. The total organic acid content was in the range of 105.4–2074.4 mg 100 g^{-1} of pomegranate juice. Citric acid predominated in most cultivars especially in sour cultivars, while sweet-sour and sweet ones were characterized by high malic acid content. Principal Component Analysis (PCA) indicated that principle component 2 was responsible for discrimination of two geographical regions. Furthermore, Factor Analysis (FA) and Hierarchical Cluster Analysis (HCA) showed the high potential in complete separation of pomegranate cultivars based on geographical origins.

Keywords: Chemometrics, Geographical origin, HPLC, Organic acid profile, Pomegranate.

INTRODUCTION

Pomegranate (*Punica granatum* L.) is one of the oldest edible and commercially important fruits extensively cultivated in many tropical and subtropical regions with over 1000 cultivars originated from different parts of the world (Arabi *et al.,* 2008; Tehranifar *et al.,* 2010; Cam *et al.,* 2009). Recently, there has been an increasing interest in Pomegranate Juice (PJ) as a rich source of functional ingredients such as phenolic compounds and anthocyanins. Chemical composition is one of the most important quality criteria for fruit juices, which are influenced by cultivar, growing area, climate, maturity, cultural practice and storage conditions (Fadavi *et al.,* 2005; Özgen *et al.,* 2008; Tezcan *et al.,* 2009). PJ contains considerable amounts of sugars, vitamins, polyphenols, minerals and organic acids, which are accountable for the color, sensory and nutritional values of pomegranate products (Hasnaoui *et al.,* 2011a). Iran is a native land for pomegranate fruit and its cultivation extended from this area throughout the world. It is grown in many parts of Iran, but the most important pomegranate producing regions are located in the Provinces of Yazd and Markazi (Sarkhosh *et al.,* 2009). In 2013, Iran was the world's largest producing country contributing 1,009,885 tons of pomegranates to the world annual production (Anonymous, 2013).

It has been reported that more than 36 organic acids exist naturally in fruit and

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vegetables. The type and amount of these natural compounds, which are extensively distributed in fruit and vegetables can affect pH, acidity, sweetness, flavors, freshness, consumer acceptability and microbial stability of PJ and the related products. In addition, it provides useful information about their origin and even the technological processes used for their production (Arabi *et al.,* 2008; Nanda *et al.,* 2001). Each type of fruit juices has a distinctive organic acid profile, which makes this property a suitable marker. Pomegranate fruits have been categorized into three varietal groups based on their sugar and organic acid ratio: sweet, sweet-sour and sour. Generally, sweet and sweet-sour cultivars are consumed as fresh fruit or concentrated juice, whereas the sour type is used for sauce, jams, jellies, syrups or other industrial applications.

There is a growing interest among consumers for the geographical origin of food. There are different reasons for the emphasis on this aspect of foodstuff including health profit, patriotism, specific organoleptic quality, concern about animal welfare and -environmentally friendly-production strategies, media attention and decreased confidence in the quality and safety of products manufactured or made outside their local region (Kelly *et al.,* 2005; Luykx and Ruth, 2008).

Multivariate data analysis or chemometrics is an interdisciplinary technique composed of several logic methods including multivariate statistics, applied mathematics and computer science, which can be used for the treatment of large amounts of multivariate data generated by spectroscopy, chromatography, etc. Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA) have been successfully applied for this purpose by many researchers (Hasnaoui *et al.,* 2011a; Hasnaoui *et al.,* 2011b; Cam *et al.,* 2009).

Since organic acid profile plays one of the major roles in the organoleptic characteristic of pomegranate fruit, the aim of this study was to discriminate the geographical origin of twenty-four Iranian pomegranate cultivars based on organic acid profile using HPLC and chemometrics analysis (PCA, FA and HCA).

MATERIAL AND METHODS

Chemicals

HPLC-grade standards (Purity ≥ 98%) of citric, malic, oxalic, tartaric, succinic, shikimik, ascorbic, acetic, fumaric and maleic acids were obtained from Supelco (USA). Water and phosphoric acid (HPLC grade) were purchased from Caledon (Canada) and Merck (Germany), respectively. Other reagents and solvents used in the experiments were of analytical grade commercially available.

Pomegranate Cultivars

Twenty-four fresh Iranian pomegranate cultivars, including ten cultivars from the Province of Markazi (M, Latitude: 35^o 2′ N, Longitude: 50^o 2′ E) and fourteen cultivars from the Province of Yazd (Y, Latitude: 32^o 0′ N, longitude: 55^o 0′ E) were purchased from the Agricultural Research Center of Saveh (province of Markazi) and Yazd, respectively. Pomegranate cultivars with similar commercial maturity (approximately 2 Kg of each cultivar) were randomly collected during September and November 2008 and transferred to the laboratory. Table 1 shows the characteristics of pomegranate cultivars including aril color and taste.

Juice Extraction

After discarding the defective ones, each pomegranate fruit was washed and drained. Then, they were peeled and cut into pieces to separate the arils manually. Next, the pomegranate's juice was extracted by a manual juicer. Subsequently, juice samples were centrifuged at 12,000 rpm for 2 minutes and filtered through a Sep–Pak C18

Table 1. The aril color and taste according to pomegranate cultivars.

Yazd region			Saveh region		
Cultivar	Aril color	Aril Taste	Cultivar	Aril color	Aril Taste
Zagh Yazdi (ZY)	Pink-red	Sour	Alake Torsh (AT)	Red	Sour
Mesri Torsh Kazeron (MTK)	Pink	Sour	Tabestani (T)	Pink	Sweet-sour
Ardestani Torsh Semnan (ATS)	Pink	Sour	Shirin Haste Bafgh (SHB)	Pink	Sour
Khoram Dizin Torsh Gorgan (KDTG)	Pink	Sour	Post Sefid Shirin (PSS)	White	Sweet
Ghorch Shahvar Yazd (GSY)	Pale-red	Sweet-sour	Bihaste Post Zard (BPZ)	Pink	Sweet
Post Siah Yazd (PSY)	White-pink	Sour	Malas Torsh (MT)	Red	Sweet-sour
Malas Yazd (MY)	Red	Sweet-sour	Post Sorkh Ravar (PSR)	Red	Sweet-sour
Vahshi Kan Tehran (VKT)	Pale-red	Sweet-sour	Post Sefid Torsh (PST)	White	Sour
Torsh Mamoli Lasjor (TML)	Pale-red	Sour	Malas Shirin (MS)	Red	Sweet
Jangali Post Ghermez Rodbar Torsh (JPGRT)	Pale-red	Sour	Agha Mohammad Ali Shirin (AMAS)	Pink	Sweet
Tab o Larz Mehrmahi (TLM)	White- pink	Sour			
Togh Gardan (TG)	Pale-red	Sour			
Shirin Shahvar Yazd (SSY)	Red	Sweet-sour			
Malas Porbarij Stahban (MPS)	Pale-red	Sweet			

cartridge (Millipore, USA) and 0.45 µm Millipore filter paper to remove suspended particles (AOAC, 1998). Finally, juice samples were filled in glass bottles and immediately kept at -20°C until further analysis.

Determination of Organic Acids

The profile of organic acids of PJs was analyzed by HPLC using Waters HPLC system equipped with Empower software, pump (Waters 600), Rheodyne 7125i six-way manual injector with 20 µl sample loop, and 2487 UV–Vis detector (Waters). Chromatographic separation was performed by a Prontosil 120-3-C_{18}AQ column (250×4.6 mm, dp 3µm) from Knauer (Germany) at room temperature using H_3PO_4 (50 mM) as mobile phase at a flow rate of 0.7 ml min^{-1}. Twenty µl of the clarified PJ was injected and the detection of organic acids was monitored at 205 nm. The amount of organic acids was calculated based on the external standard method, and they were identified by comparing their retention times with those of the pure standards. The retention time of each organic acid in PJ samples was also confirmed by spiking the samples with standard compounds.

Statistical Analysis

All experiments and analyses were carried out in triplicates and their means were reported as the results. The obtained data were compared by one-way Analysis Of Variance (ANOVA) using the SAS Software version 9.1 (USA). Statistical significance was tested at $P< 0.05$. For clustering purpose, Principal Component Analysis (PCA), Factor Analysis (FA) and Hierarchical Cluster Analysis (HCA) were applied as unsupervised exploratory data analysis methods (Bosque-Sendra *et al.,* 2012). FA (with varimax rotation) and PCA were applied to highlight the information of data matrix (with the size of 10 chromatographic variables concerning organic acids content×24 samples concerning pomegranate cultivars) using R software, version 3.0.2 (URL: http://CRAN.R-project.org/package=clusterSim). Manhattan distance and the Ward's methods were used as the dissimilarity measure and agglomerative rule, respectively, using Minitab 16.2.4 (Minitab Inc., USA).

RESULTS AND DISCUTION

Quantification of Individual Organic Acid in Pomegranate Cultivars

The profiles of organic acids (including oxalic, tartaric, malic, shikimic, ascorbic, maleic, succinic, citric, acetic and fumaric acids) of 24 fresh PJs from two geographical regions were assessed and reported based on mg/100g fruit juice in Table 2. Total Organic Acid Content (TOAC) varied from 105.4 to 2074.4 mg 100 g^{-1} fruit juice. The results showed that sour samples had higher TOAC (1033.53 mg 100 g^{-1} -ranging between 105.4 and 2074.44 mg 100 g^{-1}), followed by sweet-sour cultivars (565.35 mg 100 g^{-1} -ranging from 171.39 to 1294.7 mg 100 g^{-1}). As we expected, the lowest TOAC was determined in sweet pomegranate cultivars (296.23 mg 100 g^{-1} -ranging from 212.83 to 387.61). The average amount of organic acids (mg 100 g^{-1}) for three pomegranate groups is shown in Table 2.

Citric acid (534.9 mg 100 g^{-1}), malic acid (56.7 mg 100 g^{-1}), and tartaric acid (54.7 mg 100 g^{-1}) were the main organic acids in juices made from Iranian pomegranate cultivars. Citric acid was the major organic acid in 16 pomegranate samples. As shown in Table 2, the mean values of citric acid content in sour (843.04 mg 100 g^{-1}) and sweet-sour (316.55 mg 100 g^{-1}) pomegranate cultivars were higher than sweet (100.84 mg 100 g^{-1}) cultivars. The highest citric acid content (1611.3 ± 54.1 mg 100 g^{-1}) was found in MTK cultivar that is generally used for preparation of pomegranate paste in Iran. The citric acid content of 30 Tunisian cultivars were in the range of 0.44-31.36 (g L^{-1}) reported by Hasnaoui *et al.* (2011a) and 3.93-13.06 (mg ml^{-1}) reported by Tezcan *et al.* (2009) for seven commercial pomegranate juices from Turkey. These differences are normal and they might be originated from cultivar, growing region, climate, maturity and cultural practice.

Among three groups of pomegranate cultivars, the sweet-sour type showed the highest malic acid (76.71 mg 100 g^{-1}) and tartaric acid (61.22 mg 100 g^{-1}) content. In addition, based on the geographical region, the results showed that pomegranate cultivars from Markazi had higher amounts of malic acid (102.44 mg 100 g^{-1}) than the ones from Yazd (23.42 mg 100 g^{-1}). The highest malic acid content was found in MT (366.3 mg/100 ml), while it was not detected in MY and VKT cultivars. Malic acid was reported as the main organic acid in the commercial Turkish PJs (Tezcan *et al.,* 2009), Tunisian sweet pomegranate cultivars (Hasnaoui *et al.,* 2011b) and Spanish pomegranate cultivars (Melgarejo *et al.,* 2000). The tartaric acid concentration of pomegranate cultivars was in the range of 0-105.1 mg 100 g^{-1} and sour and sweet cultivars had similar values. However, Hasnaoui *et al.* (2011a) reported the presence and lack of tartaric acid in the sweet and sour Spanish pomegranate cultivars, respectively.

Oxalic acid noticeably existed in all pomegranate cultivars ranging from 10.3 to 45.3 mg 100 g^{-1} with the overall mean values of 27.32, 28.21 and 24.82 mg 100 g^{-1} for sour, sweet-sour and sweet cultivars, respectively. Poyrazoglu *et al.* (2002) reported the oxalic acid concentration of 13 pomegranate cultivars from the Mediterranean region of Turkey in the range of 0.2-6.72 g L^{-1} and Hasnaoui *et al.* (2011a) reported 0.3-3.89 g L^{-1} of oxalic acid for 30 Tunisian pomegranate samples. As observed, the organic acids content varies considerably, affected by genetic make-up, light, temperature and agronomic factors.

Succinic acid ranged between 0-134.4 mg 100 g^{-1} (GSY cultivar) with the overall mean

Table 2. Organic acids compositions of 24 Iranian pomegranate cultivars (Mean±SD, n= 3).[a]

No.	Cultivars	Organic Acids (mg 100 g^{-1} fruit juice)										
		Oxalic	Tartaric	malic	shikimic	ascorbic	acetic	maleic	Succinic	citric	fumaric	Total organic acid content
1	ZY	30.2 ± 1.8	64.8 ± 5.6	31.5 ± 5.3	ND	ND	ND	0.17 ± 0.01	78.4 ± 6.10	1164.3 ± 31.8	0.24 ± 0.04	1369.61
2	MTK	25.5± 2.0	61.6 ± 2.7	19.3 ± 2.1	ND	3.00 ± 0.00	ND	0.15 ± 0.00	59.3 ± 3.00	1611.3 ± 54.1	0.55 ± 0.10	1780.70
3	ATS	34.6 ± 1.9	74.5 ± 8.0	37.3 ± 4	ND	2.96 ± 0.18	ND	0.22 ± 0.01	118.4 ± 10.90	920.5 ± 17.9	ND	1188.48
4	KDTG	25.7 ± 0.8	53.6 ± 5.4	19.4 ± 0.7	1.89 ± 0.8	8.78 ± 0.95	ND	0.08 ± 0.00	38.8 ± 3.85	747.4 ± 14.0	0.28 ± 0.00	895.93
5	GSY	36.7 ± 1.9	105.1± 8.4	7.1 ± 0.9	ND	ND	ND	0.1 ± 0.00	134.4 ± 10.40	398.5 ± 11.4	0.44 ± 0.01	682.34
6	PSY	20.9 ± 0.9	66.5 ± 8.0	46.6 ± 4.4	ND	1.91 ± 0.08	30.8 ± 1.10	0.24 ± 0.00	92.6 ± 9.30	316.5 ± 6.8	0.28 ± 0.01	576.33
7	MY	23.1 ± 1.5	46.6 ± 4.6	ND	47.40 ± 1.4	ND	43.6 ± 2.10	0.04 ± 0.01	ND	140.8 ± 5.2	ND	301.54
8	VKT	17.2 ± 1.6	ND	ND	ND	5.82 ± 0.46	31.5 ± 1.80	0.08 ± 0.01	46.9 ± 1.70	1193.2 ± 64.9	ND	1294.70
9	TML	23.5 ± 2.2	51.1 ± 5.0	13.9 ± 1.3	2.82 ±0.4	ND	ND	0.15 ± 0.00	ND	13.6 ± 0.8	0.37 ± 0.00	105.44
10	JPGR	33.2 ± 1.7	78.7 ± 5.5	48.4 ± 7.7	2.89 ± 0.08	ND	76.1 ± 4.60	19.2 ± 0.80	ND	1114 ± 6.5	0.65 ± 0.02	1373.14
11	TLM	14.6 ± 0.7	25.7 ± 2.6	45.3 ± 3.5	ND	2.25 ± 0.14	ND	0.09 ± 0.00	13.4 ± 3.60	128.5 ± 11.8	0.38 ± 0.00	230.22
12	TG	23.4 ± 0.6	75.6 ± 12.7	21.3 ± 4.9	ND	2.60 ± 0.50	ND	0.19 ± 0.01	80.3 ± 12.60	667.1 ± 12.9	0.35 ± 0.03	870.84
13	SSY	17.3 ± 1.1	69.4 ± 5.2	35.5 ± 3.0	ND	ND	0.81 ± 0.02	0.36 ± 0.02	68.2 ± 1.40	394.2 ± 25.6	ND	585.77
14	MPS	10.3 ± 0.8	ND	2.3 ± 0.7	0.38 ± 0.01	ND	ND	0.37 ± 0.02	13.4 ± 3.60	278.6 ± 8.9	ND	305.35
15	AT	37.7 ± 2.2	ND	178.8 ±3.3	ND	2.00 ± 0.18	ND	0.54 ± 0.03	28.6 ± 1.60	1239.6 ± 84.6	11.37 ± 1.48	1498.61
16	T	35.5 ± 2.7	83.6 ± 2.9	31.1 ± 0.7	7.00 ± 0.00	1.72 ± 0.19	42 ± 1.30	0.11 ± 0.01	20.2 ± 6.60	38.5 ± 3.8	11.56 ± 0.23	271.29
17	SHB	29.2 ± 3.0	67.3 ± 2.8	23.1 ± 2.1	3.10 ± 1.00	2.29 ± 0.16	ND	0.22 ± 0.01	60.9 ± 6.50	1881.9 ± 76.6	6.41 ± 0.14	2074.44
18	PSS	34.8 ± 0.8	62.7 ± 0.5	24.0 ± 2.5	11.40 ± 0.60	5.11 ± 0.33	38.5 ± 2.70	0.47 ± 0.02	53.5 ± 11.50	28.5 ± 2.0	15.39 ± 0.29	274.37
19	BPZ	23.2 ± 2.2	37.4 ± 9.6	162.7 ± 8.6	ND	0.36 ± 0.07	ND	0.31 ± 0.01	ND	155.6 ± 6.4	15.39 ± 0.06	387.61
20	MT	45.3 ± 7.5	86.0 ± 4.6	366.3 ± 6.0	ND	1.11 ± 0.23	ND	0.99 ± 0.04	88.4 ± 2.30	50.7 ± 7.1	11.67 ± 1.55	650.47
21	PSR	22.4 ± 1.9	37.9 ± 1.9	97.0 ± 11.4	4.20 ± 0.30	2.47 ± 0.14	ND	ND	ND	ND	7.42 ± 0.40	171.39
22	PST	29.4 ± 0.6	ND	24.0 ± 1.6	5.00 ± 0.30	1.18 ± 0.12	42.9 ± 60.00	0.39 ± 0.04	12.4 ± 2.60	311.8 ± 5.1	11.59 ± 0.24	438.66
23	MS	38.6 ± 2.3	66.0 ± 5.7	25.5 ± 0.8	ND	2.71 ± 0.39	39.7 ± 3.80	0.25 ± 0.01	18.8 ± 1.30	14.4 ± 1.2	6.87 ± 0.94	212.83
24	AMAS	17.2 ± 0.7	97.8 ± 3.1	100.3 ± 20.3	4.20 ± 0.20	ND	ND	0.26 ± 0.01	46.6 ± 3.40	27.1 ± 3.5	7.53 ± 0.38	300.99
	Average	27.1	54.7	56.7	3.76	1.92	14.41	1.04	44.72	534.9	4.22	
	Sour	27.3	51.6	42.4	1.31	2.24	12.48	1.80	48.59	843.0	2.70	
	Sweet-Sour	28.2	61.2	76.7	8.37	1.58	16.84	0.24	51.15	316.6	4.44	
	Sweet	24.8	52.8	63.0	3.196	1.636	15.64	0.33	26.46	100.8	7.56	
	Yazd	24.0	55.2	23.4	3.95	1.95	13.05	1.53	53.15	649.2	0.25	
	Saveh	31.3	53.9	102.4	3.492	1.90	16.31	0.35	32.94	374.8	9.78	

[a] All cultivars abbreviations have been introduced in Table 1. ND: Not Detected.

content of 44.72 mg 100 g^{-1} while it was not detected in MY, TML, JPGR, BHPZ and PSR cultivars. Similar overall mean values for this acid were also reported by Poyrazoglu *et al.* (2002) for Turkish PJs. Hasnaoui *et al.* (2011b) showed that the amount of oxalic acid was much higher in the sweet than the sour Tunisian pomegranate cultivars, whereas this trend reversed regarding the succinic acid content, which might be originated from cultivar, growing region, climate, maturity and cultural practice. However, other researchers could not detect succinic acid in pomegranate fruit (Legua *et al.,* 2012).

Shikimic acid belongs to the minor group of organic acids in PJ monitored in this study and was detected in 11 pomegranate cultivars, ranging from 0.0 to 47.4 mg 100 g^{-1} (MY). Cultivars obtained from Yazd and Markazi had similar average concentrations. Its concentration in sweet-sour cultivars (8.37 mg 100 g^{-1}) was approximately three and six times higher than sweet (3.19 mg 100 g^{-1}) and sour cultivars (1.31 mg 100 g^{-1}), respectively.

Among the investigated cultivars, acetic acid was detected in only nine samples (PSY, MY, VKT, JPGR, MS, SSY, PST, T, and PSS), varied from 0.0 to 76.1 mg 100 g^{-1} (JPGR). Acetic acid is a volatile and aroma organic acid, which was not previously reported in PJs. However, Melgarejo *et al.* (2000) reported the presence of acetic acid in three Spanish cultivars (average of 0.015 g 100 g^{-1}). Also, acetic acid contents of 6.0 to 76.0 mg 100 g^{-1} have been reported in PJs from three Spanish cultivars, namely ME5, Me17, and MO6 (Legua *et al.,* 2012).

The amount of ascorbic acid ranged between 0.0- 8.78 mg 100 g^{-1} (KDTG) with the overall mean value of 1.92 mg 100 g^{-1}. Results of the current study were lower than the previous values (9.91–20.92 mg 100 g^{-1}) reported for twenty Iranian pomegranate cultivars (Tehranifar *et al.,* 2010). Maleic and fumaric acids were also determined in comparatively lower amounts in most pomegranate cultivars. This observation is common because the cultivars studied by Tehranifar *et al.* (2010) are different from cultivars studied in our research. As shown in Table 2, the highest maleic acid content was in JPGR (19.2 mg 100 g^{-1}), but it was not observed in PSR cultivar. The overall mean concentration of maleic acid was found to be 1.04 mg 100 g^{-1}. Fumaric acid concentration ranged from 0- 0.65 mg 100 g^{-1} and 6.41 to 15.39 mg 100 g^{-1} in Yazd and Markazi pomegranate cultivars, respectively (Table 2). In another report, fumaric acid was not detected in the studied pomegranate cultivars (Hasnaoui *et al.,* 2011a).

Principle Component Analysis (PCA)

PCA is the most sophisticated multivariate technique that reduces the dimension of numerical datasets and facilitates the understanding of similarities and differences among objects by generation of two or three-dimensional patterns without losing much information (Zielinski *et al.,* 2014).

PCA was applied to the determined values of organic acids of 24 pomegranate cultivars. It was possible to visualize the correlation between the analyzed organic acids and to recognize the variables characterizing the pomegranate cultivars by PCA bi-plot. As shown in Figure 1, by using two first principle components, 21.59 and 20.86% of variance could be explained. First two PCs could not distinguish well the cultivars grown in different regions. According to loading values, it was observed that PC1 highly correlated with succinic acid while PC2 greatly correlated with fumaric acid (Table 3). As shown in Figure 1, succinic, citric and ascorbic acids significantly correlated with each other. Tartaric, malic, and oxalic acids were the variables with negative loadings on PC1 and PC2, and had significant correlation with each other. Shikimic, maleic and acetic acids were the parameters with positive and negative loadings on PC1 and PC2, respectively.

In fact, similar positions of various pomegranate cultivars in the PCA plot

Table 3. Loadings, eigenvalues, and percent of cumulative variance for the first three principle components (PCs).

Variable	PC1	PC2	PC3
Oxalic	-0.377	-0.440	0.174
Tartaric	-0.362	-0.144	0.358
Malic	-0.364	-0.330	-0.285
Shikimic	0.361	-0.206	-0.0188
Ascorbic	-0.043	0.177	-0.029
Acetic	0.357	-0.407	0.347
Maleic	0.164	-0.283	0.542
Succinic	-0.519	0.209	0.239
Citric	-0.111	0.271	0.404
Fumaric	-0.152	-0.487	-0.350
Eigen value	2.15	2.08	1.67
Cumulative variance	21.59	42.45	59.21

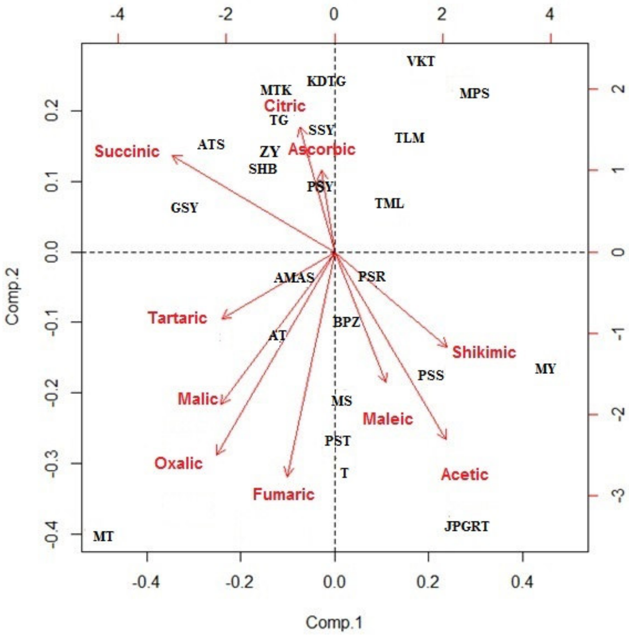

Figure 1. Principle component analysis (PCA) bi-plot (PC#1 *versus* PC#2) obtained from the organic acid profile of 24 Iranian pomegranate cultivars. All abbreviations have been introduced in Table 1.

indicated their similar organic acid profiles. Most cultivars of Yazd (including ZY, MTK, ATS, KDTG, GSY, PSY, TG, and SSY) and one cultivar of Markazi (SHB) were characterized by higher values of citric, ascorbic and succinic acid contents. Figure 1 shows high orthogonally between malic acid plus tartaric and citric acids. Some cultivars were very rich in citric acid, but very low in malic and tartaric acids (MTK, KDTG and TG), while MT and AT cultivars were rich in malic and tartaric acids, but poor in citric acid. A close association was observed among TML, TLM, MPS and VKT cultivars located in the upper right side of plot. BPZS, MS, PST and T cultivars were located close to the center of bi-plot, indicating that they could not be characterized by any of the organic acids. Shikimic, acetic and maleic acids were found in higher contents in MY, JPGR, PSS and PSR cultivars. Some cultivars from Markazi (AMAS, AT and MT) were best characterized duo to their considerable amounts of tartaric, malic,

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oxalic and fumaric acid. Based on the obtained results, it is possible to choose an interesting cultivar with specific organic acid profile for industrial applications. Acidity (especially citric acid content) affects not only the sour taste of fruit juices, but also sweetness by altering sugars/organic acid ratio. The quantity of individual organic acid is also important as citric acid covers the perception of sucrose and fructose, whereas malic acid appears to enhance sucrose perception (Lobit *et al.,* 2006). These compounds serve as good markers for sensory characteristics assessment. As shown in Figure 1, sour, sweet-sour and sweet pomegranates were not located distinctly.

In some cases, PCA does not seem to be the best statistical approach to classify the geographical origin of fruit samples based on the chemical composition. Furthermore, this method did not show enough ability to discriminate Tunisian pomegranate cultivars (Hasnaoui *et al.,* 2011a) and sweet cherry juices (Longobardi *et al.,* 2013) from different regions of Tunisia and Italy, respectively. Figure 2 shows the 3D scatter plot of the scores of PC1 versus PC2 and PC3 which together explain 59.21% of the total variance and the classification results give a satisfactory separation of two geographical regions (Table 3).

Factor Analysis (FA)

The algorithm of FA is similar to PCA except that it emphasizes on that portion of total variation that a variable shares with other variables in the data set. FA determines the interrelationship among a large number of variables and groups them based on the common original dimension. This allows one to define and identify a number of latent unique constructs and errors due to unreliability in measurement (Campbell-Platt, 2009). In this study, FA was performed using the varimax rotation method, which showed a high potential in the complete discrimination of geographical origins based on organic acid composition of pomegranate cultivars. The scores plot of FA was constructed to define different groups as shown in Figure 3-a (and 3-b). The first three factors explained approximately 50% of the total variance in the data set. Factor 2 points to the differentiation between pomegranate

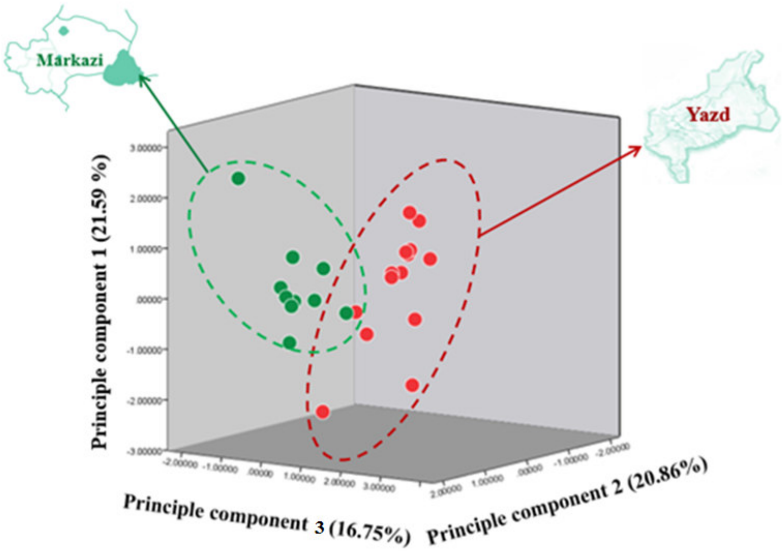

Figure 2. Three-dimensional Principle component analysis (PCA) score plot (PC#1 *versus* PC#2 and PC#3) of the 24 Iranian pomegranate cultivars.

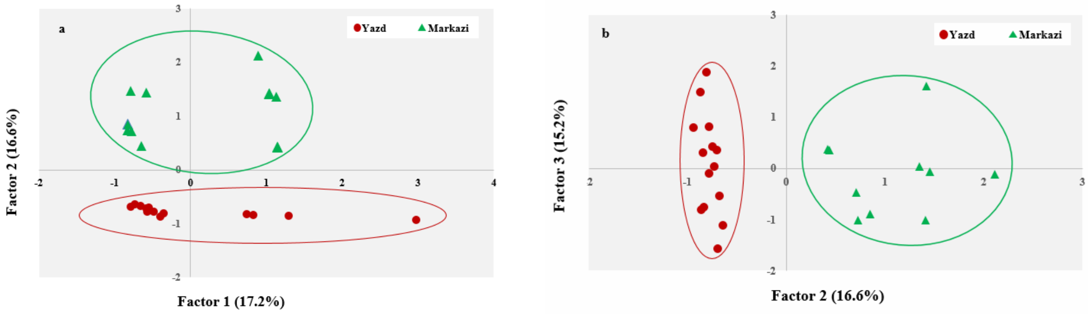

Figure 3. Score plots of factor analysis by varimax rotation: (a) F1 *versus* F2, and (b) F2 *versus* F3.

cultivars from Yazd and Markazi regions.

Hierarchical Cluster Analysis (HCA)

Unsupervised pattern recognition methods try to detect the presence of groups of samples in the data set without prior knowledge of their existence according to the similarities between them (Bosque-Sendra *et al.*, 2012). HCA analyses the inter point distances (or correlation) between all samples by means of a defined metric such as Euclidian distance, Manhattan distance, etc. (Berrueta *et al.*, 2007). Ward's technique is distinct from other methods because it uses an analysis of variance to assess the distances between clusters.

In order to better recognize the associations among Iranian pomegranate cultivars, HCA was performed using 10 identified organic acids as discriminative variables. As shown in Figure 4, three

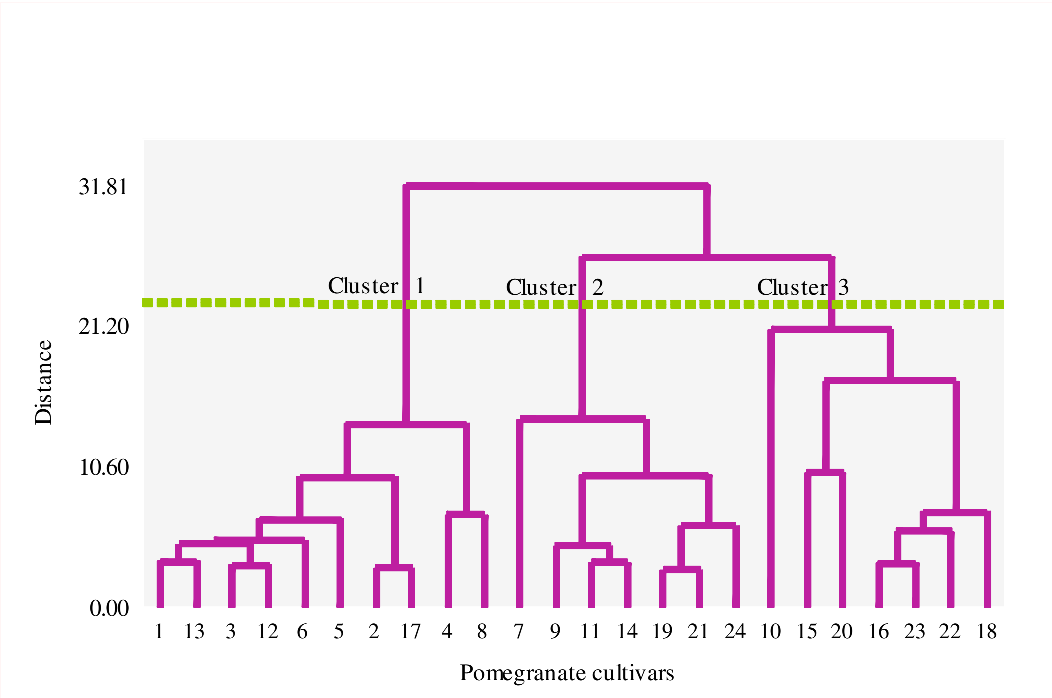

Figure 4. Dendrogram plot visualizing the clustering of the 24 pomegranate cultivars based on the organic acids profile. All cultivars numbers have been introduced in Table 1.

distinct clusters were suggested based on the similarities in the organic acid composition and it was possible to obtain clusters composed primarily of cultivars of similar geographical region. The first group included mostly the Yazd cultivars (ZY, MTK, ATS, KDTG, GSY, PSY, VKT, TG, and SSY) and just one from Markazi region (SHB), which showed great differences from other Saveh cultivars. This cluster was mostly characterized by sour and sweet-sour cultivars and contained the highest amount of citric and succinic acids. However, the cultivars from the second cluster showed no clear correlation with the origin. This cluster contained seven cultivars from both regions (three from Markazi and four from Yazd). Most cultivars in the third cluster were from Saveh (AT, T, MT, PST, MS, and PSS) and one from Yazd region (JPGR), which had a significantly different organic acid profile from other pomegranate cultivars of Yazd region. A similar fingerprint of organic acids was also observed in MY, TLM, TML and MPS cultivars (Yazd region) as well as BPZS and AMAS cultivars (Saveh region). T, PSS, PST and MS as sweet and sweet-sour cultivars were clustered within a short hierarchical distance, indicating the presence of similar profiles of organic acids among them.

According to some previous results, the Euclidean distance and Ward's method have been used to classify the Turkish PJs into three clusters based on their antioxidant capacity (Cam *et al.,* 2009). In addition, a high degree of dissimilarity has been reported for six Spanish pomegranate cultivars using linkage method based on some physicochemical attributes (Legua *et al.,* 2012). The HCA results of fatty acids composition of Iranian olive oils showed that it is possible to obtain clusters composed mostly of samples of the same origin (Piravi-Vanak *et al.*, 2012). In addition, Yudthavorasit *et al.* (2014) reported that ginger samples from identical origins (China, India, Malaysia, Thailand and Vietnam) are correctly clustered after HCA of bioactive compounds profile.

CONCLUSIONS

In the present study, 24 pomegranate samples from two different regions of Iran were characterized by organic acid profiles. The results showed a vast variance among pomegranate cultivars in terms of organic acid composition. Quantitatively, the major organic acid in all sour pomegranate cultivars was citric acid, which appears to be the major factor of sourness. Furthermore, high amounts of malic acid were found in sweet-sour and sweet cultivars. PCA and HCA of the experimental variables (organic acid content) did not completely discrete the cultivars of Yazd from the ones Saveh regions, but showed that the geographical region had an obvious effect on organic acid profiles. The best classification was obtained by FA where factor 2 pointed to the whole differentiation between cultivars from the Yazd and Saveh regions. Further research is needed to accurately distinguish the geographical origin of pomegranate cultivars because this property is affected by chemical composition and strictly correlated with consumer acceptance and industrial applications of pomegranate fruit.

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تفکیک جغرافیائی ارقام انار ایرانی بر اساس ترکیب اسیدهای آلی و تحلیل چند متغیره

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چکیده

در این بررسی، تفکیک جغرافیائی برخی از ارقام انار ایرانی با روش های آماری چند متغیره و کروماتوگرافی مورد بررسی قرار گرفت. محتوای اسیدهای آلی ۲۴ نمونه انار از ارقام مختلف (۵ رقم شیرین، ۷ رقم ملس و ۱۲ رقم ترش) از دو منطقه متفاوت (یزد و ساوه) با استفاده از HPLC/UV تعیین گردید. ده نوع اسید آلی شامل اگزالیک، تارتاریک، مالیک، شیکیمیک، آسکوربیک، مالئیک، سوکسینیک، سیتریک، استیک و فوماریک در آب میوه تازه تشخیص و اندازه گیری شدند. محتوای کل اسیدهای آلی نمونه‌های آب انار در محدوده‌ی ۲۰۷۴/۴-۱۰۵/۴ میلی‌گرم در ۱۰۰ گرم بود. سیتریک اسید، اسید آلی اصلی در اکثر ارقام و به ویژه ارقام ترش بود در حالی که در ارقام ملس و شیرین، مالیک اسید شاخص بود. تحلیل مولفه‌های اصلی (PCA) نشان داد، تفکیک ارقام دو منطقه جغرافیائی مختلف، با مولفه اصلی دوم امکان پذیر است. علاوه بر این، تحلیل عاملی (FA) و تحلیل خوشه‌ای سلسله مراتبی (HCA) توانائی بالائی برای جداسازی کامل مناطق جغرافیائی ارقام مختلف انار نشان دادند.

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