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Effect of extraction process and storage time on the quality attributes of pomegranate juice of two local pomegranate varieties

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PAPER

Abstract

This study investigates the impact of two extraction processes (squeezing the whole fruit and centrifuging the seeds) of pomegranate juice and storage on sweet and sour pomegranate quality attributes. The pH, acidity, and levels of organic acids, sugars and anthocyanin differed in both varieties and changed during the storage period. Fructose and glucose were the primary sugars, and citric acid was the dominant organic acid in the juice of both cultivars. A high level of established anthocyanin content was 15.40, 18.53, 18.03, 16.92, 16,68 and 15.47 mg/L when the storage period was 0, 5, 15, 32, 48 and 72 h, respectively, in the juice of sweet fruits obtained by squeezing the whole fruit. The juice prepared from the sweet fruits by squeezing method outscored, in all sensory quality attributes, the juice prepared by centrifuging process.

Keywords: anthocyanin; centrifugation of seeds; organic acids; sensory attributes; squeezing whole fruit

Introduction

Pomegranate (*Punica granatum*) fruit is known as 'miracle fruit' because of its vast food and pharmaceutical applications. The seeds are wrapped with juicy edible pulp and are consumed as food, pressed for juice production, and used as functional foods (Coronado-Reyes *et al.*, 2021; Hegazi *et al.*, 2021). Pomegranate fruit, peel, seeds and juice have high medicinal applications for treating and preventing various diseases such as inflammation, diabetes, diarrhea, obesity, dysentery, dental plaque and malaria (Ismail *et al.*, 2012). In addition, parts of pomegranate fruit are also used as food additives, functional food materials and active ingredients in nutraceutical products (Akhtar *et al.*, 2015). Pomegranate juice is a primary commercial product of pomegranate fruit with high consumer preferences because of its comprehensive nutritional and phytochemical components. It contains substantial amount of dietary polyphenols, tannins, anthocyanins and flavonoids with high potential antioxidant activities (Fahmy *et al.*, 2020). Topalović *et al.* (2021) identified among 97 phenolic compounds, 23 anthocyanins and their derivatives, 33 ellagitannins and derivatives of ellagic acid, 12 flavonols, 4 flavonol glycosides, 1 flavone, 17 hydroxybenzoic acids and 7 hydroxycinnamic acids and their derivatives. Cyanidin-3,5-diglucoside and cyanidin-3-glucoside are reported as primary anthocyanins in pomegranates (Kostka *et al.*, 2020). In addition, it was found that juice is a rich source of flavan-3-ols (2,650–9,820 mg/L), ellagitannins (2,010–6,420 mg/L) and hydroxybenzoic acids (720–3,390 mg/L) (Topalović *et al.*, 2021).

Therefore, pomegranate juice is favored as a healthy juice with great applications for treating and preventing obesity, diabetes, blood pressure and inflammation (Hegazi *et al.*, 2021). Factors such as cultivars, maturity stage, harvest season, climatic and agronomical conditions, post-harvest processing and juice extraction processes greatly affect the nutritional and phytochemical composition of pomegranate juice (Hegazi *et al.*, 2021; Mphahlele *et al.*, 2014).

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Optimization of these conditions is highly important to produce pomegranate juice with good quality attributes. Juice extraction is one of the critical steps that affect its functional properties, and this step is influenced by fruit genotype and other factors (Hegazi *et al.*, 2021; Mena *et al.*, 2014).

Generally, optimal time for fruit harvest and preparation process is the factor that plays an important role in the taste acceptability of consumers. In addition, the composition of organic acids and sugars in fruit juice plays a key role in flavor and sensory characteristics, such as pH, total acidity and sweetness (Ikegaya *et al.*, 2019). To date, several extraction processes have been used such as pressing the whole fruit or fruit halves by hand presser or squeezer, peeling off the fruit and extraction of juice using pressing and centrifugation, crushing the seeds and arils using the juice blender, and quartered cut fruit and pressing using rack and cloth (Hegazi *et al.*, 2021). Of these processes, the hand pressing of fruit halves gave the highest yield of phenolic contents, anthocyanins and antioxidant activity (Mphahlele *et al.*, 2016). Crushing the whole fruit or fruit halves resulted in a bitter taste because of the extraction of more tannins in the juice (Miguel *et al.*, 2004). In addition to the extraction process, fruit type (sweet, sweet–sour and sour) affected pomegranate juice's nutritional and sensory quality attributes (Hegazi *et al.*, 2021; Mphahlele *et al.*, 2014).

In Saudi Arabia, great interest has been found in recent years in producing and consuming pomegranate juice.

Consequently, cultivation of pomegranate has increased greatly, and several genotypes are cultivated throughout the country. Sweet (*Taifi*) and sour (*Bidah*) genotypes are the primary pomegranate cultivars produced in western and southern Saudi Arabia. Today, studies on production of juice from these cultivars are scarce. Therefore, the present study was conducted to investigate the effect of juice extraction process (squeezing or centrifugation) and storage period (up to 3 days) on the quality attributes of pomegranate juice of *Taifi* and *Bidah* cultivars.

Material and Methods

Materials

The samples of sweet and sour pomegranate fruits were obtained from two different locations in Saudi Arabia. Sweet pomegranate fruits were obtained from a farm of Taif city located in western Saudi Arabia whereas sour fruits were obtained from Bidah village of southern Saudi Arabia. The samples were transferred to laboratory on the same day of harvesting under controlled conditions. After sorting of samples by removing damaged fruits and selecting the same size and maturity stage fruits, they were washed thoroughly with tap water and rinsed thrice in distilled water before extraction of juice by the two processes.

Extraction of pomegranate juice

The two processes used for the extraction of pomegranate juice from both sweet and sour fruits were as follows:

1. Peels of the fruits (10 fruits of each type) were removed manually, and the seeds were separated. The juice was extracted from the seeds using an electric centrifuge (Philips Electric HR2738/01 Citrus Press Juicer, France).
2. The fruits (10 fruits of each type) were cut with knives into two halves and squeezed using juice maker (Philips Viva Collection Juicer-HR1863, France) to obtain the juice.

The extraction processes were repeated for six times, and the resulting juice was transferred into sterilized bottles and stored in the dark at 4°C for 72 h. The samples were collected at intervals of 0, 5, 15, 32, 48 and 72 h for analyzing physicochemical properties.

Determination of acidity and pH

The acidity was measured by titrating 10-mL juice against 0.1-N NaOH, and the results were expressed as percentage of citric acid (Association of Official Analytical Chemists [AOAC], 2000). The pH was measured using digital pH meter.

Determination of organic acids

Organic acids (tartaric, citric, malic and oxalic acids) of pomegranate juice samples were analyzed using the High-performance liquid chromatography (HPLC) method as described in the AOAC (2000) standard methods with some modifications. Briefly, 10-g juice sample was centrifuged at 6,000 relative centrifugal force (RCF) for 20 min, and the supernatant was collected and filtered using 0.45-µm filters (Millipore). Then, 20-µL supernatant was injected into reverse phase (RP) column (250 × 4.6 mm) and processed using 50-mM potassium phosphate buffer and 70% methanol at a flow rate of 1 mL/min. The peaks were detected at 210 nm, identified and quantified by comparing their retention time with the authentic standards of tartaric, citric, malic and oxalic acids.

Determination of sugars

The sugar contents of pomegranate juice were analyzed using the Shimadzu HPLC system (LC-10ADVP, Shimadzu, Kyoto, Japan) equipped with a Spherisorb 5 NH2 column (30 × 0.65 cm) and a 1530 refractive index detector (RID) (Shimadzu). Before analysis, 1-mL juice sample was centrifuged at 13,000 rpm for 20 min. The supernatant was collected and filtered using 0.45-μm Millipore filters. Then, 10-μL sample was injected into the column having a temperature of 35°C and separated using 75% acetonitrile as mobile phase at a flow rate of 1 mL/min. The sugars were identified by comparing their retention time with authentic standards run under the same conditions, and the concentration was calculated using the standard curves of sugars.

Determination of anthocyanin contents

The anthocyanin contents of pomegranate juice samples were determined using the HPLC system described in the AOAC (2000) standard method. Briefly, 1-mL juice sample was centrifuged (3,000 RCF, 20 min), and the supernatant was filtered using 0.45-μm Millipore filters. The filtrate (20 μL) was injected into a 100-RP 10 LiChroCart® column and separated using a linear gradient of 5% formic acid (A) and methanol from 15% to 35% (B) for 15 min, followed isocratic application to a total run time of 20 min. The flow rate was 1 mL/min, and the anthocyanin peaks were detected at 510 nm. The anthocyanin content of the samples was detected by comparing their retention time with that of *authentic* standard anthocyanins quantified from the standard curves generated using 0-, 0.01-, 0.02-, 0.04- and 0.08-mg/L of authentic standard.

Sensory analysis

The sensory analysis of pomegranate juice samples of sweet and sour fruits extracted by above-mentioned two processes was conducted using 20-point scaling method (0–4: unacceptable, 5–8: acceptable, 9–12: good, 13–16: very good and 17–20: excellent; Chen *et al.*, 1991). A panel comprising 30 trained staff of the College of Home Economics, Princess Nourahbint Abdulrahman University, Saudi Arabia, evaluated the color, smell, taste, texture and overall acceptance of pomegranate juice samples. The data were collected and subjected to statistical analysis.

Statistical analysis

The data of three experiments were collected and analyzed using One-Way Analysis of Variance (ANOVA). The mean was calculated using Student's t-test, and $p < 0.05$ was considered as statistically significant (Roscoe, 1975).

Results and Discussion

Effect of storage and extraction process on the pH and acidity of pomegranate juice

The pH and acidity values of pomegranate juice of sweet and sour varieties as affected by extraction processes and storage period are shown in Table 1.

Table 1. Effect of storage and juice production method on the pH and acidity of local sweet (*Taif*) and sour (*Bidah*) pomegranate juice.

	Storage (hours)					
Parameters	0	5	15	32	48	72
pH						
Squeezing sweet pomegranate fruit	$4.26 \pm 0.03^{a,A}$	$4.20 \pm 0.02^{a,A}$	$4.26 \pm 0.02^{a,A}$	$4.20 \pm 0.05^{a,A}$	$4.12 \pm 0.08^{a,B}$	$3.87 \pm 0.04^{a,C}$
Centrifuging sweet pomegranate seeds	$3.90 \pm 0.01^{b,A}$	$4.01 \pm 0.09^{b,A}$	$3.96 \pm 0.09^{b,A}$	$3.87 \pm 0.10^{b,A}$	$3.05 \pm 0.15^{b,C}$	$3.63 \pm 0.20^{b,B}$
Squeezing sour pomegranate fruit	$3.80 \pm 0.03^{c,A}$	$3.84 \pm 0.03^{c,A}$	$3.86 \pm 0.09^{b,A}$	$3.88 \pm 0.08^{b,A}$	$3.60 \pm 0.15^{b,B}$	$3.68 \pm 0.11^{c,B}$
Centrifuging sour pomegranate seeds	$3.60 \pm 0.06^{d,A}$	$3.66 \pm 0.05^{d,A}$	$3.60 \pm 0.05^{c,A}$	$3.67 \pm 0.029^{c,A}$	$3.44 \pm 0.05^{b,B}$	$3.50 \pm 0.07^{c,B}$
Total acidity (mg/100 mL)						
Squeezing sweet pomegranate fruit	$288.1 \pm 5.98^{c,B}$	$289.8 \pm 7.78^{c,B}$	$302.1 \pm 5.97^{c,A}$	$296.0 \pm 3.50^{c,A,B}$	$294.2 \pm 0.00^{d,A,B}$	$294.2 \pm 0.00^{d,A,B}$
Centrifuging sweet pomegranate seeds	$394.1 \pm 29.21^{b,A}$	$381.8 \pm 36.41^{b,A}$	$404.6 \pm 26.42^{b,A}$	$385.3 \pm 0.00^{b,A}$	$385.3 \pm 0.00^{c,A}$	$378.3 \pm 9.89^{c,A}$
Squeezing sour pomegranate fruit	$394.1 \pm 29.21^{b,B,C}$	$381.8 \pm 36.41^{b,B,C}$	$404.6 \pm 26.42^{b,A,B,C}$	$448.3 \pm 55.29^{a,A,B}$	$453.6 \pm 8.80^{b,A}$	$444.3 \pm 4.04^{b,A,B}$
Centrifuging sour pomegranate seeds	$449.2 \pm 16.26^{a,A}$	$453.6 \pm 23.99^{a,A}$	$452.7 \pm 24.81^{a,A}$	$458.8 \pm 4.041^{a,A}$	$462.3 \pm 5.7^{a,A}$	$462.3 \pm 13.78^{a,A}$

Means ± SD of 10 samples followed by different superscript letters are significantly different at $p < 0.05$. The small letters indicate differences in the treatments (columns), while the capital letter indicate differences in the storage time (rows).

Comparing the pomegranate types demonstrated that sweet pomegranate juice had a higher pH than that of sour juice. Similarly, a previous report indicated that sweet pomegranate varieties have higher pH values than sour varieties (Fadavi *et al.*, 2005). The pH of juice was significantly affected by the extraction process and pomegranate variety, and high pH was determined in the juice prepared by squeezed method of sweet fruit seeds, followed by that of centrifuged sweet fruit seeds, whereas the least pH was found in the juice prepared from the centrifuged sour fruit seeds ($p < 0.05$). The storage period of up to 32 h did not affect the pH of juice; however, as the storage period increased to 48 h and 72 h, the pH of both types of pomegranate juice was decreased. The change in pH during extended storage period of pomegranate fruit juice was likely due to the formation of acids because of enzymatic and microbial activities during storage. The acidity was also higher ($p < 0.05$) in juice prepared from sour pomegranate fruits than that of sweet fruits, which agreed with the previous report of the juice prepared from 10 pomegranate varieties (Fadavi *et al.*, 2005). The highest acidity and the lowest pH of pomegranate juice of sour fruit types could be due to high acid content of sour varieties (Fadavi *et al.*, 2005). In this study, the extraction process greatly influenced the acidity of juice, with the highest values being found in the juice prepared from centrifuged sour fruit seeds, followed by that of squeezed sour fruit seeds, whereas the least acidity was observed in juice prepared from squeezed sweet fruits. Again, the storage did not affect the acidity of the juice extracted by both processes of pomegranate fruits. It has been reported that the acidity of pomegranate juice obtained by centrifuging the seeds decreased after 32 h of storage whereas decrease in acidity of the juice obtained by squeezing method was less noticeable (Miguel *et al.*, 2004). Difference between the results of these studies could be due to variation in the genetic background of used pomegranate fruits.

Effect of storage and extraction process on the content of organic acids of pomegranate juice

The content of organic acids in the pomegranate juice prepared from two types of fruits and extraction processes during 72 h of storage at 4°C is presented in Table 2. Both fruit types (sweet and sour) and extraction processes (squeezing the fruit or centrifuging the seeds) affected the content of organic acids in different manners ($p < 0.05$) whereas the effect of storage period on content of organic acids was limited. Citric acid is the major organic acid in pomegranate juice. Its level was higher in the juice prepared from sour fruits than that prepared from sweet variety regardless of the extraction method, suggesting that the fruit type influenced the content of citric acid found in pomegranate juice ($p < 0.05$). Similarly, citric acid was the primary organic acid found in the juice of various pomegranate varieties, and its content was affected by these varieties as reported by other studies (Aarabi *et al.*, 2008; Türkyılmaz, 2013). Moreover, citric acid was found higher in sour cultivars than sweet ones (Ghaderi-Ghahfarokhi *et al.*, 2016). The citric acid content in sour fruit juice remained unchanged for 72 h of storage, while it was reduced to minimum values at 15 h of storage in sweet fruit juice but increased again with the progress of storage period ($p < 0.05$). Changes in citric acid during 60 days of storage differed due to variation in the fruit type, chemical changes and extraction processes used, as reported previously for various pomegranate cultivars (Aarabi *et al.*, 2008). Tartaric acid and malic acid are the second major organic acids found in pomegranate juice of different varieties, as reported by previous studies (Aarabi *et al.*, 2008). Content of tartaric acid was affected more by the extraction process than the fruit type. The highest values were observed in the juice prepared by squeezing of sweet and sour fruits ($p < 0.05$). The increased tartaric acid in the juice obtained by squeezing the whole fruits could be due to the fact that some amount of acid is found in the peels of the fruit and peeling off could lead to releasing of this amount. The storage period did not affect the tartaric acid content found in both types of pomegranate juices.

The fruit type and the extraction process also affected the content of malic acid in pomegranate juice, and a pronounced effect was observed in sour fruit juice. The highest level of malic acid was found in the juice prepared by squeezing the whole sour fruits, while the least value was found in the juice prepared from the centrifugation of sour pomegranate fruit seeds ($p < 0.05$). Variation in the content of malic acid was reported in the juice prepared from different pomegranate cultivars, suggesting the influence of cultivar on malic acid (Aarabi *et al.*, 2008; Gundogdu and Yilmaz, 2012; Türkyılmaz, 2013). Malic acid levels were gradually reduced with the storage time of the juice prepared by centrifuging the seeds of sour fruits. Decrease in malic acid during storage was likely due to its metabolism by indigenous microflora present in the juice. Decrease in the content of organic acids after pressing step was observed in pomegranate juice (Akyıldız *et al.*, 2020).

The level of oxalic acid also varied between fruit types and extraction processes of the juice, with the highest value found in the juice prepared by squeezing whole sweet pomegranate fruits, and the lowest value found in the juice prepared by centrifugation of sour fruit seeds ($p < 0.05$). Similarly, different levels of oxalic acid were reported in pomegranate juice obtained from different cultivars and by various extraction processes (Aarabi *et al.*, 2008; Gundogdu and Yilmaz, 2012; Türkyılmaz,

Table 2. Effect of storage and juice production method on the organic acids of local sweet (*Taif*) and sour (*Bidah*) pomegranate juice.

	Storage (hours)					
Parameters	**0**	**5**	**15**	**32**	**48**	**72**
Citric acid (mg/100 mL)						
Squeezing sweet pomegranate fruit	357.88 ± 65.76^{b,A,B}	354.14 ± 11.15^{b,B}	261.73 ± 9.98^{b,C}	367.57 ± 4.66^{b,A,B}	374.17 ± 9.67^{b,A}	379.32 ± 7.12^{b,A}
Centrifuging sweet pomegranate seeds	344.63 ± 6.40^{b,B}	435.10 ± 45.87^{b,A}	292.87 ± 57.08^{b,C}	369.49 ± 15.17^{b,B}	373.21 ± 20.83^{b,B}	374.31 ± 25.82^{b,B}
Squeezing sour pomegranate fruit	471.13 ± 51.66^{a,A}	476.84 ± 2.27^{a,A}	474.53 ± 3.43^{a,A}	481.34 ± 4.92^{a,A}	487.07 ± 1.363^{a,A}	484.98 ± 6.04^{a,A}
Centrifuging sour pomegranate seeds	469.36 ± 1.81^{a,A}	472.48 ± 1.45^{a,A}	477.14 ± 9.39^{a,A}	479.58 ± 7.73^{a,A}	479.48 ± 3.62^{a,A}	476.98 ± 1.40^{a,A}
Tartaric acid (mg/100 mL)						
Squeezing sweet pomegranate fruit	159.30 ± 6.69^{a,A}	185.98 ± 6.86^{a,A}	156.99 ± 7.38^{a,A}	155.11 ± 8.04^{a,A}	155.05 ± 7.13^{a,A}	154.15 ± 7.42^{a,A}
Centrifuging sweet pomegranate seeds	135.64 ± 6.12^{b,A}	134.76 ± 5.67^{b,A}	133.34 ± 6.09^{b,A}	131.93 ± 6.2^{b,A}	131.31 ± 6.02^{b,A}	129.62 ± 6.46^{b,A}
Squeezing sour pomegranate fruit	157.30 ± 0.38^{a,A}	158.79 ± 13.61^{a,A}	158.81 ± 13.58^{a,A}	152.28 ± 4.47^{a,A}	147.24 ± 0.147^{a,B}	155.32 ± 10.03^{a,A}
Centrifuging sour pomegranate seeds	133.91 ± .0.17^{b,A}	135.16 ± 0.12^{b,B}	134.87 ± 0.45^{b,B}	125.05 ± 0.28^{b,C}	124.66 ± 0.26^{b,C}	123.10 ± .0.91^{b,D}
Malic acid (mg/100 mL)						
Squeezing sweet pomegranate fruit	116.38 ± 2.16^{b,A}	116.59 ± 5.29^{a,A}	108.66 ± 11.56^{b,A}	109.34 ± 8.69^{a,A}	104.81 ± 7.87^{a,b,A}	104.75 ± 7.05^{b,A}
Centrifuging sweet pomegranate seeds	104.86 ± 10.54^{b,A}	102.35 ± 11.39^{b,A}	100.22 ± 10.95^{b,A}	97.11 ± 11.30^{b,A}	97.42 ± 9.95^{b,A}	95.28 ± 11.58^{c,A}
Squeezing sour pomegranate fruit	123.33 ± 0.57^{a,A}	122.66 ± 1.54^{a,A}	116.77 ± 0.71^{a,A}	112.61 ± 0.56^{a,B}	112.77 ± 7.24^{a,B}	123.44 ± 9.12^{a,A}
Centrifuging sour pomegranate seeds	92.77 ± 0.68^{c,A}	87.87 ± 0.46^{c,B}	88.12 ± 0.58^{c,B}	85.67 ± 0.64^{c,C}	85.15 ± 0.29^{c,C}	85.44 ± 0.81^{d,C}
Oxalic acid (mg/100 mL)						
Squeezing sweet pomegranate fruit	20.62 ± 2.61^{a,A}	20.36 ± 2.75^{a,A}	18.61 ± 2.78^{a,A}	21.91 ± 2.26^{a,A}	19.97 ± 2.25^{a,A}	20.02 ± 2.59^{a,A}
Centrifuging sweet pomegranate seeds	11.19 ± 1.41^{b,A,B}	10.61 ± 1.21^{b,A,B}	9.83 ± 0.80^{b,B}	12.06 ± 1.25^{b,A}	11.19 ± 1.08^{b,A,B}	11.47 ± 0.91^{b,A,B}
Squeezing sour pomegranate fruit	11.73 ± 0.42^{b,A,B}	11.30 ± 0.20^{b,A,B}	10.90 ± 0.86^{b,B,C}	10.64 ± 0.43^{b,C}	10.41 ± 0.59^{b,C}	12.57 ± 1.35^{b,A}
Centrifuging sour pomegranate seeds	4.33 ± 1.54^{c,C}	5.72 ± 1.61^{c,A,B}	6.45 ± 0.69^{c,A,B}	6.37 ± 0.61^{c,A,B}	5.80 ± 0.45^{c,B,C}	8.15 ± 0.54^{a,A}
Total organic acids (mg/100 mL)						
Squeezing sweet pomegranate fruit	654.18 ± 4.78^{c,A}	650.07 ± 10.81^{c,A}	454.98 ± 15.58^{c,B}	653.92 ± 4.11^{c,A}	654.00 ± 11.77^{c,A}	658.24 ± 6.6^{c,A}
Centrifuging sweet pomegranate seeds	596.32 ± 59.53^{dA}	592.81 ± 54.79^{dA}	536.25 ± 65.42$^{c\ A}$	610.59 ± 24.58^{dA}	613.13 ± 29.28^{dA}	610.68 ± 35.03^{dA}
Squeezing sour pomegranate fruit	763.49 ± 2.29^{aA}	769.60 ± 11.60^{aA}	761.00 ± 16.49^{aA}	756.87 ± 3.74^{bA}	757.48 ± 7.16^{aA}	776.30 ± 12.47^{aA}
Centrifuging sour pomegranate seeds	700.37 ± 2.48^{bB}	701.23 ± 0.69^{bB}	706.58 ± 9.04^{bB}	969.68 ± 7.62^{aA}	695.09 ± 3.33^{bB}	693.67 ± 2.34^{bB}

Means ± SD of 10 samples followed by different superscript letters are significantly different at $p < 0.05$. The small letters indicate differences in the treatments (columns), while the capital letters indicate differences in the storage time (rows).

2013). During cold storage, the oxalic acid content fluctuated except in the case of juice prepared from the whole sweet pomegranate fruits, which demonstrated no change during storage.

The highest total organic acids content was found in the juice prepared by squeezing the whole sour fruits whereas the lowest organic acids content was found in the juice obtained by centrifuging the seeds of sweet fruits.

Similarly, a higher level of organic acids was reported in the juice obtained from sour pomegranate fruits than that obtained from sweet fruits (Ghaderi-Ghahfarokhi *et al.*, 2016). Among the fruit types, the highest values of organic acids were found in the juice prepared from whole fruit, indicating that removal of peels reduced total organic acids. The content of total organic acids in pomegranate juice was not influenced by storage, except in the case of the juice prepared from whole sweet fruits, which demonstrated decrease at 15 h and then increased to the same level, and the juice prepared from sour fruit seeds, which increased to the maximum at 32 h and thereafter decreased to the initial level with progression in storage time ($p < 0.05$). The changes, i.e., increasing and decreasing trends, in the level of organic acids during storage were likely due to occurrence of different chemical and enzymatic reactions. Content of organic acids fluctuated during storage, indicating decrease in the first 15 h of storage followed by an increment with progress in storage time (Aarabi *et al.*, 2008). Variations in the levels of organic acids in pomegranate juice of different varieties and prepared by various processes have been reported in numerous studies (Aarabi *et al.*, 2008; Gundogdu and Yilmaz, 2012; Türkyılmaz, 2013). The differences in the level of organic acids of pomegranate juice among these studies were likely due to variations in the genetic makeup, growing region and conditions, season and maturity stage, cultural and post-harvest practices, processes of preparing juice, and analysis of organic acids.

Effect of storage and extraction process on the content of sugars in pomegranate juice

The content of sugars in pomegranate juice prepared from sweet and sour fruits using two extraction processes as affected by storage time is shown in Table 3. Fructose and glucose were the primary sugars found in pomegranate juice, and content of both was affected by fruit type and extraction and processing processes. Previous studies have also indicated fructose and glucose as dominant carbohydrates in pomegranate juice (Aarabi *et al.*, 2008; Fadavi *et al.*, 2005; Hasnaoui *et al.*, 2011). On 0 day of storage, the highest ($p < 0.05$) level of fructose was found in the juice prepared from seeds of sweet pomegranate fruit by centrifugation extraction, whereas the least value was observed in the juice prepared by squeezing whole sweet fruit, suggesting the influence of extraction method on the fructose content of juice. The fructose content in the juice was also affected ($p < 0.05$) by the storage time in a fluctuated manner, reaching the highest level at 15 h (juice from the seeds of sweet fruits), 32 h (juice from whole sour fruits) and 48 h (juice from whole sweet fruits and seeds of sour fruits). The increase in the content of fructose during storage could be due enzymatic hydrolysis of disaccharides and polysaccharides present in the juice. However, as the storage period extended to 72 h, chemical complexing reactions and microbial metabolism might occur, which reduced the extractability of fructose and thereby its concentration.

The content of glucose was highest in the juice obtained by centrifugation of seeds of sweet fruits ($p < 0.05$). Storage greatly affected the glucose content of the juice of both types of fruits and extraction processes. Glucose content generally increased to the maximum at 15 h (juice obtained by centrifugation process of sweet fruit seeds) and 48 h (juice of whole sweet and sour fruits and the seeds of sour fruits). The fruit type and the extraction process did not affect the total sugar level on 0 day of storage. However, as the storage time progressed, significant changes were observed in juice depending on the fruit types and extraction processes, especially at 72 h of storage, suggesting the combined effects of fruit type, extraction process and extraction time on the total sugar contents of pomegranate juice. Previous reports have also indicated that sugar content in pomegranate juice decreased at 5 h of storage and then increased as the storage time progressed to the maximum at 48 h, and reduced again by the end of storage period (Aarabi *et al.*, 2008). This was attributed to decrease in carbohydrates because of the *de novo* synthesis of anthocyanin (Aarabi *et al.*, 2008). This might be the same reason for changes in the content of sugars during storage of pomegranate juice in the present study.

Effect of storage and extraction process on the anthocyanin content of pomegranate juice

The anthocyanin content of pomegranate juice of two fruit types (sweet and sour) and two extraction processes (squeezing whole fruit and centrifuging of seeds) as affected by the storage time of the juice is shown in Table 4. The level of anthocyanin in pomegranate juice was affected by fruit type, extraction process and storage time ($p < 0.05$). The highest anthocyanin level was observed in the juice prepared from whole sweet fruits, followed by that from whole sour fruits. The least level of anthocyanin was found in the juice prepared by centrifuging of seeds of both fruit types. This could be attributed to the fact that anthocyanin is found in pomegranate fruit peels, and removing the peels reduced its content in the juice. Compared the fruit types, the highest anthocyanin content was observed in the juice prepared from whole sweet fruits than that from the whole sour ones. During storage, the anthocyanin content of the juice prepared from sweet fruits increased to the maximum at 5 h (whole fruit juice) and 15 h (seed juice). Although the anthocyanin content decreased with increase in storage time, it decreased concomitantly in the case of sour fruits with increase of storage time ($p < 0.05$). The increase in anthocyanin content

Table 3. Effect of storage and juice production method on the sugar contents of local sweet (*Taif*) and sour (*Bidah*) pomegranate juice.

	Storage (hours)					
Parameters	**0**	**5**	**15**	**32**	**48**	**72**
Fructose (g/100 mL)						
Squeezing sweet pomegranate fruit	7.22 ± 0.34^{c,C}	7.56 ± 0.58^{a,B}	7.67 ± 0.32^{b,B}	6.57 ± 1.44^{d,D}	8.82 ± 2.55^{a,A}	7.65 ± 0.34^{a,b,B}
Centrifuging sweet pomegranate seeds	7.86 ± 0.12^{a,A,B}	7.95 ± 0.08^{a,A,B}	8.10 ± 0.24^{a,A}	7.73 ± .010^{c,B,C}	7.50 ± 0.39^{a,C}	7.73 ± 0.19^{a,B,C}
Squeezing sour pomegranate fruit	7.71 ± 0.01^{b,A}	7.82 ± 0.04^{a,A}	7.94 ± 0.04^{a,b,A}	8.05 ± 0.03^{a,A}	7.88 ± 0.12^{a,A}	6.98 ± 0.81^{b,B}
Centrifuging sour pomegranate seeds	7.69 ± 0.00^{b,B}	7.68 ± 0.02^{a,B}	7.81 ± 0.02^{a,b,A,B}	7.84 ± 0.03^{b,A,B}	7.89 ± 0.04^{a,A}	7.81 ± 0.08^{a,A,B}
Glucose (g/100 mL)						
Squeezing sweet pomegranate fruit	6.93 ± 0.21^{b,A,B}	7.12 ± 0.45^{a,b,A,B}	7.37 ± 0.41^{b,A,B}	6.25 ± 1.19^{d,B}	8.66 ± 2.45^{a,A}	7.36 ± 0..20^{a,A,B}
Centrifuging sweet pomegranate seeds	7.45 ± 0.17^{a,B}	7.56 ± 0.31^{a,B}	7.98 ± 0.03^{a,A}	7.55 ± 0.09^{a,B}	7.47 ± 0.29^{a,B}	7.62 ± 0.25^{a,B}
Squeezing sour pomegranate fruit	7.07 ± 0.01^{b,C}	7.16 ± 0.03^{a,b,B}	7.32 ± 0.01^{b,A}	7.35 ± 0.03^{b,A}	7.38 ± 0.05^{a,A}	6.67 ± 0.73^{b,D}
Centrifuging sour pomegranate seeds	6.89 ± 0.01^{b,D}	6.99 ± 0.03^{b,C}	7.13 ± 0.03^{c,B}	7.16 ± 0.02^{c,B}	7.26 ± 0.03^{a,A}	7.24 ± 0.05^{a,b,A}
Total sugars (Brix)						
Squeezing sweet pomegranate fruit	16.0 ± 0.00^{a,C}	16.8 ± 0.00^{b,A}	16.5 ± 0.00 ^{b,B}	16.0 ± 0.00^{c,C}	15.0 ± 0.00^{b,D}	8.5 ± 0.00 ^{c,E}
Centrifuging sweet pomegranate seeds	16.0 ± 0.00^{a,B}	17.3 ± 0.50^{a,A}	17.0 ± 0.00^{a,A,B}	17.3 ± 0.29^{a,A}	16.5 ± 0.58^{a,A,B}	17.3 ± 0.50^{a,A}
Squeezing sour pomegranate fruit	16.0 ± 0.00^{a,B}	16.0 ± 0.50^{b,A,B}	16.0 ± 0.00^{b,B}	16.5 ± 0.00^{b,A}	16.0 ± 0.41^{a,B}	9.3 ± 1.50 ^{c,C}
Centrifuging sour pomegranate seeds	16.0 ± 0.00^{a,A}	16.0 ± 0.00^{b,A}	16.0 ± 0.00^{b,A}	16.6 ± 0.25^{b,A}	15.8 ± 0.29^{b,B}	13.1 ± 0.85^{b,C}

Means ± SD of 10 samples followed by different superscript letters are significantly different at $p < 0.05$. The small letters indicate differences in the treatments (columns), while the capital letters indicate differences in the storage time (rows).

Table 4. Effect of storage and juice production method on the anthocyanin contents of local sweet (*Taif*) and sour (*Bidah*) pomegranate juice.

	Storage (hours)					
Parameters	**0**	**5**	**15**	**32**	**48**	**72**
Alkali treatment						
Squeezing sweet pomegranate fruit	15.40 ± 0.49^{a,C}	18.53 ± 0.43^{a,A}	18.03 ± 0.20^{a,A}	16.92 ± 0.44^{a,B}	16.68 ± 0.035^{a,B}	15.47 ± 6.86^{a,C}
Centrifuging sweet pomegranate seeds	11.07 ± 1.65^{c,C}	14.32 ± 2.81^{b,A}	14.92 ± 0.07^{b,A}	14.12 ± 0.53^{b,A,B}	13.55 ± 0.68^{b,B}	12.15 ± 0.58^{a,C}
Squeezing sour pomegranate fruit	14.74 ± 0.77^{b,A}	12.79 ± 0.09^{c,B}	12.47 ± 0.08^{c,B}	11.99 ± 0.11^{c,C}	11.70 ± 0.12^{c,D}	11.11 ± 0.36^{a,E}
Centrifuging sour pomegranate seeds	9.78 ± 0.82^{c,A}	9.48 ± 1.00^{d,A}	9.15 ± 1.09^{d,A}	8.64 ± 1.14^{d,A,B}	8.32 ± 1.13^{d,A,B}	7.19 ± 0.87^{bB}
Acid treatment						
Squeezing sweet pomegranate fruit	0.56 ± 0.02^{a,B}	0.76 ± 0.11^{a,A}	0.75 ± 0.14^{a,A}	0.71 ± 0.00^{a,A}	0.70 ± 0.01^{a,A}	0.68 ± 0.01^{a,A}
Centrifuging sweet pomegranate seeds	0.34 ± 0.06^{c,B}	0.50 ± 0.00^{b,A}	0.54 ± 0.25^{b,A}	0.53 ± 0.00^{b,A}	0.54 ± 0.03^{b,A}	0.51 ± 0.05^{b,A}
Squeezing sour pomegranate fruit	0.47 ± 0.00^{b,A}	0.46 ± 0.12^{b,A}	0.45 ± 0.00^{b,A}	0.43 ± 0.00^{c,A}	0.43 ± 0.00^{c,A}	0.42 ± 0.01^{c,A}
Centrifuging sour pomegranate seeds	0.30 ± 0.00^{c,A}	0.30 ± 0.05^{c,A}	0.29 ± 0.02 ^{c,A}	0.28 ± 0.01^{d,A,B}	0.27 ± 0.01^{d,B}	0.26 ± 0.01^{d,B}

Means ± SD of 10 samples followed by different superscript letters are significantly different at $p < 0.05$. The small letters indicate differences in the treatments (columns), while the capital letters indicate differences in the storage time (rows).

at the beginning of storage (5 h and 15 h) could be due to the *de novo* synthesis of anthocyanin from carbohydrates (Aarabi *et al.*, 2008) whereas decrease in its concentration with increase in storage time could be likely due to chemical reactions and microbial metabolism. Previous studies have also demonstrated the varying levels of anthocyanin in pomegranate juice of different origins such as Tunisian (Hasnaoui *et al.*, 2011), Iranian (Alighourchi *et al.*, 2008) and Turkey (Türkyılmaz, 2013) varieties. These studies established the influence of genotypes, environmental conditions, maturity stage, post-harvest processing and juice extraction processes on the levels of anthocyanin found in pomegranate juice. In addition, similar changing trend during storage of pomegranate juice extracted by two processes (squeezing and centrifugation) was also reported by Aarabi *et al.* (2008).

Sensory attributes of pomegranate juice

The sensory attributes of pomegranate juice prepared from two local varieties (sweet and sour) using two

Table 5. Sensory attributes of local sweet (*Taif*) and sour (*Bidah*) pomegranate juice produced by two different methods and from two different types of local verities.

Treatments	Sensory attributes					
	Stability	Color	Flavor	Texture	Smell	Overall acceptance
Squeezing sweet pomegranate fruit	17.7 ± 2.78[a]	17.5 ± 2.92[a]	17.76 ± 2.82[a]	17.00 ± 3.83[a]	17.66 ± 3.06[a]	17.96 ± 2.39[a]
Centrifuging sweet pomegranate seeds	15.93 ± 3.03[ab]	15.76 ± 3.6[ab]	16.70 ± 3.14[ab]	14.93 ± 4.20[ab]	16.86 ± 3.54[a]	16.26 ± 2.80[a]
Squeezing sour pomegranate fruit	15.16 ± 3.75[b]	14.36 ± 4.24[b]	15.20 ± 4.12[ab]	13.70 ± 5.03[ab]	16.63 ± 2.97[a]	15.26 ± 2.91[a]
Centrifuging sour pomegranate seeds	13.50 ± 4.99[ab]	14.53 ± 5.42[ab]	14.13 ± 5.09[b]	13.16± 5.65[b]	16.36 ± 3.66[a]	14.20 ± 4.72[a]

Means ± SD of 10 samples followed by different superscript letters are significantly different at $p < 0.05$.

extraction processes are shown in Table 5. Generally, the juice prepared from sweet fruits outscores the one prepared from sour fruits in all sensory attributes. This is due to the above-mentioned results, which indicated the superiority of sweet pomegranate juice over sour variety considering the levels of chemical components, namely sugars, organic acids and anthocyanins. Similarly, it has been reported that the juice prepared from sweet pomegranate fruits received higher preferences than that prepared from sweet–sour and sour fruits (Mayuoni-Kirshinbaum *et al.*, 2013). The scores of overall acceptability and all sensory attributes of all juice types were higher than 13, suggesting that the panelists ranked all juice types as very good and excellent, with the highest preference to the juice prepared from whole sweet fruits and the least preference for the juice prepared from the seeds of peeled off fruits. Previous studies have demonstrated that the sensory attributes of pomegranate juice depended on the factors such as genotype, maturity, season, agronomical practices, climatic conditions and processing methods (Borochov-Neori *et al.*, 2009; Mayuoni-Kirshinbaum *et al.*, 2013; Vázquez-Araújo *et al.*, 2014). Overall, the produced juices could have high consumer acceptability regardless of the fruit type and the extraction process.

Conclusion

This study investigated the production of pomegranate juice from local varieties using two different extraction processes (squeezing whole fruit, and centrifuging fruit seeds after removal of fruit peels). The results established that the quality attributes of the produced juice were affected by genotype, extraction method and storage period at different magnitudes. The juice prepared from the sweet variety had higher pH and sensory acceptability, and lower acidity and anthocyanin level than the juice prepared from sour fruits. Concerning the processing methods, the juice prepared by squeezing the whole fruit outscore, in most of quality attributes, the juice prepared by centrifuging the seeds after removal of fruit peels. Storage duration affected the quality attributes in a fluctuated manner. Overall, squeezing unpeeled pomegranate fruit is the most economical and easy process to produce acceptable and stable juice, especially from sweet pomegranate fruits. The future studies must specifically address the effect of sterilization and long storage conditions on the quality attributes of pomegranate juice.

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