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Development and quality assessment of new drinks combining sweet and sour pomegranate juices

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Abstract

This study was carried out to evaluate the physicochemical properties and the suitability of some parameters for the design of a new beverage combining sour and sweet pomegranate juices as an alternative use for neglected varieties. Sweet pomegranate juices from varieties Zaghouani and Tounsi and sour juices from varieties Mezzi and Garsi were used in this study. Higher titratable acidity (1.6% calculated as citric acid) with a pH of 2.73–2.82 was registered in Garsi and Mezzi juices. The contents of ascorbic acid, anthocyanins and total phenols were in general high, 20-25 mg ascorbic acid, 37.7-56 mg cyanidin and 164.4-181.8 g gallic acid per 100 ml juice, respectively. Zaghouani and Mezzi varieties gave the cleanest juices, while Tounsi gave the less colored juice. The highest antioxidant activity was registered in sour varieties. New polyphenol-rich beverages were prepared using sour and sweet pomegranate juices in different proportions (reaching pH 4.0). The results of sensory tests suggest that mixed beverages are preferred to pure sweet juices. Garsi (18.2%)-Zaghouani (81.8%) mixture provided the most appreciated beverage with good organoleptic properties (color, clarity and flavor) followed by Garsi (16.7%)-Tounsi (83.3%) mixture which was well appreciated for its taste and improved color. Further research is needed to establish new pomegranate juice combinations and to examine the stability of characteristics and bioactive proprieties of mixtures after cold storage in order to develop new healthy beverages.

Key words: Pomegranate, Juice quality, Antioxidant activity, Beverage, Sensory evaluation

Introduction

Over the past few decades, food consumption habits have changed immensely. Consequently, consumers have a broad range of choices for the basic food bundle. The development of healthy food was rated as the most important area of research, followed by developing natural foods (Katz, 2000). The rising interest in maintaining and improving human health and life justify the increasing demand of fruits. One of the most frequently utilized fruit production technologies is juice processing. Consumers demand original juice with minimal processing, a juice with no sugar added, and also a juice which that closely emulates the raw fruit from which it is derived.

Fruits of pomegranate species are not only consumed fresh but also used to produce jam, jelly, syrup and several types of soft drinks (Maestre et al., 2000; Vardin and Fenercioglu, 2003). Recently, there is a huge demand for industrial processing to obtain pomegranate juice because of its benefits toward healthy living. The juice is a rich source of natural components as polyphenols, anthocyanins and mineral nutrients that play an important role in maintaining juice quality and determining nutritive value. Moreover, pomegranate fruit juice has other properties such as antioxidant, antinflammatory, and antiatherosclerotic against some diseases (Malik et al., 2005; Neurath et al., 2005; Sumner et al., 2005). A diet high in pomegranate juice is linked to lower risk for several chronic degenerative diseases, including certain cancers and cardiovascular disease (Lansky and Newman, 2007; Viuda-Martos et al., 2010). In Tunisia, pomegranate has been cultivated traditionally since ancient times and local germplasm is very diverse containing numerous varieties with sweet fruits and others with sour fruits which were more and more neglected. Our research was focused on providing an alternative use of these varieties. The study of organoleptic, chemical and antioxidant properties of some Tunisian varieties and the design

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of new beverages combining sour juice and sweet juice was carried out in this work.

Material and Methods

Plant material

Ripe fresh fruits were collected from four pomegranate varieties growing in the same *ex-situ* collection in Gabès (south Tunisia) (Mars and Marrakchi, 1998). The varieties selected for this study are: Garsi, Mezzi, Tounsi and Zaghouani. Representative fruits samples were collected, at harvesting maturity, for each variety.

Preparation and analysis of raw juice

Fruits were washed in cold tap water and drained. They were manually cut-open and the outer leathery skin was removed. The arils were manually separated from the fruits and juices were obtained by pressing the arils. The following attributes related to juice were evaluated.

Juice color was assessed visually according to grading scale of color intensity (2: light pink: the lightest color) to 18 (reddish purple: the darkest color) according to its intensity. Juice clarity was also evaluated visually through a note scale going from 1 (very hazy) to 5 (limpid).

The pH measurements were performed using a JENWAY model 4320 pH-meter at 20°C. Titratable acidity (TA) was determined according to the AOAC methods (1984) using 0.1N NaOH to the end point of pH 8.1 and expressed as grams of citric acid per liter (Ender et al. 2002). The soluble solids content (TSS) was measured with manual refractometer Atago (Tokyo, Japan) at 20°C and expressed as °Brix. Maturity index was calculated by dividing TSS to TA. Total sugars were determined by the Lane–Eynon (1923) method and expressed as g of sugar per 100 g of juice.

Formol index number (IF), indicator of free amino acids (Fry et al., 1995), was determined using a DL 58 titrator (Mettler-Toledo, Greifensee, Switzerland). The results were expressed in ml 0.25M NaOH 100 ml^{-1} juice. Potassium (K) and sodium (Na) levels were determined using a Jenway flame spectrophotometer after centrifugation in a 201M Sigma centrifuge (AFNOR, 1996). Calcium (Ca) was determined by complexometry using EDTA solution (AFNOR, 1996). All results were expressed in mg 100^{-1} g of juice.

Vitamin C content was determined with iodine and sodium thiosulfate using starch as an indicator according to Arya et al. (2000). Anthocyanin content was determined according to Porter et al. (1989). To 0.5 ml of juice were added 6 ml of n-butanol-HCl (95 : 5, v/v) solution and 0,2 ml of $NH_4Fe(SO_4)_2 12H_2O$-HCl 2M (2%, m/v) solution. The absorbance was determined using an UV/Visible spectrophotometer Philips PU 8800. Results were expressed as mg of cyanidin per 100 ml of juice. Total phenolic content was determined with the Folin–Ciocalteu method (Arnous et al., 2001). Reaction was followed with a spectrophotometer and results were expressed as mg of Gallic acid/100 ml of juice.

Antioxidant activity

The antioxidant activity of pomegranate juices was determined using the stable 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) (Espín et al., 2000; Llorach et al., 2004). All samples were centrifuged at 10,480 g (model EBA 21; Hettich Zentrifugen) for 5 min at room temperature.

The reaction mixture consisted of 2 µl of the diluted sample and an aqueous methanolic solution (250 µl) containing the free radical (DPPH•). The reduction of the DPPH radical was determined by measuring the absorption at 515 nm after 50 min of reaction, using 96-well microplates (Nunc, Roskilde, Denmark) and Infinite®M200 microplate reader (Tecan, Grödig, Austria). The DPPH• radical scavenging activity was expressed as mmol L^{-1} Trolox.

Juice mixture

New beverages were prepared using sour and sweet pomegranate juices in different proportions (reaching pH 4.0) in order to reduce the acidity of sour juices (Carpenter et al., 2000; Mars, 2001).

Juice sensorial analysis

The flavor of a nutriment is a property established using a multitude of sensorial attributes (Martinez et al., 2006). Juice sensorial analysis was conducted by untrained panel of tasters. Fresh pomegranate juices extracted from all the cultivars and the beverages obtained from the mixture were evaluated for their color, taste and the overall appreciation. A hedonic scale going from 1 to 8 was adopted for the taste (1: very acid, 2: acid, 3: acid with a sweet aftertaste, 4: less acid with a sweet aftertaste, 5: less sweet with an acid aftertaste, 6: sweet with an acid aftertaste, 7: sweet, 8: very sweet). Pomegranate juices were served at room temperature (about 20ºC) together with the appropriate questionnaire, one at a time and waiting 5 min between samples. Unsalted crackers and water were provided to consumers for palate cleansing between samples. In each questionnaire, consumers were asked, using 5-point hedonic scales (from 1: dislike, 2: neither like nor dislike, 3: like moderately, 4: like very much and 5: like extremely), about the overall liking of the sample and their satisfaction degree about pomegranate juice sensory attributes (color and taste).

Statistical analyses

All data were subjected to ANOVA using SPSS 13.0 software. The data shown are mean values and the significance of the differences between juices and mixtures was determined using the Student-Newman and Keuls (SNK) test ($P < 0.05$).

Results and Discussion

Organoleptic characteristics

Color of fruit juice represents key elements of its total appearance. All evaluated pomegranate juices exhibited red color. Mezzi and Garsi juices were rather reddish than Zaghouani while Tounsi gave a red-pink juice. Turbidity is also an important index of clear juice that is a natural juice that is pulpless and do not have cloudy appearance (Sin et al., 2006). Mezzi juice was the clearest one followed by Garsi and Zaghouani, however Tounsi juice is relatively hazy (Table 1).

Regarding TSS content, Garsi yielded the lowest mean of total soluble solids content (14.08 °Brix), and Mezzi had the highest mean (16.28 °Brix). TSS content for all varieties ranged higher than the minimum threshold generally required for commercial use (>12%) and felled into the range of other pomegranate cultivars grown under Spanish (Martinez et al., 2006) and Iranian (Tehranifar et al., 2010) conditions. Total sugars content of studied varieties ranged from 12.56 to 14.23 g/100 g (Table 1). The highest amount was observed for Mezzi and the lowest was in Garsi (Table 1). Similar results were reported by Poyrazoglu et al. (2002), however, Melgarejo et al. (2000) found high amounts over 15 g/100g in some Spanish sweet varieties.

Juice pH variation was high ranging from 2.74 for Garsi to 4.02 for Zaghouani (Table 1). As regards to titratable acidity (expressed as % citric acid), statistically significant differences were noted among varieties. Zaghouani scored the lowest acidity (0.23%) and Garsi the highest one (2.04%). According to Chace et al. (1981), pomegranate was appropriate for fresh market when its acidity content was lower than 1.8%. Hence, Garsi could be considered inappropriate for fresh consumption. Muradoglu et al. (2006) reported that among 53 Turkish cultivars, some of them had an acidity superior to 0.9%.

The maturity index (TSS/TA) is one of the important factors influencing the taste and flavor of pomegranate, which some researcher used to classify the pomegranate cultivars (Kulkani and Aradhya, 2005; Tehranifar et al., 2010). This classification has been optimized for Spanish cultivars: maturity index (MI) = 5-7 for sour, MI = 17-24 for sour-sweet, and MI= 31-98 for sweet cultivars (Martinez et al., 2006). The maturity index values varied from 6.91 to 65.96 (Table 1). According to Martinez et al. (2006), cultivars are classified as: Garsi as sour, Mezzi as sour-sweet and Tounsi and Zaghouani as sweet varieties.

Nutritional properties

There are many mineral elements present in pomegranate juice (Figure 1). Potassium is the most abundant of all the elements investigated in pomegranate juice. The average overall potassium content was 283.88 mg/100g. No cultivar differences were observed for potassium contents. Only juice from Garsi contained markedly higher potassium content. Sodium was also present in pomegranate juices abundantly with amounts ranging from 63 to 72.2 mg/100 g. The highest content was revealed in Tounsi 1 juice while Mezzi 2 has the lower one. As for potassium content, no significant differences were noted between cultivars for their content of calcium. Tounsi1 contained the highest amount (20.52 mg/100g). Thus, pomegranate juice can be a good source of nutrients and variation could originate from the pomegranate cultivar, and agro-climatic conditions (Al-Maiman and Ahmad, 2002). Most of the findings regarding the mineral components of the pomegranate juice are consistent with the results of the other studies (Al-Maiman and Ahmad, 2002; Fischer-Zorn and Ara, 2007).

Table 1. Characteristics of four Tunisian pomegranate varieties.

Variety	JC	TURB	TSS (°Brix)	TS (g/100 g)	PH	TA (%)	MI
Mezzi (Sour-sweet)	14.80±1.79a	4.80±0.45a	16.28±5.40a	14.23±2.23a	2.85±0.12d	1.69±0.59b	9.62b
Garsi (Sour)	13.20±1.79a	4.00±0.0b	14.08±7.56b	12.56±3.99b	2.74±0.03c	2.04±0.14a	6.90c
Tounsi (Sweet)	8.00±0c	3.00±0.0c	14.92±7.95b	13.04±4.64b	3.83±0.10b	0.26±0.12c	56.99a
Zaghouani (Sweet)	10.00±0b	4.00±0.0b	15.00±4.69b	13.07±2.53b	4.02±0.02a	0.23±0.08c	65.96a
Significance level	**	**	**	**	**	**	**

**Means with different letters, in the same column, indicate significant differences at $P< 0.01$.

JC: Juice color, TURB: Juice turbidity, TSS: Total soluble solids content, TS: Total sugars content, PH: juice pH, TA: Titratable acidity, MI: maturity index

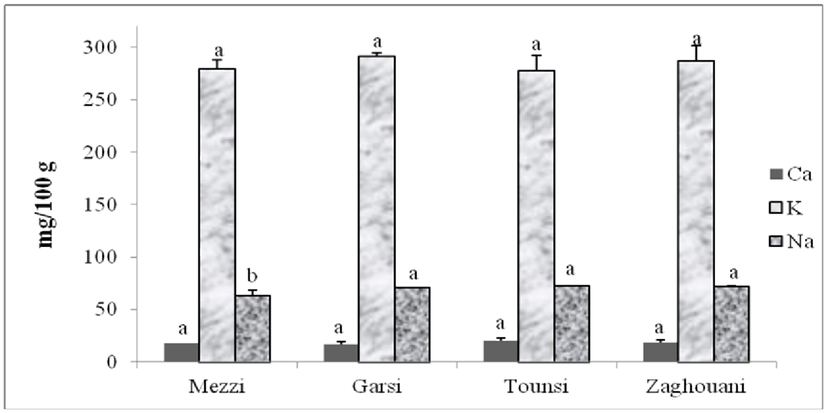

Figure 1. Mineral composition of pomegranate juices prepared from four cultivars.
Means with different letters indicate significant differences at $P \leq 0.01$ according to Duncan range test. Ca: Calcium amount; K: Potassium amount; Na: Sodium amount

Formol index which reflects the amount of free amino acid varied from 2.19 to 3.15 mL 0.1N NaOH/ 100 mL. Our findings related to formal index are lower than those reported by Ekşi and Özhamamci (2009). Ascorbic acid, abundant in pomegranate juice, has biological functions in fruits which include roles in redox control and antioxidant activity that prevent the browning of tissues (Kulkarni and Aradhya, 2005). The concentration of ascorbic acid varied from 20 to 25 mg/100 ml (Table 2). Sour varieties showed higher amounts of ascorbic acid as compared to sweet ones. Our pomegranates exhibited values higher than those reported by Zarei et al. (2010) for Iranian cultivars.

Anthocyanins are water-soluble pigments primarily responsible for the attractive color of many fruits, including pomegranate juices, and they are well known for their antioxidant activity (Noda et al., 2002; Mena et al., 2011). Examination of the total level of anthocyanins in different juices demonstrated that sour varieties exhibited higher content (over than 47 mg/100 ml) than sweet ones (Table 2). This could be explained by dependence of the intensity and stability of the anthocyanin on juice pH (Wilska-Jeszka and Korzuchowska, 1996). The destruction of anthocyanin pigments increases with increase in pH (Laleh et al., 2006). The levels of anthocyanin found with our juices were higher than those reported for Iranian (Alighourchi et al., 2008) and Chilean cultivars (Sepúlveda et al., 2010).

The content of total phenolics is one of the most important parameters for appraising the characterization of pomegranate cultivars, with respect to their nutritional value and potential use for different products. A variation in terms of total phenolic content were observed among the pomegranate varieties (164.47-181.84 mg Gallic acid/100 ml) (Table 2), but no significant differences were revealed between them. Ozgen et al. (2008) reported similar levels of total phenolics for Turkish cultivars (124.5-207.6 mg/100g) whereas, Gil et al. (2000) found higher level exceeding 180 mg/100 ml for Wonderful variety. According to these results, as being a good source of total phenolics, pomegranate can be considered as an important nutrient for human health.

Antioxidant activity

The DPPH method is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors, and to evaluate antioxidant activity of foods. The use of this method DPPH free radical is advantageous because it is more stable than the hydroxyl and superoxide radicals. The degree of discoloration indicates the scavenging potentials of the antioxidant extract. The DPPH scavenging activity of pomegranate juices was significantly different (Table 2). The sour variety Mezzi showed the highest antioxidant activity value (22.50 mmol L^{-1} Trolox) while Tounsi showed the lowest values (16.01 mmol L^{-1} Trolox). These results are closer to those obtained for Iranian (Mousavinejad et al., 2009), Turkish (Ozgen et al., 2008) and Spanish varieties (Mena et al., 2011).

Plant phenolics constitute one of the major groups of antioxidants acting as free radical terminators (Samarth et al., 2008). This result showed a good relationship with the total phenolic content. Many studies reported that high polyphenols content contributes towards high radical scavenging activity (Mena et al., 2011). Nevertheless, antioxidant capacity cannot only be related to the phenolics content but it is the result of multiple factors (Arazo et al., 2011).

Table 2. Formal index, ascorbic acid, total phenolics, total anthocyanins contents and antioxidant capacity in aril juices prepared from 4 Tunisian pomegranate varieties.

Variety	FI	AAC (mg/ 100 ml)	TAC (mg/ 100 ml)	TPC (mg/ 100 ml)	DPPH (mmol L^{-1} Trolox)
Mezzi	3.15±0.11[a]	24.21±0.01[a]	51.01±2.40[a]	181.84±16.71[a]	22.50±3.27[a]
Garsi	3.15±0.14[a]	25.10±0.01[a]	47.66±2.16[b]	174.55±11.30[a]	17.59±2.39[b]
Tounsi	2.64±0.10[b]	21.22±0.01[b]	34.21±1.93[d]	171.21±14.10[a]	16.01±3.76[b]
Zaghouani	2.19±0.04[c]	20.42±0.01[b]	43.11±1.81[c]	164.47±36.19[a]	17.58±3.25[b]
Significance level	**	**	**	NS	**

**Means with different letters, in the same column, indicate significant differences at P< 0.01; NS: Non significant at P>0.05.
FI: Formal index; AAC: Ascorbic acid content; TAC: Total anthocyanins content; TPC: Total phenolics content

Table 3. Volumes of sweet juices added to sour juices.

Sour juice	Tounsi volume added (ml)	Tounsi volume percentage (%)	Zaghouani volume added (ml)	Zaghouani volume percentage (%)
Mezzi (100 ml)	450	81.8	400	80.0
Garsi (100 ml)	500	83.3	450	81.8

Juice mixture

The acidic taste and related flavor are some of the important attributes of pomegranate juice, which contribute to its strong appeal in the food and beverage industry. In this regard, sour varieties represent diversity in local pomegranate genetic resource (Mars, 2001) with very low sugar: acid ratio that is suited for formulation of a wide range of food and beverage products. Providing new beverages mixed with sour pomegranate juices is likely to increase their consumption. Thus, we mixed both sour and sweet juices in different ratios in order to obtain a final pH of 4. With such pH value the juice is considered acceptable (Carpenter et al., 2000). The volumes added were presented in Table 3.

Sensory evaluation

Color

Attractive color is one of the most important sensory characteristics of pomegranate arils and juice products. Significant differences for beverages color acceptance were found (P < 0.05). The sensory analysis regarding beverage color recorded mean values between 2.66 (like moderately) and 3.94 (like very much). Zaghouani-Garsi juice mixture was the most preferred according to its color followed by Zaghouani-Mezzi juice mixture and Zaghouani juice (Figure 2).

Flavor

Significant difference was found for the flavor of beverages (P > 0.05). Mean values stayed between 1 (very acidic) and 7.66 (very sweet). Mezzi is considered by the panelists as acidic with a sweet aftertaste. The mixture Mezzi-Tounsi was less sweet with an acidic aftertaste while Mezzi-Zaghouani mixture was considered sweet with an acidic aftertaste. Tounsi juice as well as Garsi-Tounsi and Garsi Zaghouani beverages were found sweet. The average scores for flavor acceptance was between 1 (dislike), which can be regarded as unsatisfactory result, considering the bitter flavor of Garsi juice and 5 (like extremely) considering Zaghouani juice as very sweet beverage (Figure 2).

Overall preference

Low overall liking was associated with juices with high intensities of sourness. Mixing the juices elaborated with sour or sour-sweet pomegranates with sweet ones is a solution to simultaneously decrease the sourness of the products and increase its acceptability by consumers. Panelists easily detected significant differences in taste and color. They preferred in general Zaghouani juice beverage as well as beverages made of Garsi juice mixed with Zaghouani which provided good organoleptic properties (color, clarity and flavor) or Garsi mixed with Tounsi which was esteemed for its taste and improved color. The panelists gave such products high ranking (Figure 2).

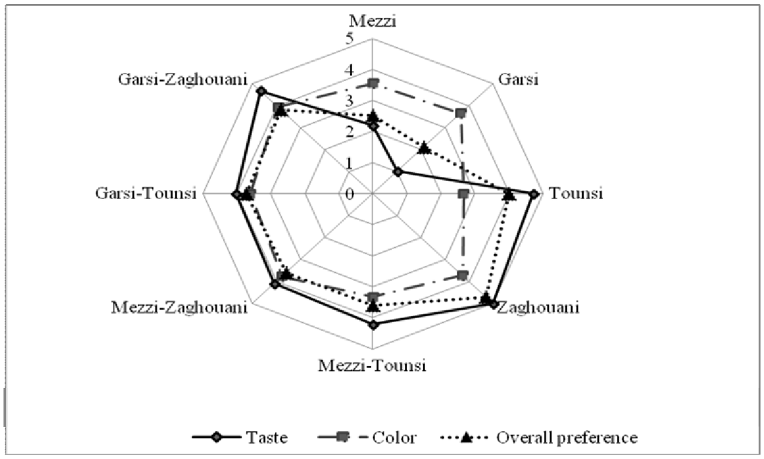

Figure 2. Satisfaction degree scores of consumer panel for main sensory attributes of fresh pomegranate beverages according to taster's hedonic rating.

Conclusion

Though the picture is far to complete, the study has shown that Tunisian pomegranate varieties particularly sour varieties (Mezzi and Garsi) were rich in polyphenols as well as anthocyanins, ascorbic acid and mineral components. This study provides a useful insight into production for new beverages with nutritional intake. Sensory analysis which is often used to determine the acceptability of a newly developed product showed that beverages made by mixing sour and sweet pomegranate juices were well appreciated by the untrained panelists. Further research is needed to establish new pomegranate juice combinations and to examine the stability of characteristics and bioactive proprieties of mixtures after cold storage in order to develop new healthy beverages.

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