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in

Melgarejo P. (ed.), Valero D. (ed.).
II International Symposium on the Pomegranate

Zaragoza : CIHEAM / Universidad Miguel Hernández
Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 103

2012
pages 205-208

Changes in some free sugars and phenolic contents of pomegranate fruits (*Punica granatum* L.) in three development stages

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Abstract. In this study, some free sugars and phenolic contents at three development stages (immature, unripe and full ripe) of pomegranate cv. Hicaznar fruits grown in Antalya, Turkey were determined. While total sugar contents showed a regular increment, total phenol content decreased from the immature fruit to full ripe fruit. The predominant sugars quantified were fructose and glucose, while sucrose was minor sugar in all development stages. Fructose contents in immature fruits to mature fruits varied from 3.53 to 5.78 g per 100g fresh weight basis, while glucose contents varied from 3.44 to 6.37 g per 100g fresh weight. On the other hand, sucrose contents varied from 0.21 to 0.41 g per 100g fresh weight. On the contrary to sugars, the lowest levels of total phenolics and flavanoids were determined in full ripe fruits. Results indicate that both free sugars and phenolic contents of pomegranate were strongly influenced by development stages.

Keywords. Pomegranate fruit – Sugar – Phenolic – HPLC.

I – Introduction

Pomegranate is grown mainly in Mediterranean and Asian countries. Among these countries, India, Iran, China and Turkey are main producers. Other important producers are Spain, Tunisia, Morocco, Afghanistan, China, Pakistan, Azerbaijan, Armenia, Cyprus, Egypt, Israel and Saudi Arabia (Ercisli *et al.*, 2007; Gozlekci *et al.*, 2011). The number of pomegranate orchards in Turkey has been increasing rapidly in recent years because of high profit earnings. The cultivar 'Hicaznar' is the most important cultivar for export to European countries (Gozlekci *et al.*, 2011).

Pomegranate fruit is rich in polyphenols including ellagitannins, gallotannins, ellagic acids, gallagic acids, catechins, anthocyanins, ferulic acids, and quercetins. Phenolic compounds and flavonoids are unique category of plant phytochemicals especially for their vast potential in health-benefiting properties. They represent the most abundant and the most widely represented class of plant natural products (Fraga, 2010). Many studies focused on the physico-chemical characteristics of pomegranate fruits at maturity stage. We aimed to compare physico-chemical characteristics of pomegranate on three development stages.

II – Material and methods

Pomegranate cv. 'Hicaznar' sampled at three development stages: 1st stage; immature fruit (May 15), 2nd stage; un-ripe fruit (July 15); 3rd stage; full ripe fruit (October 15). A total of 30 fruits were used for physical measurements (Table 1). Pomegranate juice was obtained from pomegranate arils by a hand press, and homogenized for total phenolic, total flavonoid and sugar extractions. Total soluble solid, pH and acidity were determined directly on juice (AOAC, 1995). Total phenolics were estimated in Folin-Ciocalteu reagent according to Singleton *et al.*,

(1999). Total flavonoids were estimated according to Miliauskas *et al.* (2004). All determinations were carried out in quadruplicate and the mean values were used. Sugars in the samples were determined by HPLC analysis (Miron and Schaffer, 1991).

Table 1. Physical properties of pomegranate fruits at development stages (mean±SE)

Fruit characteristics	Fruit development stages		
	Immature	Un-ripe	Full ripe
Fruit weight (g)	12.51±2.27 ^{c}	128.21±2.27^{b}	418.62±2.12^{a}
Fruit diameter (mm)	27.76±0.66^{c}	64.31±0.66^{b}	95.18±0.62^{a}
Fruit length (mm)	52.75±1.52^{c}	80.41±1.52^{b}	106.38±1.42^{a}
Shape index (FD/FL)	0.52 ±0.02^{c}	0.80±0.02^{b}	0.90±0.01^{a}
Calyx length (mm)	24.05±1.16^{ns}	24.14±1.16	25.74±1.08
100-aril weight (g)	0.28±0.33^{a}	7.56±0.33^{b}	32.08±0.31^{c}
Aril yield (%)	13.22±1.05^{c}	46.09±1.05^{b}	56.72±0.99^{a}
Juice yield (%)	8.23±0.85^{c}	26.97±0.85^{b}	49.37±0.80^{a}
Seeds in aril (%)	35.81±2.14^{a}	41.48±2.14^{a}	16.48±2.01^{b}
Skin thickness (mm)	5.13±0.13^{a}	4.78±0.13^{a}	4.12±0.12^{b}
Skin color parameters			
a*	21.90±1.58^{c}	-8.71±1.58^{b}	39.88±1.48^{a}
b*	20.08±1.15^{c}	36.41±1.15^{a}	27.01±1.08^{b}
Lightness (L*)	38.50±1.43^{c}	54.51±1.43^{b}	59.21±1.34^{a}
Chroma (C*$^{)}$	29.83±1.64^{c}	37.48±1.64^{b}	48.27±1.53^{a}
Hue angle (H$^{o)}$	42.11±1.62^{a}	-76.58±1.62^{c}	34.41±1.52^{b}
Aril color parameters			
a*	-0.66±0.21^{c}	2.55±0.21^{b}	5.97±0.20^{a}
b*	13.95±0.35^{a}	11.74±0.35^{b}	2.04±0.33^{c}
Lightness (L*)	61.08±0.70^{a}	48.37±0.70^{b}	31.22±0.66^{c}
Chroma (C*$^{)}$	13.97±0.39^{a}	12.02±0.39^{b}	6.31±0.36^{c}
Hue angle (H^{o})	-87.22±0.64^{c}	77.65±0.64^{a}	18.89±0.60^{b}

Means in each row followed by different letters are significantly different (P≤ 0.05); ± standard error.

All the statistical analyses were carried out using SPSS statistical software (SPSS 19.0, 2010) and results were expressed as mean ± standard error. ANOVA was performed by ANOVA procedures. Significant differences among the means were determined by 'Duncan's multiple range test' at a level of p<0.05.

III – Results and discussion

Table 1 shows physical properties of pomegranate fruits at different development stages and demonstrated significant differences on most characteristics (*P*<0.05). Average fruit mass of 'Hicaznar' ranged from 12.51g (immature fruit) to 418.62 g (full ripe fruit). The fruit length and diameter values were determined between 52.75 mm and 106.38 mm and 27.76 mm and 95.25 mm in immature and full ripe stages, respectively. Fruit shape (expressed as diameter/length ratio), of immature fruits were oblong and full ripe fruits were oblate. There were significant differences (P<0.05) in aril ratio and juice yield among the development stages. Full ripe fruits had the highest aril and juice yield (56.72% and 49.37%) (Table1). In previous studies, a wide variation in fruit mass of pomegranate cultivars varying between 150 and 568 g were reported, (Ercan *et al.*, 1992; Yilmaz *et al.*, 1992; Tehranifar *et al.*, 2010). Fruit length and width of full ripe

pomegranate cultivars grown in different countries ranged from 61 to 91 mm and 36 to 104 mm, respectively (Yilmaz *et al.*, 1992; Amaros *et al.*, 1998; Barone *et al.*, 2001). Fruit skin and aril colors of pomegranate fruits at different stages of development are shown in Table 2. Skin color of fruits varied from green to dark red color during fruit development. Skin and aril colors expressed as luminosity and chroma were ≈42.11 and 29.83 for immature fruit and ≈34.41 and 48.27 for full ripe fruits, respectively. Red aril color is one of the most important sensory characteristics of pomegranate pulp and juice (Celik and Ercisli, 2009).

As shown in Table 2, significant statistical differences (P<0.05) among all studied chemical parameters in different fruit development stages were observed. Soluble solid content values showed an increase during fruit development stages which is the highest (16.08%) in full ripe fruits. Titratable acidity was significantly lower in full ripe fruits (1.64%) compared to unripe fruits (3.98%). Dark red aril color, high SSC and relatively high acidity of pomegranate arils are considered to be a good choice for both fresh fruit and juice markets (Ozgen *et al.*, 2008).

Table 2. Chemical properties of pomegranate juice at development stages (mean±SE)

Juice characteristics	Fruit development stages		
	Immature	Un-ripe	Full ripe
Titratable acidity (%)	1.16±0.08^{c}	3.98±0.08^{a}	1.64±0.08^{b}
pH	4.60±0.07^{a}	2.37±0.07^{c}	3.19±0.06^{b}
Soluble solid content (%)	5.79±0.11^{c}	9.03±0.11^{b}	16.08±0.10^{a}
T. phenolics (mg GAE/ L)	2920.0±0.16^{a}	2340.0±0.16^{b}	986.7±0.16^{c}
T. flavonoids (mg GAE/ L)	800.0±0.03^{a}	330.0±0.03^{b}	580.0±0.03^{c}
Fructose (g/100 ml)	3.54±0.29^{c}	5.04±0.29^{ab}	5.78±0.29^{a}
Glucose (g/100 ml)	3.44±0.25^{c}	5.00±0.25^{b}	6.37±0.25^{a}
Sucrose (g/100 ml)	0.21±0.18^{c}	0.32±0.18^{b}	0.41±0.18^{a}
T. sugars (g/100 ml)	7.19±0.60^{c}	10.40±0.60^{b}	12.56±9.60^{a}

The values shown are mean ± standard error of three replications. Significant differences among the means were determined by Duncan's multiple range test ($P \leq 0.05$) within parameters in each line; means with the same letter do not differ significantly.

There were significant differences in total phenolics and total flavonoid contents in juice obtained from different fruit development stages at $P<0.05$ (Table 2). Pomegranates showed a rapid and significant depletion in total phenolics during the fruit development from initial stage to full ripe stage. Total phenolics and total flavonoids ranged from 986.7 (full ripe fruit) to 2920.0 mg GAE/L (immature fruit) and 330.0 (un-ripe fruit) to 800.0 mg GAE/L (immature fruit) in juice. Karadeniz *et al.* (2004) reported total phenolics (2408 mg kg^{-1}) and total flavonoids (459 mg kg^{-1}) in pomegranate.

Fructose concentration varied from 3.54% (immature fruit) to 5.78% (full ripe fruit), while glucose values were between 3.44% (immature fruit) and 6.37% (full ripe fruit) in juice (Table 2). Ozgen *et al.* (2008) reported an average of 6.4% and 6.8%, and Al-Maiman and Ahmad (2002) of 6.66% and 7.72% fructose and glucose contents. Glucose and fructose were the dominant sugars in juice at fruit development stages. Glucose, fructose, sucrose and total sugar values increased with the advance in maturity. The increase in total and individual sugars in fruit juice can be due to hydrolysis of starch into simple sugars as it was similarly reported by Biale (1960).

IV – Conclusion

Physical and chemical properties of the pomegranate cultivars indicated that the developmental

stage of fruit for 'Hicaznar' is an important factor in determining fruit quality. The study provided important data for physical and compositional changes of the fruits at different development stages of pomegranate, emphasizing that pomegranate fruit can be a good source of nutrients at different maturation stages.

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