Scientific Papers. Series A. Agronomy, Vol. LXI, No. 1, 2018
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

EVALUATION OF SOME MORPHOLOGICAL, CHEMICAL PARAMETERS AND ANTIOXIDANT CAPACITY OF POMEGRANATE

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Abstract

The aim of this study is the evaluation of some morphological and compositional characteristics of three pomegranate fruit samples. For pomegranate juice samples (PJ), total soluble solids (TSS) varies between 14.6 and 16.3°Brix, titratable acidity (TA) between 0.28 and 1.13(%), total phenolic contents (TPC) from 221 to 323.3 (mg/100 mL) and DPPH radical scavenging activity shows EC50 values between 35.2 and 48.3 (mL PJ/g). For peel methanol extracts (PE), TPC was 198.2-279.8 and EC50 3.7-5.6 (µg/mL).

Key words*: pomegranate, phenolic contents, DPPH.*

INTRODUCTION

Punica granatum L. (*Punicaceae*) has been used for centuries in the folk medicine of many countries (Kumar et al., 2013) for the prevention and treatment of a wide number of health disorders such as inflammation, diabetes, diarrhea, dysentery, dental plaque and to combat intestinal infections and malarial parasites (Ismail et al., 2012, Rosenblat et al., 2006).

Pomegranate fruit juice, peel and leaf extracts have been reported to possess strong antioxidant activity (Zhang et al., 2008), and can help prevent or treat various disease risk factors including high blood pressure, high cholesterol, oxidative stress (Aviram et al., 2001), hyperglycemia, inflammatory activities (Lansky and Newman, 2007) and disorders of the digestive tract (Seeram et al., 2005).

The scientific studies on the antioxidant activities, bioactive constituents, and pharmacological properties of pomegranate have increased considerably in the last decade (Kalaycıoglu and Erim, 2017).

Figure 1 shows the number of studies on pomegranate recorded in Science Direct between 2008 and 2017.

The main objective of this research was to characterize three pomegranate samples with various origins, to quantify phenolics content in pomegranate juice and methanolic extract and to evaluate free radical scavenging activity.

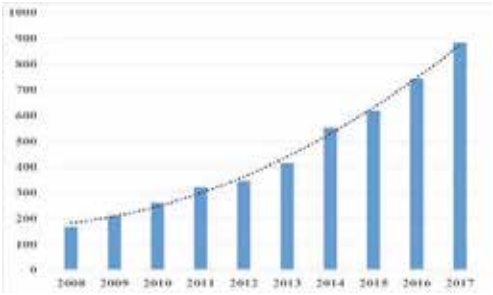

Figure 1. Number of publications recorded in Science Direct between 2008-2017 for „pomegranate"

MATERIALS AND METHODS

Plant material

We used three pomegranate (*Punica granatum* L.) fruits samples weighing about 2 kg each. Two samples (S1 and S2) were purchased from local markets (imported from Turkey), the third sample (S3) being obtained from Spain.

From each sample were selected healthy fruits with no visible external cuts or spoilage. The

fruits were rinsed with tap water and cut at the equatorial zone. Arils were manually extracted and squeezed through a metal sieve. The resulting juice was filtered through filter paper.

Pomegranate peel extraction. Pomegranate peels were dried to brittleness (hot air oven 45°C, 40 h) and powdered to 40 mesh (Grindomix GM200 knife mill). Peel powder (20 g) was extracted for 4 h with MeOH solution (MeOH: water 4:1) in a Soxhlet extractor (Buchi B811). Methanol extracts were concentrated under reduced pressure (Rotavapor Bucchi R215) and lyophilized.

TSS and TA measurements. The total soluble solids (TSS) were determined with a digital refractometer (Mettler-Toledo, 30 PX). The titratable acidity (TA) was obtained by titration with NaOH 0.1 N to pH 8.2 (g citric acid/100 mL). The maturity index was calculated as the ratio of TSS/TA.

Determination of total phenolic content (TPC). TPC of the extracts were estimated using the Folin-Ciocalteu colorimetric method reported by Singleton et al. (1999). After appropriate dilution, the samples were mixed with 1.0 mL of 10-fold diluted Folin-Ciocalteu reagent and 0.8 mL of a 7.5% sodium carbonate solution. The mixture was kept for 30 min at room temperature and after that, the absorbance was measured at 765 nm using a UV-Vis spectrophotometer (JASCO V 630). The results were expressed as mg of gallic acid equivalents (GAE) per g of powder extract.

DPPH radical scavenging activity. The free radical scavenging activity was determined using DPPH (2,2-diphenyl-picryl-hydrazil) test (Blois,1958). A DPPH solution (0.1 mM in ethanol, 4 mL) was mixed with 1 mL sample, containing different concentrations of extract. After 30 min, absorbance at 517 nm was recorded. The antiradical activity (%) was calculated using the relation:

$$\begin{pmatrix} DPPH\ radical \\ scaveging\ activity\ \% \end{pmatrix} = \frac{A_c - A_s}{A_c} \cdot 100$$

A_c- absorbance of DPPH solution;
A_s- absorbance of sample.

The value corresponding to 50% inhibition (EC50) was obtained from the graph of anti-oxidant activity (%) *vs*. extract concentration.

All results (mean ± standard error) were the mean of three determinations.

RESULTS AND DISCUSSIONS

The weights of the three pomegranate samples as well as their diameter, arils weight and juice volume are presented in Table 1. As it can be observed, there are no significant differences between S1 and S2 samples for any of the studied parameters.

The S3 sample presents significant differences versus S1 sample considering the fruit weight, arils weight proportion and the volume of the juice obtained.

Our results correspond with those presented for Turkish cultivars by Gözlekçi et al. (2011) (arils %: 42.3-52.85; juice volume %: 37.16-48.69), Durgaç et al. (2008) (arils %: 36.9-59.4). For Spanish cultivars Martinez (2006) has obtained for pomegranate juice values between 50.25% and 64.17%.

Table 1. Morphological parameters of pomegranate fruits

Sample	Total weight (g)	Equatorial diameter (mm)	Arils (%) (g/ 100 g FW)	Juice (mL/100 g FW)
S1	296.94± 13.67[a]	81.0± 4.0 [a]	52.4± 6.8 [a]	31,4± 3.2[a]
S2	317.9± 19.9[a]	83.0 ±4.0 [a]	56.1± 4.8 [a,b]	38.6± 5.0[ab]
S3	377.3± 19.4[b]	85.7± 4.04 [a]	66.2± 4.3 [b]	46.4± 4.5[b]

In each column, values with the same letter are not significantly different (Tukey simultaneous tests for differences between means - $P \leq 0.05$).

Because pomegranate fruit external skin color does not indicate the extent of ripening degree or its readiness for consumption (Holland et al., 2009), another parameter such as color of aril, total soluble solids, titratable acidity, maturity index are usually considered for fruit quality assessment (Martinez et al., 2006).

Sugar content determined as total soluble solids (TSS) varies between 14.6 and 16.3°Brix, pH value between 3.12 and 4.10, titratable acidity between 0.28 and 1.13.

The results of the chemical analyzes for pomegranate juice presented in Table 2 clearly distinguish the three samples. Similar results were communicated by Hernandez (1999) for

Spanish cultivars, pH: 2.89-4.42, TSS: 13.48-16.51, TA: 0.23-2.03, and by Nuncio-Jáuregui et al. (2014), pH: 3.55-5.42, TSS: 14.80-16.53, TA: 0.23-2.14.

The polyphenols content of pomegranate juice varied between from 221 to 324 mg gallic acid equivalents per 100 mL juice and from 198.2 to 279.8 mg gallic acid equivalents per g extract.

Table 2 Chemical analysis of the juice from the pomegranate fruits

Sample	TSS (°Brix)	pH	TA (g/100 mL)	Maturity index
S1	14.6± 0.11[a]	4.10± 0.02[a]	0.28± 0.01[a]	52.1
S2	15.3± 0.20[b]	3.12± 0.01[b]	1.13± 0.01[b]	12.4
S3	16.3± 0.05[c]	3.49± 0.03[c]	0.69± 0.00[c]	23.6

In each column, values with the same letter are not significantly different (Tukey simultaneous tests for differences between means - $P \leq 0.05$).

Table 3. Total phenolic content (TPC) and DPPH radical scavenging activity (EC50) in juice and peel methanol extracts

Sample	Juice		Peel Extract	
	TPC (mg/100 mL)	EC50 (mL PJ/g)	TPC (mg/g)	EC50 (µg/ mL)
S1	221± 3.0a	48.3± 0.9a	198,2± 3.4a	5.6± 0.3 a
S2	243± 2.0b	35.2± 1.1b	248,6± 4.5b	3.7± 0.2 b
S3	323.3± 2.1c	40.2± 1.4c	279,8± 4.1c	4.2± 0.4 b

In each column, values with the same letter are not significantly different (Tukey simultaneous tests for differences between means - $P \leq 0.05$).

Data presented in Table 3 prove that no relation can be clearly established between the total content of phenolic compounds and the free radical scavenging activity. As the antioxidant activity increases with the decrease of EC50 value, it would has been expected the S3 sample, having the highest value for polyphenols, 324 mg gallic acid equivalents per 100 mL juice, to have the lowest value for EC50, but our experimental results showed that the lowest EC50 value was obtained for the S2 sample with a medium value for TP, 243 mg /100 mL. Future experiments will be conducted to verify this result and to get additional evidence.

It could be also observed that samples with high polyphenol content in the juice, will have also have a high phenolic content in peels. High majority of the authors of the scientific papers in the area used for the determination of the phenolic compounds content the Folin-Ciocalteu regent method. For this reason, the results reported by different teams can be easily compared. Özgen et al. (2008) reported values starting from 124.5 to 207.6 mg GAE/100 mL for the concentration of phenolic compounds in six cultivars grown in Turkey, while Çam et al. (2009) in experiments conducted with eight cultivars, obtained for the same characteristic, values between 208.3-343.6 mg GAE/100 mL. The experiments done by Çalıskan and Bayazıt (2012) with 76 accesions grown in Turkey revealed values of the content of phenolic compounds between 1080 - 9449 mg GAE/kg. Similar results were also communicated by other scientists for Spanish cultivars: 150-450 mg GAE/100 mL (Mena et al., 2011), 267.4-421 mg GAE/100 mL (Nuncio-Jáuregui et al., 2014), 113.62-358.11 mg GAE/100 mL (Vegara et al., 2014). On the other hand, the values of the antioxidant activity are more difficult to be compared with data from the scientific literature, as this parameter is determined through different analytical methods.

However, we can mention the results presented by Kulkarni et al. (2005), who obtained a value EC50 of 8.33 µg/mL for a methanolic extract of peels, working with a Ganesh variety, cultivated in India, or the results of Fernandes et al. (2015) reporting an EC50 value of 16.33 µg/mL for the methanolic extract of peels for the variety Mollar de Eiche.

In scientific publications (Fawole et al., 2013, Mphahlele et al., 2014; Hmid et al., 2016) it is demonstrated that the chemical parameters of the pomegranate juice depend on cultivar, geographic origin, harvest time and post-harvest practices. For these reasons, the values obtained by us would not be considered as characteristic for pomegranate varieties. However, these results are important because they represent characteristics of the fruit reached on the consumer's table.

CONCLUSIONS

Based on the results experimentally obtained, one can conclude that the only use of morphological parameters (fruit weight, aril weight, juice volume) does not permit the sample differentiation. The chemical characteristics (TSS, pH, TA, Maturity Index), on the other hand, are different enough to make a difference between the three analyzed samples. All three samples have high values for both TPC and free radical scavenging activity. The samples with high concentration of polyphenolic compounds in juice, also present high YPC values in the methanol extract. However, we cannot establish so far a direct correlation between TPC values and the antioxidant activity expressed as free radical scavenging activity.

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