Bulgarian Chemical Communications, Volume 51, Issue 1, (pp. 113 – 116) 2019

Polyphenols content and antioxidant activity of various pomegranate juices

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Received July 16, 2018; Revised October 29, 2018

The aim of the study was the determination and analysis of pH, content of common phenols, antioxidant activity (radical trapping activity), common anthocyanins and acidity of pomegranate (*Punica granatum*) and their relation to the soil composition and altitude. Methods for systematic approach and critical analysis of the available scientific periodicals were applied. Spectrophotometric method was used for determination of adsorption with gallic acid standard; DPPH method was used for determining the antioxidant activity (radical trapping activity). It was found that the value of pH index ranges from 3.05 to 4.29. The lowest level of common phenols 67.71 ± 0.12 mg GAE/100 ml was observed in the Red Filk variety. Total anthocyanins were within the range of 0.60 ± 0.01 mg/100 ml (Red Filk) to 35.80 ± 0.65 mg/100 ml (Pink Satin). Antioxidant activity (DPPH) was: 966.13 ± 37.67 mmol TE/100 ml (Red Filk) to 2271.72 ± 26.91 mmol TE/100 ml. Titratable acidity was within the range: 0.47 ± 0.10 g/100 mL for Red Filk and up to 1.64 ± 0.10 g/100 mL for Pink Satin. It was concluded that there is a direct correlation between the antioxidant activity and the altitude at which the pomegranate is growing. There is a direct correlation between the common phenols and the antioxidant activity, excluding variety Wonderful.

Keywords: Pomegranate, Antioxidant activity, Phenols, Anthocyanins.

INTRODUCTION

The pomegranate is a type of deciduous tree or a bush of the genus *Punica*. The plant is dry and “comes” from Asia, Africa, Europe and Middle East. Pomegranate fruits are mainly consumed fresh but they are also used in the preparation of fresh juices, canned beverages, jellies, jams, etc. In recent years there has been increasing interest in pomegranate (number of published materials grew 7 times) as medical and food industry product. The pomegranate tree especially its fruit, has a long history and a bunch of healing properties. Depending on the characteristics and composition, pomegranates may be intended for fresh consumption, industrial processing or medical purposes. It is therefore important to know the characteristics of different pomegranate varieties in order to gain more knowledge about their application [1-3]. Pomegranate juice has powerful antioxidant properties. They may be associated with anti-inflammatory effects on a plant-based basis. Phytochemistry and pharmacological actions of all components of *Punica granatum* suggest a wide range of clinical applications for cancer treatment and prophylaxis [2, 4-10]. As a result of the increased attention given to pomegranate, the number of countries producing pomegranate has increased and new varieties appeared [3-8, 11]. The synergistic effect of pomegranate ingredients is better than that of the individual components. Over the last decade, numerous studies have been published on the antioxidant, anticancer and anti-inflammatory properties of pomegranate ingredients for the treatment and prevention of cancer, cardiovascular disease, diabetes, dental conditions, erectile dysfunction, bacterial infections, antibiotic resistance and skin damage [4, 11-13].

Pomegranate juice has been proven to be extremely effective in the prevention of atherosclerosis [14, 15]. Gil *et al*. determined the antioxidant activity of pomegranate juice by 4 different methods and found that it is 3 times higher than that of red wine and green tea [16]. Hmid *et al*. investigated the pomegranate varieties grown in Morocco and found high concentration of phenolic compounds, antocyanins and organic acids [17].

The aim of this study was the determination and analysis of pH, total polyphenols, antioxidant activity (radical trapping activity) total anthocyanins and acidity of pomegranate (*Punica granatum*) and their dependence on the altitude.

METHODS AND MATERIALS

Fruit sample

The antioxidant characteristics of pomegranates from different varieties grown in Bulgaria (Garnet Sash and Wonderful), Greece (Red Filk), Macedonia (Garnet Sash) and Turkey (Pink Satin) were studied. Pomegranate varieties from Bulgaria

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Delchev. The altitude of the town of Petrich is 168 m, and of Gotse Delchev 540 m. The Red Filk variety from Greece was grown in the Kavala area at an altitude of 53 m. Rosso Gigante from Macedonia is from the town of Doiran (148 m above sea level). At an altitude of 200 m above sea level near the town of Bursa is the variety Pink Satin.

Juice extraction, pH and titratable acidity analysis

To obtain the juice, pomegranate fruits were peeled by hand and the seeds were liquefied using a Philips food processor. The obtained juice was pre-filtered and then centrifuged at 3000 rpm for 15 min. pH was measured potentiometrically with a pH meter (WTWinoLab pH 7110, Germany). Titratable acidity, expressed as percentage of malic acid, was performed by titrating 10 mL of pomegranate juice with a 0.1 M NaOH to a pH point of 8.1. The results were expressed in g malic acid per 100 g juice.

Total phenolics content (TPC)

The total phenolic contents were meas-ured using a Folin-Ciocalteu assay. Folin- Ciocalteu reagent (1 mL) (Sigma) diluted five times was mixed with 0.2 mL sample and 0.8 mL 7.5% Na2CO3. The reaction was performed for 20 min at room temperature in darkness. Then the absorbance of the sample was measured at 765 nm against blank sam-ple, developed by the same way but without extract. The results were expressed in mg equivalent of gallic acid (GAE) per 100 g juice, according to calibration curve, build in range of 0.02 - 0.10 mg gallic acid (Sigma) used as a standard.

Total monomeric anthocyanins analysis

The total monomeric anthocyanins content was determined using the pH differential method described by [17]. 2 g juice were extracted with 8 mL ethanol at ultrasound bath for 15 min. The pH of juice samples was brought to 1.0 with potassium chloride and 4.5 with sodium acetate buffers. The dilutions were then allowed to equilibrate for 5 min at room temperature. The absorbance of equilibrated solutions at 520 nm for anthocyans content and 700 nm for haze correction was measured on a VIS spectrophotometer (Camspec M107, UK) with 1-cm path-length disposable cuvettes. All absorbance measurements were carried out at room temperature against distilled water as a blank. Pigment content was calculated as cyanidin-3-glucoside (cyanidin-3-glucoside) equivalents with a molecular weight of 449.2 and an extinction coefficient of 26 900 L/(cm.mol).

Antioxidant activity (DPPH assay)

Each analyzed extract (0.15 mL) was mixed with 2.85 mL of freshly prepared 0.1 m mol solution of 1,1-diphenyl-2- picrylhydrazyl radical (DPPH, Sigma) in methanol (Merck). The reaction was performed at 37 °C in darkness and the absorption at 517 nm was recorded after 15 min against methanol. The antioxidant activity was expressed as m mol Trolox equivalents (TE) per 100 g juice by using a calibration curve built with 0.05, 0.1, 0.2, 0.3, 0.4 and 0.5 mmol 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox®, Fluka) dissolved in methanol (Sigma).

RESULTS AND DISCUSSIONS

Pomegranate (*Punica granatum* L.) is one of the oldest edible fruits. It is widely grown in different parts of Asia, North Africa, the Mediterranean areas and in the Middle East. The pomegranate fruit is pressed to prepare commercial juices. A large number of bioactive compounds would be expected to be extracted from juices and would have high antioxidant properties. The values obtained for pH, common phenols, total anthocyanins, antioxidant activity and titratable acidity during the test are presented in Tables 1 and 2. The results for juice titrable acidity (TA), and pH for the different cultivars are presented in Table 1. Significant differences were revealed among the pomegranate cultivars for all parameters. The pH values ranged between 3.0 and 4.2. The pH values obtained in the current study are in agreement with pomegranate cultivars grown in Morocco, Tunisia and Turkey [17-19]. The titrable acidity content varied from 0.47 to 1.60 g/100 mL. Similar results were also reported by Hmid *et al.* [17], whereas the values reported by Hasnaoui *et al.* were different [19]. According to the results, cultivar type plays an important role in terms of pH and titrable acidity of the pomegranate juice [17-19]. Phenolic compounds of pomegranates juice were presented as ellagic acid, gallic acid, chlorogenic acid, caffeic acid and ferulic acid [17]. It is evident from Table 2 that the highest content of total phenols has the variety Pink Satin (193.16 ± 1.12 mg GAE/ 100 mL), followed by Wonderful, Garnet Sash, Rosso Gigante and Red Filk. Additionally, many investigators reported that pomegranate juice has a high free radical scavenger capacity. Our results revealed that pomegranate juice of the Wonderful variety has the highest antioxidant activity (2271.72 ± 26.91 m mol TE/ 100 mL). In accordance, it was established that the total polyphenol content of 18 cultivars varied from 138 to 947 mg GAE/ 100 mL of pomegranate juice [17]. In addition, six anthocyanins - 3-glucosides and 3,5-diglucoside of delphinidin, cyanidin and pelargonidin has been identified in the pomegranate juice [18, 19].

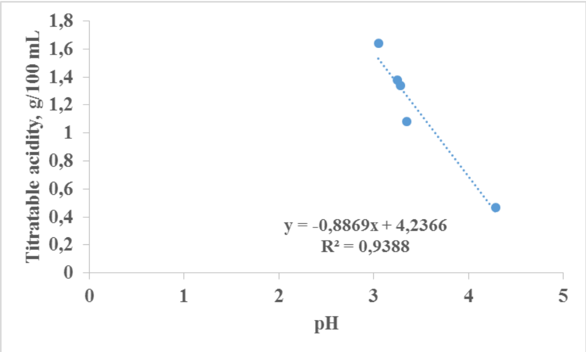

Figure 1. Correlation between pH and titratable acidity.

Turkyılmaz [18] established significant differences between total anthocyanins contents (2–44 mg/100 mL juice) in different varieties of pomegranate. Similar result were obtained in our study (Table 2). This indicator is the lowest for the Red Filk variety. The Pink Satin, Garnet Sash and Rosso Gigante varieties are approximately evenly distributed. As shown in Table 2, the total anthocyanins in different juices could be arranged in the following order: Pink Satin>> Rosso Gigante > Garnet Sash > Wonderful > Red Filk. The highest content of polyphenols coincides with the highest anthocyanins content in Pink satin variety.

The major organic acid reported in pomegranate juice is citric acid (66–74%), followed by malic acid (6–12%) [18, 19]. The relationship between pH and titratable acidity is decreasing, which corresponds to the theoretical descriptions of the scientific periodicals and the practical expectations of the study. The linear dependence between pH and antioxidant activity (Figure 1) is disturbed by the Wonderful variety - 2271.72 ± 26.91 m mol TE / 100 ml.

Table 1. pH and titratable acidity of different pomegranate juices

Variety	pH	Titratable acidity (g malic acid /100 mL)
Rosso gigante	3.25 ± 0.04	1.38 ± 0.10
Wonderful	3.35 ± 0.05	1.08 ± 0.10
Red filk	4.29 ± 0.03	0.47 ± 0.10
Garnet sash	3.28 ± 0.04	1.34 ± 0.10
Pink satin	3.05 ± 0.05	1.64 ± 0.10

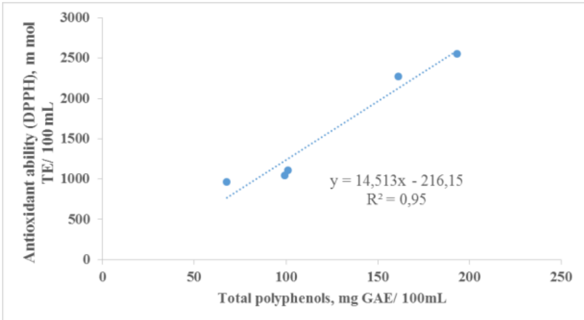

Figure 2. Correlation relation between total polyphenols and antioxidant ability.

CONCLUSIONS

The results provide important information of the polyphenols content and antioxidant capacity of pomegranate juice, which can be useful for developing fruit processing industries for commercial available pomegranate varieties. The pomegranate juice was determined as a very valuable food for people with chronic illnesses, because of its high antioxidant activity. In addition, a positive correlation between antioxidant activity and altitude of pomegranate varieties, as well as between total polyphenols and antioxidant activity was found. This study enriched the information about pomegranate fruits and demonstrated the potential beneficial health effect of this plant.

Table 2. Total phenols, total anthocyanins and antioxidant activity of different pomegranate juices

Variety	Total phenols (mg GAE/100 ml)	Total anthocyanins (mg/100 ml)	Antioxidant activity (DPPH) (m mol TE/100 ml)
Rosso gigante	99.40 ± 0.52	7.81 ± 0.10	1047.00 ± 33.63
Wonderful	161.04 ± 0.51	2.40 ± 0.03	2271.72 ± 26.91
Red filk	67.71 ± 0.12	0.60 ± 0.01	966.13 ± 37.67
Garnet sash	101.15 ± 0.91	5.10 ± 0.05	1109.77 ± 48.37
Pink satin	193.16 ± 1.12	35.80 ± 0.65	1558.09 ± 83.93

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