Comparison of Chemical Composition and Radical Scavenging Activity of Pomegranate Extracts from Different Growing Areas

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Abstract

The pomegranate (Punica granatum L.) is a promising source of functional food, which contains several phytochemicals that perform important roles in reducing the risk of pathological diseases. Chemical compositions, such as the total sugar, uronic acid, polyphenols, and anthocyanin contents, and radical scavenging activity were determined and compared among PEs from six different cultivation areas. Total anthocyanin contents (TAC) and total polyphenol contents (TPC) from various growing areas were detected in the following order, respectively: Spain (19.08 μg/mL) > Turkey (12.91 μg/mL) > Iran-A (6.67 μg/mL) > Taiwan (4.77 μg/mL) > Uzbekistan (1.88 μg/mL) and Turkey (639.52 μg/mL) > Uzbekistan (502.19 μg/mL) > Spain (306.40 μg/mL) > Iran-B (249.20 μg/mL) > Taiwan (162.78 μg/mL) > Iran-A (143.93 μg/mL). PEs from six different cultivation areas were determined to vary in ellagic acid content from 8.90 μg/mL to 332.52 μg/mL. The amounts of total sugars in PE from Iran-A evidenced relatively high total sugar contents, but low uronic acid levels (11.92 mg/mL). DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activities were as follows: Turkey > Uzbekistan > Spain > Iran-B > Iran-A > Taiwan. ABTS [2,2’-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)] radical scavenging activities were detected in the following order: Turkey > Uzbekistan > Spain > Iran-B > Iran-A > Taiwan. These results indicate that the PEs from Turkey with higher levels of TPC, TAC, ellagic acid, and higher radical scavenging activity may constitute a promising functional source for the prevention of several degenerative diseases.

Key words: pomegranate, growing area, chemical composition, antioxidant activity, ellagic acid

INTRODUCTION

The pomegranate (Punica granatum L.) is one of the oldest edible fruits, and is grown widely throughout many tropical and subtropical countries (1). Over 1000 cultivars of Punica granatum are known to exist, originating from the Middle East, extending throughout the Mediterranean, eastward to China and India, and on to the American Southwest, California and Mexico in the New World (2). Interest in this fruit is growing, as it is considered to be a functional product of potentially great benefit in the human diet, as it contains several groups of substances that are useful in disease risk reduction (1).

The edible portion of the fruit contains a considerable quantity of sugars, vitamins, polysaccharides, polyphenols, and minerals. Despite its importance as a semi-arid cultivar, little effort has been exerted in the study of the chemical composition of the edible portion of the pomegranate. The chemical composition of fruits differs depending on the cultivar, growing region, climate, maturity, harvest practices, and storage (3). Significant variations in the sugars, water-soluble vitamins, and mineral composition of pomegranates have been described by various researchers over the years (4). Owing to the influence of environmental and cultivar differences on the nutritional values of the fruit, more work in this area is warranted (1,5,6). Therefore, the consumption of pomegranate as a juice has increased considerably among the general Korean population, because of its reported health benefits.

The principal objective of this study was to quantify the chemical components, including total sugar, uronic acid, ellagic acid, total polyphenol, and total anthocyanin contents of PEs obtained from six different cultivation areas. Additionally, the radical scavenging activities of...
six PEs against DPPH and ABTS radicals were also evaluated and compared.

MATERIALS AND METHODS

Materials
Commercial PE (60 brix) from six growing areas (Iran A & B, Spain, Taiwan, Turkey, Uzbekistan) were generously provided from Komax (Seoul, Korea). ABTS [2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)], DPPH (1,1-diphenyl-2-picrylhydrazyl), ellagic acid and Folin-Ciocalteu phenol reagent were purchased from Sigma (St. Louis, MO, USA). All other chemicals were of analytical grade.

Total anthocyanin content (TAC)
The TACs of PEs were determined via the pH differential method using two buffer systems: potassium chloride buffer, pH 1.0 (0.025 M) and sodium acetate buffer, pH 4.5 (0.4 M) (7). In brief, 0.4 mL of PE sample was mixed with 3.6 mL of corresponding buffers and read against water as a blank at 510 and 700 nm. Absorbance (A) was calculated as:

\[ A = (A_{510} - A_{700}) \times \frac{100}{510 - A_{700}} \]

The total anthocyanin content of the samples (mg cyanidin-3-glucoside/100 mL of PE) was calculated via following equation:

\[ \text{TAC} = \frac{(A \times \text{MW} \times \text{DF} \times 100)}{\text{MA}} \]

where A: absorbance; MW: molecular weight (449.2); DF: dilution factor (10); MA: molar extinction coefficient of cyanidin-3-glucoside (26,900).

Assay of polyphenol contents and ellagic acid contents
The total polyphenol contents (TPC) of PEs were spectrophotometrically determined via the Folin & Ciocalteu assay as described by Vinson et al. (8). The 1 mL PE sample was mixed with 1 mL of 6 M HCl and 5 mL of 75% methanol/water solution in a screw-capped tube. The tube was vortexed and placed in a 90°C water bath and then shaken for 2 hr. The tube was then allowed to cool to room temperature and diluted with distilled water to a volume of 10 mL. 1 mL of this solution was mixed with 5 mL of Folin & Ciocalteu reagent, previously diluted 10-fold. 15 mL of Na2CO3 (7 g/100 mL) was added to this mixture to establish basic conditions.

The ellagic acid contents were determined in the extract by HPLC with UV detection via the method developed by Poyrazoğlu et al. (9). The mixture was then diluted with distilled water to 100 mL. The absorbance versus the prepared blank was read at 760 nm until it achieved steady state. The same procedure was applied for six standard solutions of ellagic (50~300 mg/100 mL). The final results were expressed as mg ellagic equivalent per 100 mL of PE. Extracted pomegranate juice was diluted (1:1) with distilled water and filtered through 0.45 mm Millipore filters and subjected to HPLC. The HPLC elution was carried out at room temperature and utilized as solvent A, with a mixture of formic acid and water (5:95, v/v), and as solvent B, with methanol. The elution profile was, at a flow rate of 1.0 mL/min, 15% solvent B isocratic for 5 min followed by a 15~30% linear gradient for 15 min and 30~50% linear gradient for 10 min with solvent B and holding for an additional 10 min with 50% solvent B, and finally followed by a 50~15% linear gradient with solvent B for 10 min. The chromatogram was simultaneously monitored at 280 and 320 nm at a 2 nm bandwidth, with spectra obtained continuously throughout the elution. The calculation of concentrations was based on the external standard method.

Analysis of total sugar, uronic acid and carbohydrate components
The juice samples were defrosted and centrifuged (2 min at 12,000 rpm), then filtered through a Sep-Pak C18 cartridge (Millipore, Milford, USA) and a 0.45 μm Millipore filter to remove interferences and particles. Total sugar and uronic acid contents were determined with phenol-H2SO4 (10) and m-hydroxydiphenyl (11), respectively, using galactose and galacturonic acid as the respective standards.

Carbohydrate components were also determined via HPLC using a Varian HPLC system and a refractive index detector. A column (High-Performance Carbohydrate Cartridge, 5 μm, 4.6×250 mm) was utilized for the separation. 20 μL of clarified juice was injected into the column. HPLC elution was conducted at room temperature using distilled water at a flow rate of 1.5 mL/min as a mobile phase. Concentrations were calculated by the external standard method.

Radical scavenging activity
The DPPH scavenging activity was measured according to the method described by Quang et al. (12), with some modifications. In brief, 0.4 mL of 0.2 mM DPPH ethanolic solution was mixed with 0.1 mL of sample. The mixture was then vigorously shaken and left to stand for 10 min under subdued light, after which the absorbance was measured at 520 nm.

The ABTS radical scavenging activities of PEs were determined as described by Wang and Xiong (13) and Almajano et al. (14) with a slight modification. The ABTS+ solution was prepared with final concentrations
of 7 mM ABTS and 2.45 mM potassium persulfate. The mixture was then left in darkness at room temperature for 12~16 hr prior to use. Before the assay, the ABTS\(^+\) solution was diluted with 0.2 M in sodium phosphate-buffered saline (pH 7.4) to an absorbance of 0.70±0.02 at 734 nm. Then, 40 μL of sample (containing 40 mg protein/mL) was added to 4 mL of diluted ABTS\(^+\) solution. The mixture was shaken vigorously for 30 sec and left in darkness for 6 min. The absorbance of the resultant solution was then measured at 734 nm. The results were expressed as a percentage of scavenged nitric oxide with respect to the negative control without any added antioxidant. The percentage of scavenging was calculated as the ratio of the absorption of the sample relative to the control without the extract.

**Statistical analysis**

Data were statistically analyzed via analysis of variance (ANOVA) and differences among the means were determined for significance at p<0.05 using Duncan’s multiple range test (SPSS 15.0 for Windows, SPSS Inc., IL, USA).

**RESULTS AND DISCUSSION**

**Total anthocyanin content (TAC), total phenolic content (TPC) and ellagic acid content**

Table 1 shows the antioxidant compositions of six PEs, respectively. The TAC of PE from Spain was more than was seen in the PEs from other growing areas. The TACs of PEs from Spain, the second-highest TAC level among our tested samples, was approximately 1.48-fold higher than that of the PE from Turkey. TAC according to various growing areas were detected in the following order: Spain (19.08 μg/mL)> Turkey (12.91 μg/mL)> Iran-A (6.67 μg/mL)> Taiwan (4.77 μg/mL)> Uzbekistan (1.88 μg/mL)> Iran-B (0.76 μg/mL).

The highest TPC levels were detected in the PE from Turkey (732.55 μg/mL) and the lowest in the PE from Iran-A (143.93 μg/mL). TPC levels were detected in the following order: Turkey (639.52 μg/mL)> Uzbekistan (502.19 μg/mL)> Spain (306.40 μg/mL)> Iran-B (249.20 μg/mL)> Taiwan (162.78 μg/mL)> Iran-A (143.93 μg/mL).

Gil et al. (15) previously reported that the TAC and TPC of the “Wonderful” pomegranate cultivar were 38.7 and 248.7 mg/100 mL, respectively. In another study, the TAC and TPC of the “Suruc” cultivar were reported as 8.9 and 156.4 mg/100 mL, respectively (16). These variations are attributable to differences among cultivars, growing seasons, agricultural practices, and variations in applied total phenolic determination assays.

Red fruit juices have received a great deal of attention due to their phenolic contents and antioxidant activities. To determine the relative phenolic content of PEs, we compared their TPC values with those corresponding to other red fruit juices, as determined via the same methodology. The TPC levels of PEs were higher than those of the other juices, including turnip juice (77.2 mg/100 mL), sour-cherry juice (79.7 mg/100 mL) (17), red grape juice (172.8 mg/100 mL), and red wine (186.9 mg/100 mL) (18).

It has been reported that pomegranate juice or extract is one of the most important sources of anthocyanins (cyanidin, delphinidin, and pelargonidin), which give the fruit and aril their red color, and some of the phenolics and tannins (such as punicalin, pedunculagin, punicalagin and ellagic acid) (19). Thus, the ellagic acid contents of PEs were assayed in six PEs from different growing areas (Fig. 1). Ellagic acid content in the PEs varied from 8.90 μg/mL to 332.52 μg/mL (Fig. 1). The contents

**Table 1. Total polyphenols and total anthocyanin contents of various pomegranate extracts**

<table>
<thead>
<tr>
<th>Pomegranate</th>
<th>Iran-A (^{(1)})</th>
<th>Iran-B (^{(1)})</th>
<th>Spain (^{(1)})</th>
<th>Taiwan (^{(1)})</th>
<th>Turkey (^{(1)})</th>
<th>Uzbekistan (^{(1)})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total polyphenols (μg/mL)</td>
<td>143.93±1.63 (^{(2)})</td>
<td>249.20±1.96 (^{(2)})</td>
<td>306.40±2.88 (^{(2)})</td>
<td>162.78±1.63 (^{(2)})</td>
<td>639.52±1.96 (^{(2)})</td>
<td>502.19±1.89 (^{(2)})</td>
</tr>
<tr>
<td>Total anthocyanin (μg/mL)</td>
<td>6.67±0.38 (^{(2)})</td>
<td>0.76±0.05 (^{(2)})</td>
<td>19.08±0.22 (^{(2)})</td>
<td>4.77±0.18 (^{(2)})</td>
<td>12.91±0.46 (^{(2)})</td>
<td>1.88±0.30 (^{(2)})</td>
</tr>
</tbody>
</table>

\(^{(1)}\)The name for each sample was growing area. The results are presented as means±SD. Same alphabet doesn’t mean different value significant at p<0.05 by Duncan’s multiple range tests.
of PEs from Turkey evidenced high content levels. The ellagic acid contents according to various growing areas were detected in the following order: Turkey > Iran-B > Iran-A > Spain > Uzbekistan > Taiwan.

The extracts from red raspberry leaves or seeds, pomegranates, or other sources have been demonstrated to contain high levels of ellagic acid, and are available as dietary supplements in capsule, powder, or liquid form. A recent profusion of pomegranate nutraceutical products, "standardized to 40% ellagic acid", has appeared in the marketplace (20). Ellagic acid has been detected in significant quantities thus far in 46 fruits, including pomegranate, raspberries, strawberries and cranberries, and also in nuts, walnuts, pecans, pomegranates, and other plant foodstuffs (21). In order to evaluate the importance of pomegranate as dietary sources of ellagic acid for use as nutraceuticals, it is important to determine the ellagic acid contents in PEs cultivated in different areas.

**Total sugar, uronic acid and sugar composition**

Although people are generally aware of the interrelationship between diet and health, other quality factors must be present in a health-promoting food, particularly superior flavor, before widespread consumption of the food can become feasible (22). The combination and the ratio of sugars and organic acids have been previously related to the flavor quality of fruits. The amounts of total sugars and uronic acid in the pomegranate cultivars are provided in Fig. 2. The PE from Iran-A evidenced a high level of total sugar content (2.25 g/mL). However, the PE evidenced low levels of uronic acid (11.92 mg/mL). The PE from Turkey was determined to harbor a medium level of total sugar, but high levels of uronic acid. Acidic polysaccharides obtained from plant sources have been demonstrated to exhibit a variety of biological activities, including immunostimulatory, antioxidant, antitumor, and antiviral properties (23,24).

The quantities of fructose, glucose, sucrose and total sugars in the pomegranate according to the growing area are provided in Fig. 3. Fructose and glucose were identified as dominant sugars in all analyzed PEs. The fructose and glucose concentrations were measured to be 94.68 ~ 212.15 mg/mL and 137.4 ~ 278.18 mg/mL, respectively. However, the sucrose levels were relatively low, at below 98.80 mg/mL. Ozgen et al. (25) also identified fructose and glucose as dominant sugars in all analyzed cultivars. The fructose and glucose concentrations averaged 6.4 and 6.8 g/100 mL, respectively. The sucrose levels in their samples proved almost negligible. This bolsters the previously reported results for other pomegranate cultivars (1,22). Sucrose was detected only in trace amounts, and thus sucrose was not addressed in the present study. PEs from Turkey and Uzbekistan evidenced almost negligible levels of sucrose.

**DPPH and ABTS radical scavenging activity**

The results of the radical scavenging activities are summarized in Fig. 4. Based on our results of DPPH and ABTS radical scavenging activities, the PEs from Turkey exhibited relatively high DPPH and ABTS radical scavenging activities. PEs obtained from Spain, Taiwan, and Iran evidenced relatively low levels of DPPH and ABTS radical scavenging activity. The DPPH radical scavenging activities were detected as follows: Turkey > Uzbekistan ≥ Spain ≥ Iran-B ≥ Iran-A ≥ Taiwan. ABTS radical scavenging activities were detected in the following order: Turkey ≥ Uzbekistan ≥ Spain ≥ Iran-B ≥ Iran-A ≥ Taiwan.
The phenolic content is correlated with reduced cardio- and cerebrovascular diseases and cancer mortality (28). The beneficial effects of polyphenol compounds may be attributable to free radical scavenging. Pomegranate juice has recently become more popular, because it has been loosely identified to exhibit important biological properties (29). Thus, the antioxidant and antitumoral activity of pomegranate bark tannins (punicacortein) (30) and the phenolic compounds, especially tea flavonoids, are powerful antioxidants using an in vitro oxidation model for heart disease. J Agric Food Chem 43: 2800-2802.

Pomegranate has favorable antioxidant capacity and is an effective scavenger of several reactive oxygen species, primarily as the result of its high levels of flavonoids and other polyphenolic compounds (32). The results of our study also demonstrated that selected cultivars evidenced high total polyphenol levels.

In this study, the PEs from Turkey also evidenced high levels of radical scavenging activities and of TPC and TAC. We evaluated a commercial pomegranate extract because of increasing interest in pomegranate among the general Korean population. PE from Turkey evidenced high levels of TPC and TAC, and also exhibited relatively profound radical scavenging activities.

**REFERENCES**


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