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Research Article

Chemical Compositions and Antioxidant Activities of Thirty-Seven Typical Pomegranates Grown in China

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The chemical compositions of juice and peel from 37 cultivars of pomegranates grown in China were identified using UPLC-Q TOF MS. The total phenolic contents and antioxidant activities of pomegranate juice and peel samples were also determined to make comparisons of their potential nutraceutical values. A total of 20 and 25 compounds were tentatively identified from the juice and peel samples. For the antioxidant activities of juice samples, No. 32 from Italy showed greatest abilities in scavenging DPPH and ABTS free radicals, and a registered novel cultivar No. 24 showed greatest value in scavenging oxygen radical. For the peel samples, No. 8 from Anhui Province of China showed generally greater values in all the three assays. All these results showed that different cultivars of pomegranate have different amounts of bioactive components and antioxidant activities, and development about the byproduct of these pomegranates after industrial processing should be focused in the future.

1. Introduction

Pomegranate (*Punica granatum* L.) has a long cultivation history and has been widely consumed and welcomed as fresh fruit or good source of juicing fruit globally for thousands of years. The edible part of the pomegranate (50%) consists of 40% arils and 10% seeds, and the edible portion contains various bioactive chemical components, including phenolic acids, tannins, flavanols, and anthocyanins [1–3]. It is widely accepted that the beneficial health effects of pomegranate are helpful in preventing several diseases, including cardiovascular disease, diabetes, prostate cancer, and other chronic diseases [1, 3–5]. *In vitro* studies also proved that pomegranate and its products contain various polyphenols, anthocyanins, and other bioactive compounds, thus effectively contributed to their antioxidant, antimicrobials, and other bioactivities [6–8]. Due to the bioactivities of pomegranate, global demand of pomegranate and pomegranate products increased for years [9], and different cultivars of pomegranate have significant different chemical compositions and bioactive effects [6, 10, 11], thus selecting appropriate cultivars of pomegranate for certain producing regions is important and necessary.

Although no official reported data from Food and Agriculture Organization of the United Nations (FAO), the global market size of pomegranate will increase more than

25% in the next five years in the prediction of economic report. Major growing regions including the origin grown regions of pomegranate around Mediterranean (Middle East, North Africa, and South Europe) and other main cultivate and export counties like India, the United States, some South America counties, and China all have their own major cultivars [6]. As one of the largest pomegranate-producing and pomegranate-consuming countries, China has not only the biggest pomegranate consumption marker but also the leading imported value of pomegranate at over 850 million US dollar in 2018. For the cultivation of pomegranate, China has more than 200 of cultivars that cultivated mainly in its central, northwest, and southwest provinces [8, 11, 12]. China has the planting area of pomegranate for more than 120,000 hectares, with a production exceeding 1.0 million tons in 2020. In the whole world, pomegranate producers include Iran, India, Tunisia, and the United States. Iran and India have the greatest productions at 650,000 and 500,000 tons, respectively [13]. These different cultivars showed significant different chemical compositions and bioactivities, like a commercial cultivar of pomegranate. Qingpi mainly from Shandong Province of China showed the greatest bioactivities and levels of bioactive components [8, 11]. But these differences might be due to their different genotypes or cultivars, different growing conditions (climate, cultivating way, fertilizing way, water, pesticides, soil, etc.), and/or storage approaches. For example, the annual average temperatures range from 12.2 to 15.8°C among Henan, Anhui, Shaanxi, and Shandong provinces, while the annual average rainfalls range from 560 to 900 mm in these regions, which could result completely different biosynthesis of bioactive components in the final products of pomegranates [14, 15]. Study with controlled variables is needed to better compare the chemical and biological differences of major pomegranate cultivars in China.

Beside the edible arils, pomegranate also has other functional parts like peel, endocarp, and seed. The peel of pomegranate was recognized as agri-byproduct in the juice, jam, or wine industry in the past. However, increasing studies demonstrated that pomegranate peel contained considerable bioactive components, even greater than that in pomegranate arils or juice [16–19]. So the comparison of chemical compositions in peel of different pomegranate cultivars might be meaningful in investigating appropriate cultivars for pomegranate-processing industry.

Present study targeted major growing cultivars of pomegranates in China, harvested them in the same growing condition in 2021 fall. The chemical profiles and compositions of anthocyanins and other phenols in the juice and peel of selected cultivars of pomegranates were evaluated with UPLC-Q TOF MS. Total phenolic contents, antioxidant activities, and other bioassays were also conducted to determine the overall bioactivities of these cultivars of pomegranate. These studies are aimed at clarifying the chemical compositions of major bioactive components and overall nutraceutical effects of all the 37 pomegranate samples, thus selecting appropriate cultivars of pomegranate grown in China that are most suitable for fresh consumption, juice and/or wine industry, or ornamental purpose.

2. Materials and Methods

2.1. Samples and Chemicals. A total of 111 pomegranate samples (37 cultivars, 3 pomegranates per each cultivar) were gifted from Zhengzhou Fruit Research Institute, Chinese Academy of Agricultural Sciences (Table S1). Among all these 37 cultivars, 22 are local cultivars that have been cultivated in different locations of China for a long time (Nos. 1-22), including 8 cultivars from Anhui Province (Nos. 1-8), 1 cultivar from Fujian Province (No. 9), 8 cultivars from Shandong Province (Nos. 10-17), 1 cultivar from Shaanxi Province (No. 18), 1 cultivar from Sichuan Province (No. 19), 2 cultivars from Yunnan Province (Nos. 20-21), and 1 old cultivar without certain cultivate location (No. 22). Cultivars Nos. 23-26 are recently breed novel cultivars and have been registered in China. Cultivars Nos. 27-33 are imported cultivars, including 1 from the United States (No. 27), 1 from Tunisia (No. 28), 1 from Turkmenistan (No. 29), 1 from Israel (No. 30), and 3 cultivars from Italy (Nos. 31-33). The remaining 4 cultivars are developing cultivars that are still being breed and have not registered yet (No. 34-37). The seeds of all of these 37 cultivars were collected from their major cultivation areas and unified cultivating and harvesting the experimental fields of Henan Academy of Agricultural Sciences under similar growing and harvesting conditions.

Gallic acid, 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), and fluorescein (FL) were purchased from Sigma-Aldrich (St. Louis, MO). The Folin-Ciocalteu reagent was obtained from Macklin reagent (Shanghai, China). Acetonitrile, methanol, isopropanol, and formic acid are all in LC-MS grade and obtained from Merck (Darmstadt, Germany). Water was purified with Millipore-Q 10 system and used for all experiments. All the other chemicals were of analytical grade, purchased from Sigma-Aldrich (St. Louis, MO), and used without further purification in this study.

2.2. Sample Preparation. Pomegranate fruits were washed with ultrapure water and dried at ambient temperature. The edible portion was manually separated from the pomegranate fruit, and pomegranate juice was prepared with a manual squeezer and used directly in the antioxidant assays and chemical composition analyses after appropriate dilution. The fresh out layer peel of pomegranate was ground with a home used blender (Baijie, Zhejiang Province, China), and then, 1 gram of peel sample was mixed with 10 mL of 50% acetone, vortexed 1 min, and sonicated 30 min to extract the phenolic compounds. The pomegranate peel extractions were used for antioxidant assays and chemical composition analyses. Each pomegranate sample was prepared in triplicate, and results of three pomegranates from one cultivar were used to represent the overall condition of certain cultivar of pomegranate.

2.3. Identification of Chemical Compositions in Pomegranate Juice and Peel Extract Samples. Major chemical components, especially anthocyanins and other phenols in juice and peel

extract from pomegranate samples, were identified using Waters UPLC-Xevo G2 Q TOF MS system (Milford, MA). Both juice and peel extract samples were hydrolyzed with 12 M HCl with the ratio 4 : 1 (sample/acid, *v*/*v*) at 55°C water bath for 2 hours. After cool down to ambient temperature, acetone from peel extract was removed by nitrogen gas, and the water layer was extracted by 4 mL of ether/ethyl acetate (1 : 1, *v*/*v*) 3 times. Three factions of extractions were combined, and organic solvent was evaporated with nitrogen gas again. The residue was dissolved with LC-MS grade methanol, filtered with a 0.22 μm GHP syringe filter (Waters, Milford, MA), and injected into the LC-MS system. A Waters Acquity UPLC BEH C_{18} column (150 mm × 2.1 mm i.d., 1.7 μm) was utilized in the separation with the oven temperature at 40°C. The flow rate was 0.4 mL/min, with the injection volume 2 μL for each sample. The mobile phase A was 0.1% formic acid in ultrapure water and B was 0.1% formic acid in acetonitrile. The gradient elution was used, with the initial rate of 10% B; 6.00 min, 22% B; 6.10 min, 100% B; 8.00 min, 100% B; 8.10 min, 10% B; and 10.00 min, 10% B. Both ESI positive and negative modes were tested, and the positive mode was proved with better resolution in the following conditions: collision voltage 35 V, source temperature 120°C, desolvation temperature 450°C, cone gas flow rate 150 L/h, and desolvation flow rate 800 L/h.

Chromatograms and MS spectra were acquired by MassLynx 4.1 (Waters, Milford, MA, USA). Identification of chemical compounds in pomegranate was based on the accurate molecular weight and mass fragment information obtained from MS1 and MS2 data, theoretical and experimental isotopic patterns, cleavage law of compounds, and retention time, as well as the compounds and fragment information reported in previous literatures. Online databases, including SciFinder and PubChem, were consulted for structure identifications.

2.4. Total Phenolic Content and Antioxidant Assays

2.4.1. Total Phenolic Content (TPC). The total phenolic contents of both pomegranate juice and peel extract were determined based on our previous used lab protocol [20]. One aliquot of 50 μL of appropriately diluted phenolic compound extracted from the edible portion or peel of pomegranate was mixed with 250 μL of the Folin-Ciocalteu reagent, and then, 3 mL of ultrapure water and 750 μL of saturated sodium carbonate solution were consequently added to initiate the reaction. The whole reaction was kept in darkness at ambient temperature for 2 hours, and the total phenolic contents were measured with a Tecan M1000 pro spectrophotometer (Tecan Trading AG, Switzerland) at wavelength 765 nm, then compared with gallic acid as standard, and calculated as gallic acid equivalent per mL of pomegranate juice or per gram of fresh pomegranate peel.

A total of three different antioxidant assays were processed to evaluate the overall conditions of antioxidant activities of pomegranate juice and peel extracts, including relative $DPPH^{\bullet}$ scavenging capacity (RDSC), $ABTS^{\bullet+}$ scavenging capacity (ABTS), and oxygen radical absorbance capacity (ORAC) assays. All the three assays were processed based on our previous published manuscript with a standardized lab protocol [20, 21].

2.4.2. Relative $DPPH^{\bullet}$ Scavenging Capacity (RDSC). The RDSC assay was used to determine the effects of pomegranate samples in competitively inhibit DPPH free radical. One aliquot of pomegranate juice or peel extract was mixed with the same volume of 0.2 mM DPPH fresh solution. The kinetics of mixture was observed with plate reader in 1.5 hours after being mixed, with the wavelength at 515 nm in every minute. Trolox was selected as the standard, and the RDSC values of pomegranate samples were showed as Trolox equivalent per mL of pomegranate juice or per gram of fresh pomegranate peel.

2.4.3. $ABTS^{\bullet+}$ Scavenging Capacity (ABTS). The ABTS assay was conducted based on a laboratory protocol. Briefly, 2 mL of $ABTS^{\bullet+}$ working solution was added to the test tubes. Then, 160 μL of either solvent, standard solution (5 to 300 μmol/L Trolox), or sample was added and vortexed for 30 s. After another 60 s reaction, the absorbance at 734 nm was read by spectrophotometer. The results were expressed as μmol Trolox equivalent per mL of pomegranate juice or per gram of fresh pomegranate peel (μmol TE/g).

2.4.4. Oxygen Radical Absorbance Capacity (ORAC). One aliquot of 30 μL of sample was mixed with 225 μL of freshly prepared 81.63 nM fluorescein (FL) in a 96-well plate and preheated at 37°C for 20 minutes before the initial of reaction. Then, 25 μL of fresh prepared 0.36 mM AAPH working solution was added into each plate, and the fluorescence intensities at λ_{ex} of 485 nm and λ_{em} of 535 nm were measured every minute for 2 h at 37°C. Results were reported as milligrams of Trolox equivalent per mL of pomegranate juice or per gram of fresh pomegranate peel.

2.5. Statistical Analysis. The mean value for triplicate tests of each pomegranate was calculated, and data of three pomegranate samples from same cultivar were reported as the mean ± standard deviation (SD) for certain cultivar of pomegranate. One-way analysis of variation (ANOVA) and Tukey's post hoc test were employed to identify differences in means. The *t* test was used to identify different values on both the TPC and antioxidant activity assays. The Pearson correlation was used to identify the correlations. Statistics were analyzed using SPSS for Windows (version release 10.0.5, SPSS, Inc., Chicago, IL).

3. Results and Discussion

The chemical profiles and bioactivities play important roles in the overall qualities of pomegranate cultivars. As the major byproduct in pomegranate-related food industry, the bioactive components and total bioactivities in the peel of pomegranates might also be an important fact in determining the whole processing value of certain cultivar of pomegranate. In addition, relationship about the antioxidant activities between pomegranate juice and their corresponding peel, no matter positive or negative correlation, could increase the understandings about these major cultivars

Table 1: Identification of chemical components from pomegranate juice (QC sample).

No.	RT (min)	Exptl. mass (m/z)	Calc. mass (m/z)	Difference (ppm)	Adducts	Chemical formula	Tentative identification	Fragments
1	2.29	331.0659	331.0665	-1.8	M-H	$C_{13}H_{16}O_{10}$	Monogalloyl hexoside	125, 169, 271
2	2.55	399.1505	399.1503	0.5	M-H	$C_{15}H_{28}O_{12}$	(2-Hydroxy-propyl) sucrose	353
3	2.60	649.0684	649.0677	1.1	M-H	$C_{27}H_{22}O_{19}$	Galloyl-HHDP-glucoside (lagerstannin C)	301
4	2.74	627.1569	627.1561	1.3	M-H	$C_{27}H_{32}O_{17}$	Albizinin	
5	2.82	541.0251	541.0254	-0.6	M-2H	$C_{48}H_{28}O_{30}$	Punicalagin	275, 301, 532, 601, 781
6	2.91	783.0695	783.0681	1.8	M-H	$C_{34}H_{24}O_{22}$	Bis-HHDP-hexoside (pedunculagin I)	229, 275, 391, 483, 632
7	2.93	329.0879	329.0873	1.8	M-H	$C_{14}H_{18}O_9$	1-O-Vanilloyl-beta-D-glucose	167
8	2.94	341.0874	341.0873	0.3	M-H	$C_{15}H_{18}O_9$	1-Caffeoyl-beta-D-glucose	161,179
9	2.96	541.0251	541.0254	-0.6	M-2H	$C_{48}H_{28}O_{30}$	Punicalagin isomers	275, 301, 532, 601, 781
10	3.00	449.1060	449.1084	-5.3	M+	$C_{21}H_{21}O_{11}$	Cyanidin 3-O-beta-D-glucoside	287
11	3.10	525.1613	525.1608	1.1	M-H	$C_{24}H_{30}O_{13}$	Mudanpioside E	363
12	3.13	525.1613	525.1608	1.1	M-H	$C_{24}H_{30}O_{13}$	Mudanpioside E isomers	363
13	3.17	785.0853	785.0837	2.0	M-H	$C_{34}H_{26}O_{22}$	Digalloyl-HHDP-gluc (pedunculagin II)	301, 483, 633, 765
14	3.27	463.0522	463.0513	1.9	M-H	$C_{20}H_{16}O_{13}$	Ellagic acid hexoside	301
15	3.30	355.1043	355.1029	3.9	M-H	$C_{16}H_{20}O_9$	1-O-Feruloyl-beta-D-glucose	135, 175, 193, 217, 236
16	3.36	305.0673	305.0661	3.9	M-H	$C_{15}H_{14}O_7$	Gallocatechin	
17	3.48	507.1486	507.1503	-3.4	M-H	$C_{24}H_{28}O_{12}$	Syringetin hexoside	295, 312, 315, 327, 343, 345, 441, 471
18	3.53	415.1619	415.1604	3.6	M-H	$C_{19}H_{28}O_{10}$	Benzyl alcohol beta-D-rutinoside	
19	3.58	475.1803	475.1816	-2.9	M-H	$C_{21}H_{32}O_{12}$	Kanokoside A	429
20	3.94	425.0523	425.0509	3.3	M-H	$C_{21}H_{14}O_{10}$	Ellagic derivative	301

grown in China. In the present study, major chemical components in both peel and juice of pomegranate were identified. The total phenolic contents, as well as the antioxidant activities of all the 37 cultivars of pomegranates, were also clarified to compare their potential developing values.

3.1. Identification of Chemical Components from Juice and Peel of Pomegranate. The UPLC-Q TOF MS negative and positive chromograms of a typical pomegranate juice sample are shown in Figure S1A. Based on their accurate mass weights and major fragment information, a total of 20 major chemical components were tentatively identified (Table 1). Peak No. 6, with a retention time of 2.91 min, was selected as a representative to illustrate the compound identification progress (Figure 1). In the negative ion mode, the experimental measured molecular weight of Peak 6 is 783.0764 Da, leading to the calculation of the chemical formula as $C_{34}H_{24}O_{22}$. The major fragment ions in MS2 analysis, including molecular fragments at 632, 541, 483, 391, and 275 Da, were identified as the de-monosaccharide, de-disaccharide, and dehydrated reduction of the certain compound. Considering previous publications, Peak 6 was tentatively identified as pedunculagin I. By using a similar strategy, the chemical formulas of all the major peaks have been determined, and 20 of them have been identified. Among the identified compounds are saponins of different phenols (Peaks 7, 8), anthocyanins (Peak 10), flavonoids (Peak 16), and some characteristic ellagitannin compounds in pomegranate (Peaks 5, 6, 13, etc.). These findings are consistent with previous results, indicating that components such as cyanidin 3-O-glucoside (Peak 10) and pedunculagin I and II (Peaks 6, 13) are representative compounds in pomegranate juice.

The UPLC-Q TOF MS negative and positive chromograms of a typical pomegranate peel sample are shown in Figure S1B. Using a similar identification strategy as that used for identifying the chemical profiles in pomegranate juice, 25 compounds were tentatively identified from the pomegranate peel samples (Table 2). Among all the identified compounds, some major compounds are similar to those found in the juice samples. However, the typical anthocyanin cyanidin 3-O-glucoside is not detected in the pomegranate peel samples. This is consistent with previous findings that certain anthocyanins are mainly present in the aril/juice of pomegranate rather than in the peel section. Additionally, there are also some components identified from the peel that are not detectable in the juice sample, such as certain organic acids (Peaks 2, 17) and pentacyclic triterpenoids (Peaks 23-25). The presence of

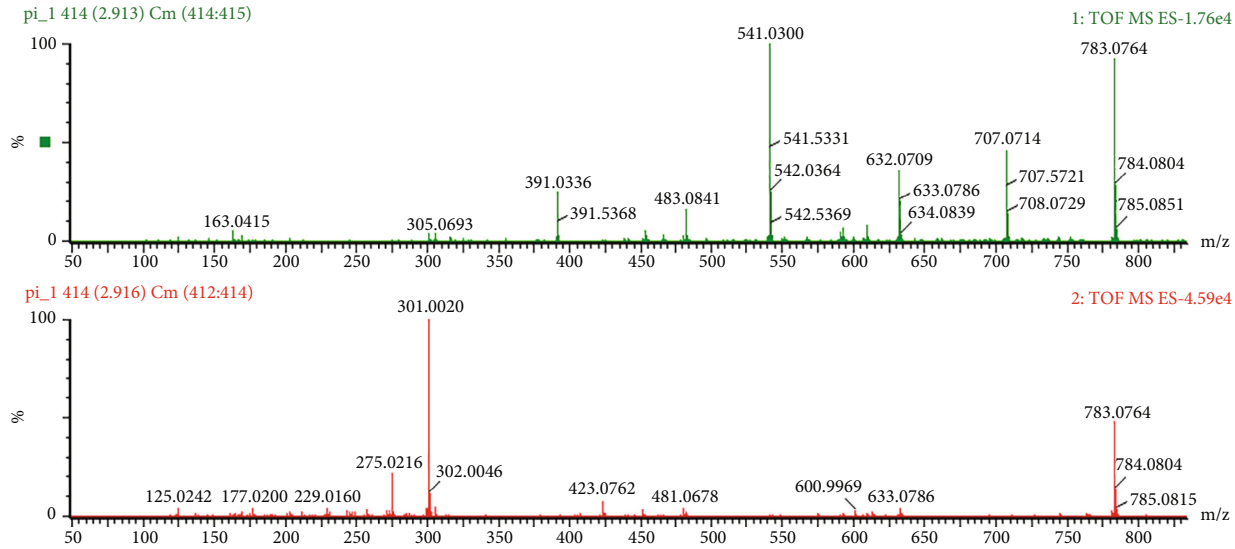

Figure 1: Respective spectrum of pedunculagin I in UPLC-Q TOF MS negative ion mode: MS1 (upper); MS 2 (lower).

these phenols may contribute to the potential bioactivities of both the aril/juice and peel of pomegranate. Determining the total phenolic compounds and antioxidants can effectively enhance our understanding of different cultivars of pomegranate.

3.2. Total Phenolic Contents (TPC). The total phenolic contents are widely used to show the overall concentrations of phenolic compounds in food samples. In this study, the TPC values of pomegranate juice and peel samples are shown in Figure 2. As shown in Figure 2, the TPC values of pomegranate juice samples range from 0.57 to 2.16 mg gallic acid equivalent (GAE) per milliliter. The significantly greatest TPC in this study is pomegranate No. 32, which is an imported cultivar from Italy, followed by No. 11 from Shandong Province (1.76 mg GAE/mL), No. 35 developing cultivar (1.58 mg GAE/mL), and No. 23 novel cultivar (1.56 mg GAE/mL). Beside the two lowest TPC values from No. 17 and No. 6, all the other pomegranate juice samples have the TPC values over 0.7 mg GAE/mL, and the average TPC value of all the samples is 1.08 mg GAE/mL. In this group of pomegranate samples that were grown and harvested in the same condition, Anhui Province and Shandong Province from China are the two major sources, and each province provided 8 cultivars (Nos. 1-8 from Anhui, No. 10s-17 from Shandong Province). While comparing TPC values of samples from these two provinces, the average TPC values are 0.96 and 1.12 mg GAE/mL for Anhui and Shandong, and no statistical difference was observed. On the other hand, the second greatest and the lowest TPC values are from Shandong Province, which showed greater derivation between samples in Shandong than that in Anhui Province. These TPC results of pomegranate juice samples showed similar trends compared with previous studies about pomegranate juice. In 2017, Conidi et al. reported the TPC value of raw pomegranate juice at 2.636 mg GAE/mL, and the filtered pomegranate juice has the TPC at 2.457 mg GAE/mL after nanofiltration [22], which were in the same level as the present TPC values. In 2011, Mena et al. reported the TPC values of industrial use of pomegranate cultivars grown in Spain, with most the TPC values from about 1.5 to 2.5 mg GAE/mL of pomegranate juice samples except one series of experimental cultivars with greater TPC values initially labeled with letter "W" [10]. Besides, there are also some studies reporting the TPC values in GAE per gram pomegranate juice or gram pomegranate aril. For example, Yan et al. indicated that the TPC values of 6 cultivars of pomegranate grown in China range from 0.39 to 1.39 mg GAE/gram of juice [11], considering the possible density of pomegranate juice; these results are quite similar as the present TPC results. In a study about the antioxidant activities and TPC values of 62 fruits, Fu et al. reported that the TPC value was 1.469 mg GAE/gram of pomegranate arils [23]. Present results about TPC values of pomegranate juice samples showed that the total phenolic contents from different cultivars of pomegranates are quite different. Relatively great standard derivation numbers showed in the error bars indicated that individual pomegranate fruits might also have distinct TPC values, even in the same cultivar. Such phenomenon could also be observed in the following results, which might be attributed to the regular individual difference, or resulted from the abnormal heavy rain during the whole summer of 2021 in Henan Province (where all these pomegranate samples were grown).

The TPC values of pomegranate peel samples showed that the overall phenolic contents were greater in the peel of pomegranate than that in the juice, which could be agreed by previous review articles [1, 6]. The TPC values of pomegranate peels range from 2.20 to 10.84 mg GAE/g, with the greatest value from Anhui Province (No. 8) and the lowest one was a novel cultivar (No. 24), and the average TPC value of pomegranate peel samples is 5.27 mg GAE/g. Previous studies also investigated the TPC values of pomegranate peel samples, but most of them were reported in dry weight of peel, and the TPC values were about less than one hundred to two hundred milligram GAE/g dry peel [6, 11]. Considering that the moisture content of pomegranate was reported

Table 2: Identification of chemical components from pomegranate peel (QC sample).

No.	RT (min)	Exptl. mass (m/z)	Calc. mass (m/z)	Difference (ppm)	Adducts	Chemical formula	Tentative identification	Fragments
1	0.76	481.0635	481.0618	3.5	M-H	$C_{20}H_{18}O_{14}$	HHDP hexoside	301,302
2	1.16	191.0192	191.0192	0.0	M-H	$C_6H_8O_7$	Citric acid	111
3	1.40	481.0635	481.0618	3.5	M-H	$C_{20}H_{18}O_{15}$	HHDP hexoside isomers	301,302
4	2.28	331.0683	331.0665	5.4	M-H	$C_{13}H_{16}O_{10}$	1-O-Galloyl-beta-D-glucose	169
5	2.61	609.1261	609.1244	2.8	M-H	$C_{30}H_{26}O_{14}$	Gallocatechin-epigallocatechin	
6	2.75	305.0666	305.0661	1.6	M-H	$C_{15}H_{14}O_7$	Epigallocatechin	
7	2.82	541.0251	541.0254	-0.6	M-2H	$C_{48}H_{28}O_{30}$	Punicalagin	275, 301, 532, 601, 781
8	2.94	541.0251	541.0254	-0.6	M-2H	$C_{48}H_{28}O_{30}$	Punicalagin isomers	275, 301, 532, 601, 781
9	2.99	449.1060	449.1084	-5.3	M+	$C_{21}H_{21}O_{11}$	Cyanidin 3-O-beta-D-glucoside	287
10	3.06	799.0631			M-H		Ellagic acid derivative	301, 479, 781
11	3.15	289.2713	289.0712	0.3	M-H	$C_{15}H_{14}O_6$	Catetein	179,205,245
12	3.19	633.0729	633.0728	0.2	M-H	$C_{27}H_{22}O_{18}$	Corilagin	301, 463
13	3.28	463.0522	463.0513	1.9	M-H	$C_{20}H_{16}O_{13}$	Ellagic acid hexoside	301
14	3.37	951.0758	951.0740	1.9	M-H	$C_{41}H_{28}O_{27}$	Galloyl-HHDP-DHHDP-hex (granatin B)	301, 445, 613, 933
15	3.54	433.0406	433.0407	-0.2	M-H	$C_{19}H_{14}O_{12}$	Ellagic acid pentoside	301
16	3.56	447.0583	447.0564	4.3	M-H	$C_{20}H_{16}O_{12}$	Ellagic acid deoxyhexoside	301
17	3.69	300.9993	300.9984	3.0	M-H	$C_{14}H_6O_8$	Ellagic acid	185, 229, 257, 283
18	3.74	595.1638	595.1663	-4.3	M+	$C_{27}H_{31}O_{15}$	Cyanidin-3-O-rutinoside	287,449
19	3.85	447.0933	447.0927	1.3	M-H	$C_{21}H_{20}O_{11}$	Kaempferol 3-O-glucoside	227,255,284
20	4.02	447.0933	447.0927	1.3	M-H	$C_{21}H_{20}O_{11}$	Quercetin hexoside	151, 179, 301
21	4.05	435.1287	435.1291	-0.9	M-H	$C_{21}H_{24}O_{10}$	Phlorizin	273
22	4.25	435.1287	435.1291	-0.9	M-H	$C_{21}H_{24}O_{10}$	Phlorizin isomers	167, 273
23	8.84	487.3441	487.3423	3.7	M-H	$C_{30}H_{48}O_5$	Asiatic acid	
24	9.35	485.3272	485.3267	1.0	M-H	$C_{30}H_{46}O_5$	Actinidic acid	
25	9.49	487.3441	487.3423	3.7	M-H	$C_{30}H_{48}O_6$	Asiatic acid isomers	

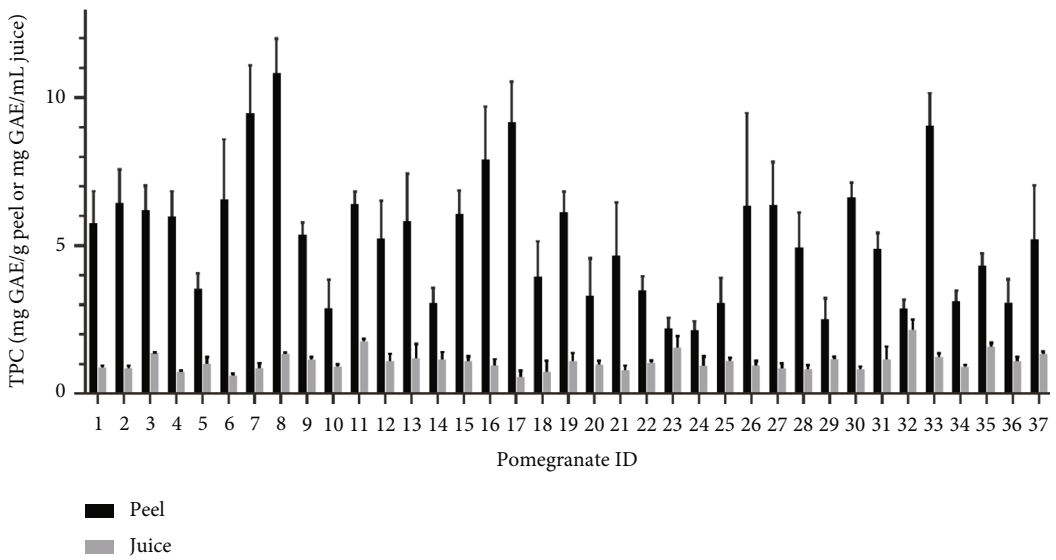

Figure 2: TPC of the pomegranate peel and juice samples. GAE stands for gallic acid equivalent. The vertical bars represent the standard deviation ($n = 3$) of each cultivar of pomegranate.

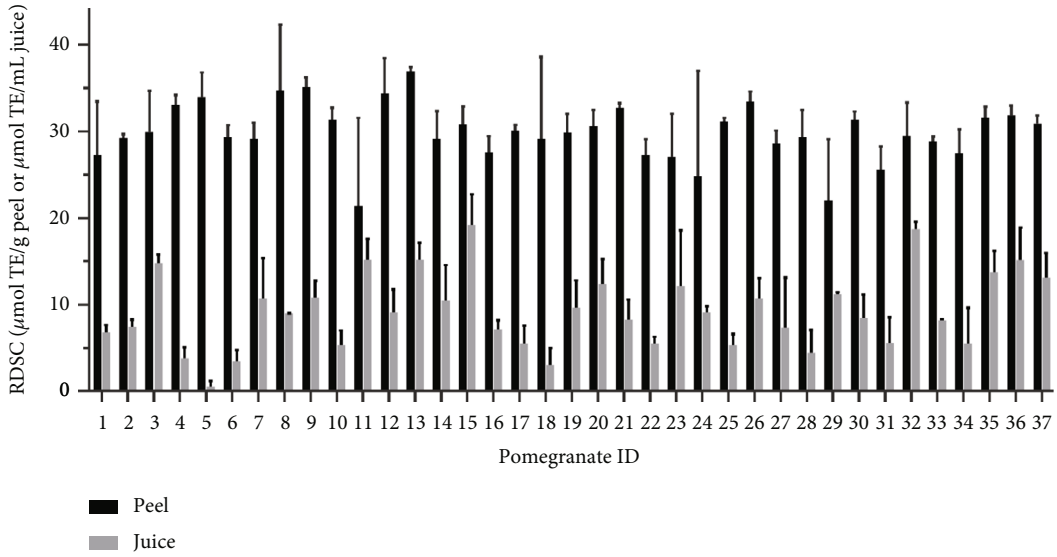

Figure 3: Relative DPPH scavenging capacity (RDSC) of the pomegranate peel and juice samples. TE stands for Trolox equivalent. The vertical bars represent the standard deviation ($n = 3$) of each cultivar of pomegranate.

at 73.6% [24], previous results about TPC values of pomegranate peel were still about three- to fourfold greater than the present results. This might due to different water contents in different samples, or the release of more phenolic compounds during drying progress.

Interestingly, average TPC value of peels from Anhui Province (6.85 mg GAE/g) is nonsignificantly greater than that from Shandong Province (5.82 mg GAE/g), which shows just the opposite trend compared with the TPC values of pomegranate juice samples from these two provinces. While enlarging the scale to all the TPC values between pomegranate juice and peel samples, this opposite trend could also be found in some of the sample. For example, No. 17 has the lowest juice TPC but the third greatest peel TPC, and No. 32 has the greatest juice TPC but the third lowest peel TPC. The correlation efficiency between the overall TPC values of juice and peel is -0.1886, which showed slightly negative relation between the TPC of juice and peel. Similar trends could also be observed in some antioxidant results, with specific discussion in the antioxidant section.

3.3. Antioxidant Activities. Antioxidant activity assays are the most widely used tests to evaluate the bioactivity of food or botanical samples. Different assays might target the scavenging capacities of different free radicals; thus, only one antioxidant assay is insufficient to determine the overall antioxidant ability of sample. In this study, three different antioxidant assays, including relative DPPH radical scavenging capacity (RDSC), ABTS radical cation scavenging activity, and oxygen radical absorbance capability (ORAC), have been conducted to verify the antioxidant abilities of both pomegranate juice and peel samples.

3.3.1. Relative DPPH Radical Scavenging Capacity (RDSC). The RDSC values of pomegranate juice are shown in Figure 3. Among all the samples, No. 15 from Shandong Province, No. 32 imported from Italy, and No. 13 from Shandong Province are the top three juice samples that have greatest RDSC values at 19.19, 18.69, and 15.18 μmol Trolox equivalent (TE) per milliliter juice, respectively, but no statistical difference could be observed until the 9th greatest RDSC value. Juice from pomegranate No. 5 originally from Anhui Province showed the lowest RDSC value at 0.58 μmol TE/mL. As two provinces that contributed most to this group of pomegranate samples, samples from Anhui and Shandong provinces have distinct average RDSC values at 7.07 and 10.90 μmol TE/mL, which again confirmed that the average DPPH radical scavenging capacity of this group of pomegranates from Shandong Province might be greater than that from Anhui, similar as the previous TPC results. Imported pomegranate samples also showed different RDSC values, and No. 32 labeled "wonderful" from Italy contained great and stable DPPH radical scavenging capacity, similarly like the trends of imported samples in TPC. In all the four developing cultivars, the last three of them showed remarkable RDSC values except No. 34, indicating potential developing possibilities of these cultivars. Compared with previously published data about DPPH scavenging data reported by Yan et al. that pomegranate juice samples from China range from 1.4 to 8.0 mg/g juice [11], the average RDSC value of domestic samples in the present study is 8.79 μmol TE/mL, which is about 2.2 mg/mL juice and in the same level as the previous results. Mena et al. reported the DPPH scavenging capacity of Spanish pomegranate juice samples at 7.01-15.30 μmol TE/mL [10], which was almost the similar level as the present results. Another study by Li et al. reported the RDSC values of pomegranate juice samples from China at about 0.1-0.2 mg gallic acid equivalent or 0.1-0.25 mg vitamin C (ascorbic acid) equivalent [8]. Although there is no direct converted equation between Trolox and gallic acid/vitamin C, previous study demonstrated that the overall order of antioxidant abilities was gallic acid > vitamin C > Trolox [25], and present RDSC results about pomegranate juice are reasonable.

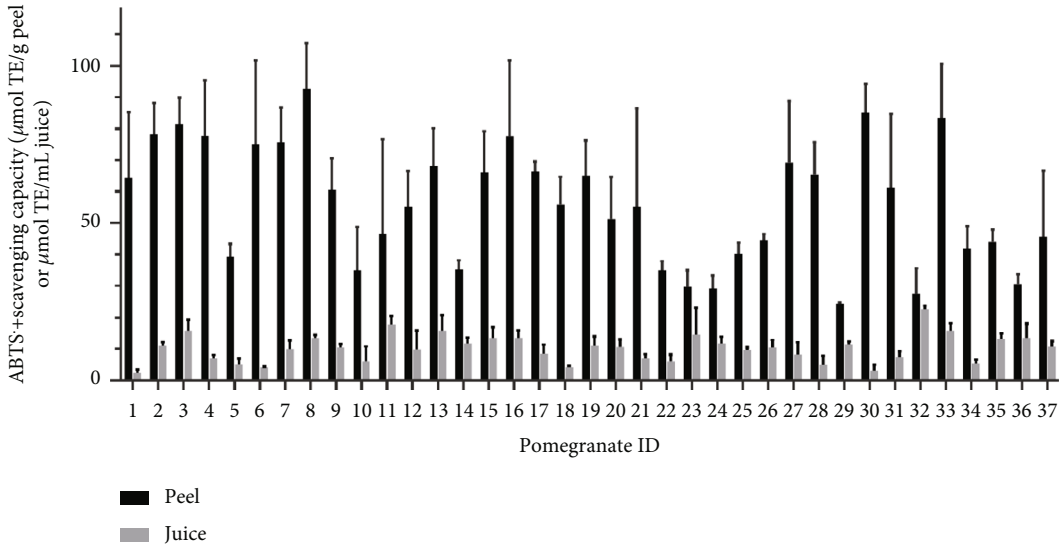

Figure 4: ABTS cation radical scavenging activity of the pomegranate peel and juice samples. TE stands for Trolox equivalent. The vertical bars represent the standard deviation ($n = 3$) of each cultivar of pomegranate.

The RDSC values of pomegranate peel samples were visually unified (Figure 3). The mean RDSC value of all the samples is 29.90 μmol TE/g fresh peel, with the lowest and greatest values ranging from 21.41 to 36.96 μmol TE/g. And the RDSC results between juice and peel samples showed almost no correlation, which might be because of the similar RDSC values of pomegranate peel samples.

3.3.2. ABTS Radical Cation Scavenging Activity. This assay showed the capacities of pomegranate juice and peel samples in scavenging ABTS radical. As shown in Figure 4, these 37 pomegranate juice samples showed quite different activity in scavenging ABTS radical, ranging from 2.45 to 22.60 μmol TE/mL, which is about 10-fold variation. Again, the average ABTS values of samples from Anhui and Shandong are 8.60 and 12.08 μmol TE/mL, respectively, which showed similar trends as the TPC and DPPH results, but nonsignificant difference could be found between these two groups. The average ABTS value for all the juice samples is 10.22 μmol TE/mL, with the greatest five samples: No. 32 (22.60 μmol TE/mL, Italy), No. 11 (17.75 μmol TE/mL, Shandong), No. 13 (15.89 μmol TE/mL, Shandong), No. 3 (15.88 μmol TE/mL, Anhui), and No. 33 (15.77 μmol TE/mL, Italy). Previous results also reported the ABTS values of pomegranate juice samples in 1.3-5.2 mg TE/g juice [11], which could be calculated into 5.2-20.8 μmol TE/g, just in the same range as the present results. Another study reported the ABTS value of pomegranate aril extract at 40.61 μmol TE/g arils [23], which could also confirm the reliability of the present results.

The ABTS values of pomegranate peel samples are shown in Figure 4, which have similar trends as the TPC results between juice and peel results. Generally, the ABTS values of peel samples are 5-10-fold greater than the corresponding juice samples when ignoring the small differences from their different units. Just as the opposite result from juice results, the average ABTS values of Anhui pomegranate peel samples (73.12 μmol TE/g) are nonsignificantly greater than that of Shandong samples (56.32 μmol TE/g), which is also similar as the TPC results.

3.3.3. Oxygen Radical Absorbance Capability (ORAC)DIRE NA. ORAC could be utilized to evaluate the activity of samples in absorbing the oxygen radical ($O_2^{\bullet}$). The ORAC values of pomegranate juice samples are 3.63-15.88 μmol TE/mL (Figure 5), with the average value at 10.56 μmol TE/mL. The novel cultivar No. 24 and local cultivar No. 10 from Shandong are the two juice samples with greatest ORAC values, at 15.88 and 15.83 μmol TE/mL, respectively. But cultivars No. 10 and No. 23 have abnormal standard derivation, indicting great individual differences in these two cultivars. Similarly like the results in TPC and above two antioxidant assays, the average ORAC value of all the 8 Shandong juice samples (11.15 μmol TE/mL) is not only greater than its Anhui competitor (7.48 μmol TE/mL), but also greater than the overall average value (10.56 μmol TE/mL). And the novel cultivars, imported cultivars, and the developing cultivars showed better and unified ORAC activities compared with the domestic locally grown cultivars.

Compared with juice samples, pomegranate peel samples have greater ORAC values, which is similar like all the assays mentioned above (Figure 5). The three greatest ORAC values are all from Anhui samples, including No. 8 (53.83 μmol TE/g), No. 7 (38.94 μmol TE/g), and No. 6 (37.93 μmol TE/g). And the average ORAC value of Anhui pomegranate peel samples is 29.53 μmol TE/g, which is greater than either the average value of Shandong samples (21.31 μmol TE/g) or the average value of all the samples (22.00 μmol TE/g). Besides, the ORAC values between juice and correlated peel samples showed negative correlation, with the correlation efficiency at -0.34. Combined with similar trends that appeared in TPC values, it could be assumed that the phenolic compounds and antioxidant abilities might have a dynamic equilibrium that the greater

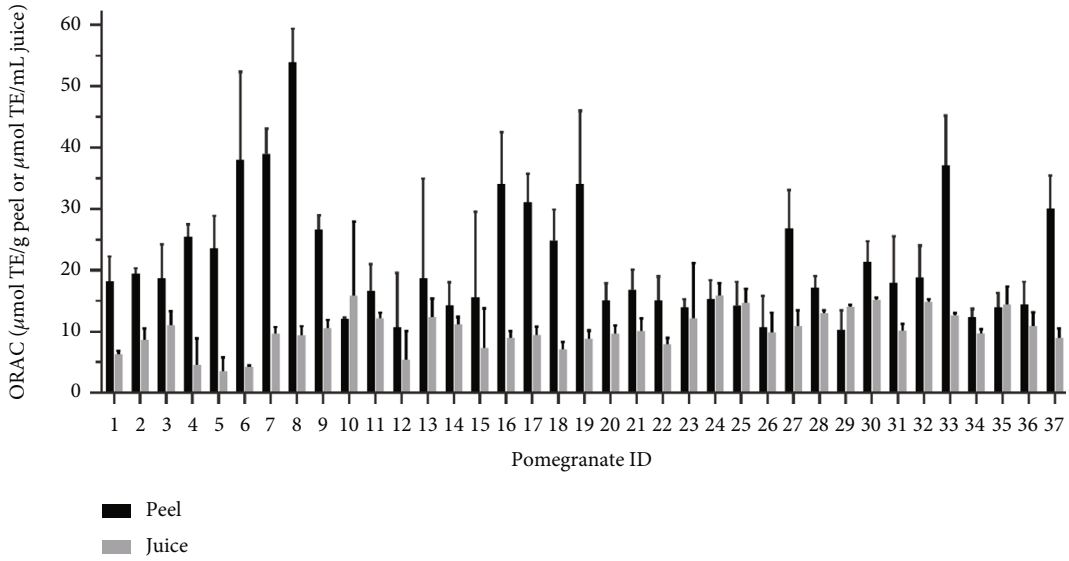

Figure 5: ORAC of the pomegranate peel and juice samples. TE stands for Trolox equivalent. The vertical bars represent the standard deviation ($n = 3$) of each cultivar of pomegranate.

in juice (arils), the less in peel and vice versa. But more data are needed to verify this phenomenon, and possible mechanisms are also under investigation.

In conclusion, the chemical profiles of juice and peel of 37 cultivars of pomegranate were determined. Peel samples generally contained more identifiable chemical components than the juice counterparts, and some specific compounds could only be detected in either juice (anthocyanins) or peel (pentacyclic triterpenoids) parts. For the total phenolic contents and antioxidant activities, different cultivars of pomegranate showed remarkable differences in both juice and peel parts. In order to discuss the possible trends of different cultivars, the average values of TPC and antioxidant activities will be used in the following discussion. For the juice samples, local cultivars originally grown in Shandong Province have nonsignificantly greater TPC value (1.09 mg GAE/mL juice) than that in Anhui Province (0.96 mg GAE/mL juice). But both values are lower than the average value of registered novel cultivars (1.14 mg GAE/mL juice), imported cultivars (1.18 mg GAE/mL juice), and unregistered novel cultivars (1.23 mg GAE/mL juice), which might indicate the necessity of promoting novel pomegranate cultivars. Antioxidant activities showed similar trends that local grown cultivars generally had lower bioactivities than the novel or imported cultivars. Results from ABTS assay might be the only exception that the average value from Shandong samples is the greatest (3.02 μmol TE/mL juice), followed by registered novel cultivars (2.91 μmol TE/mL juice), unregistered novel cultivars (2.69 μmol TE/mL juice), and imported cultivars (2.63 μmol TE/mL juice), and samples from Anhui Province still showed lowest activities (2.01 μmol TE/mL juice) in scavenging ABTS free radicals. For the peel samples, quite opposite trends could be observed compared with the juice results. Anhui samples showed nonsignificantly greatest average values in TPC, ABTS, and ORAC assays, while the registered or unregistered novel cultivars showed the lowest ones. Such interesting trends that pomegranate juice and peel might have trade-off total phenolic contents and antioxidant activities raise further research focus on the plant physiological properties of pomegranate. And present results about these major cultivars indicate the potential possibility of upgrading cultivars of pomegranates grown in China, thus enhancing the nutraceutical values of pomegranate aril and subsequent byproduct development of pomegranate peel in the future.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare no competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Authors' Contributions

Wenhao Zheng and Boyan Gao contributed equally to this work.

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Supplementary Materials

Table S1: sample information about thirty-seven pomegranate samples. Figure S1: representative UPLC-Q TOF MS chromatograms of pomegranate: (A) peel and (B) juice sample. *(Supplementary Materials)*

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