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RESEARCH ARTICLE

Comparison of Phenolic Compound Contents and Antioxidant Capacities of Loquat (*Eriobotrya japonica* Lindl.) Fruits

Hong-xia Xu, Xiao-ying Li, and Jun-wei Chen

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Abstract The characteristics, phenolic compound contants, and antioxidant capacities of 6 cultivars of loquat fruit grown in China were evaluated. HPLC was used to identify and quantify phenolics. Chlorogenic acid, neochlorogenic acid, 4-*O*-caffeoylquinic acid, protocatechuic acid, 4-hydroxybenzoic acid, caffeic acid, ferulic acid, ellagic acid, and *o*-coumaric acid were the main phenolic compounds of mature loquat fruits. Contents of chlorogenic acid, neochlorogenic acid, 4-hydroxybenzoic acid, protocatechuic acid, and *o*-coumaric acid were all significantly correlated with antioxidant capacities determined using DPPH, ABTS radical scavenging activity, and ferric reducing/antioxidant power assays. The 'Taxiahong' cultivar contained the highest amounts of total phenolics and flavonoids and the highest antioxidant capacity, while 'Taipingbai' showed the lowest. The high level of phenolic compounds and antioxidant capacities of some cultivars indicates that these cultivars can be sources of bioactive compounds that are relevant to human health.

Keywords: antioxidant, flavonoid, fruit, loquat, phenolic

Introduction

Loquat (*Eriobotrya japonica* Lindl.), a member of the Rosaceae family, is native to China and has been commercially cultivated in many subtropical countries. Loquat is consumed mainly as a fresh fruit that has a delicious taste and also contains nearly all of the essential nutrients, particularly minerals, carotenoids (vitamin A) (1), and polyphenols (2). Both loquat fruit and leaves are often included in Chinese herbal remedies for cough and asthma (3) as they contain many phenolics and some triterpenes that exhibit anticancer, anti-inflammation, and hypoglycemic effects (4-6).

Interest in dietary fruit phytochemicals has largely focused on phenolic compounds which, for their ubiquity and diversity, are considered the most important antioxidant components in fresh fruit. As a consequence, considerable efforts have been devoted to quantification of the phenolic contents of the most common species (7-9). However, information available about the phenolic content of loquat fruit is restricted to total phenolics, and only a few reports have characterized the complete phenolic pattern (5,10,11).

In several previous studies, correlation analyses were performed to aid understanding of the antioxidant role of different bioactive components. Hong *et al*. (12) indicated that the antioxidant capacity of loquat leaves showed high correlation coefficients with flavonoids and phenolics. Zhou *et al*. (13) obtained similar results from loquat flowers. Xu *et al*. (9) also found that there was a significant positive correlation between the antioxidant capacity and the total phenolic and total flavonoid contents of loquat fruit. However, there are no reports on the relationship between specific phenolic compounds and the antioxidant capacity of loquat fruits. Which phenolic components are the main contributor to the antioxidant capacities of loquat fruits is unknown.

Commercial acceptance of a genotype depends on fruit size, color, organic acids, sugars, flesh firmness, and contained antioxidant components. Therefore, loquat has been the subject of horticultural improvement regarding production capacity, the organoleptic characteristics of the fruit, and the vital nutrient content. In this study, the main organoleptic characteristics of 6 loquat cultivars were compared using different methods to evaluate the antioxidant capacity.

Hong-xia Xu, Xiao-ying Li, Jun-wei Chen (✉)
Institute of Horticulture, Zhejiang Academy of Agricultural Sciences, Hangzhou, Zhejiang 310021, China
Tel: +86-571-86417302; Fax: +86-571-86404018
E-mail: chenjunwei@mail.zaas.ac.cn

Since the polyphenolic composition of loquat fruits from China has not yet been investigated, the aim of the present work was to identity the relative abundance of polyphenolic compounds in loquat fruit cultivars using HPLC. Simultaneously, relationship between antioxidant capacity and the flavonoid and phenolic contents were determined to provide a scientific basis for research and exploitation of loquat fruit resources.

Materials and Methods

Materials and chemicals Fruit samples of the following 6 loquat (*E. japonica* Lindl.) cultivars were analyzed in this study: 'Ninghaibai', 'Taipingbai', 'Daguotaipingbai', 'Taxiabai', 'Taxiahuang', and 'Taxiahong'. The fruits were harvested from an orchard in Lishui, Zhejiang Province, China in May 2012 at commercial maturity. A total of 50 fruits of each cultivar without blemish or damage were used for analyses. DPPH, ABTS, TPTZ, Trolox, and rutin were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). HPLC-grade protocatechuic acid, neochlorogenic acid, 4-hydroxybenzoic acid, chlorogenic acid, 4-*O*-caffeoylquinic acid, caffeic acid, ferulic acid, ellagic acid, and *o*-coumaric acid were also purchased from Sigma-Aldrich. All other chemicals and organic solvents used in this study were of analytical grade.

Determination of color, total soluble solids, and acidity The skin color of 30 fruits for each cultivar was determined using a chromameter (ADCI-60-C; Chentaike, Beijing, China). Color readings were recorded 4 times from the equatorial region of each fruit and averaged to obtain a mean value for each fruit. Color measurements were recorded using the CIE L^*, a^*, b^* color space (14). Chroma $[C^*=(a^{*2+}b^{*2})^{1/2}]$ and hue angle $[H_{ab}=\tan^{1} (b^*/a^*)^{1}]$ values were calculated according to methods previously reported (15). Fifteen fruits of each cultivar were peeled and juiced respectively to measure the amount of total soluble solids (TSS) with a hand-held refractometer (Pocket PAL-1; Atago, Tokyo, Japan). The remained 15 fruits of each cultivar were peeled and cut into small pieces. A 5 g pulp sample was homogenized in 5 mL of distilled water using a mortar and pestle. The homogenate was filtered through a Whatman no. 4 filter, washed with distilled water and diluted to 25 mL. The titratable acidity (TA) of the filtrate was measured using titration with 0.01 M NaOH and phenolphthalein as an indicator. The remaining twenty fruits were peeled, frozen in liquid nitrogen, and stored at −80ºC until analysis.

Extraction and separation of phenolic compounds Phenolic compounds were extracted according to the procedure of Ding *et al*. (10) with some modification. A 5 g frozen pulp sample was homogenized in 20 mL of cold methanol (95%) using a Waring blender. Homogenates were kept at 4ºC for 12 h, then centrifuged at 12,000×*g* for 15 min. Supernatants were collected and the residue was subjected to extraction again twice using cold methanol (80%). All supernatants were combined and evaporated under a vacuum at 35ºC to remove methanol. Concentrates were then subjected to extraction 3 times using hexane to remove lipids, carotenoids, and chlorophyll. The aqueous phase was evaporated again to remove hexane. Finally, water was added to the extracts to constitute a total of 10 mL. Extract solutions were used for quantitative analysis of phenolic compounds and the antioxidant activity.

For HPLC analysis, a 2 mL extract aliquot was passed through a C^{18} Sep-Pak cartridge (Waters, Milford, MA, USA) to remove sugars. The absorbed fraction was eluted from the cartridge using methanol, and the first 1 mL of the eluate was collected and filtered through a 0.45-μm membrane filter before use. Polyphenols were separated using HPLC (1525; Waters). A photodiode-array UV-vis detector (2487; Waters) was used for characterization of each peak at 280 and 325 nm. A Waters Atlantis T_3 C^{18} (4.6×250 mm) column with an operating temperature at room temperature was used. The mobile phase was composed of (A) acetic acid/water (5:95, v/v), and (B) acetic acid/ acetonitrile/water (5:80:15, v/v). During analysis, the solvent gradient was programmed from 0 to 50% B in A for 50 min at a flow rate of 1.0 mL/min. UV absorbance (for phenolic acid, flavanone, and flavonol) was measured at 280 nm and, for flavone, at 325 nm.

Total phenolic content analysis The total phenolic content was measured using the Folin-Ciocalteu reagent assay with some modification (16). A volume of 0.1 mL of sample aliquot and 0.9 mL of water were pipetted into a test tube, then mixed with 5 mL of 0.2 M Folin-Ciocalteu reagent. The solution was allowed to stand at 25ºC for 5 min before addition of 4 mL of 15% Na_2CO_3. The mixture was kept for 90 min at room temperature at which time a plateau was reached. The absorbance was measured at 765 nm. Results were expressed as μg gallic acid equivalent (GAE)/g fresh weight (FW).

Total flavonoid content analysis The flavonoid content was measured following the method of Jia *et al*. (17). In brief, 2.0 mL of fruit extract, 3.0 mL of distilled water, and 0.3 mL of 5% $NaNO_2$ were mixed in a 10 mL volumetric flask. The mixture was kept for 5 min at room temperature, followed by addition of 0.6 mL of 10% $AlCl_3$. After 6 min, 2.0 mL of 1.0 M NaOH was added to the flask, followed by addition of 2.1 mL of distilled water to increase the volume to 10 mL. After incubation for 15 min at room

temperature, the absorbance was read at 510 nm. The flavonoid content was expressed as μg rutin equivalent/g FW.

Antioxidant capacity determinations

***DPPH assay*:** The free radical scavenging activity of extracts was determined using the stable DPPH radical (18). Briefly, an aliquot (0.1 mL) of the loquat extract was added to 3 mL of a 0.1 mM methanolic DPPH solution. After 30 min of incubation in the dark at room temperature, the absorbance at 517 nm was then measured. Results were expressed as μmol Trolox equivalent (TE)/g FW.

***ABTS assay*:** The ABTS radical scavenging ability of extracts was evaluated according to the procedure of Re *et al*. (19). An ABTS* stock solution (7 mM) was prepared using $K_2S_2O_8$ (2.45 mM, final concentration) as the oxidant agent. The working solution of ABTS* was obtained by diluting the stock solution in ethanol to an absorption value of 0.70±0.02 at 734 nm. Then, 0.2 mL of extract was added to the 2.0 mL ABTS* working solution and the absorbance were measured at 734 nm after 15 min. Results were expressed as μmol TE/g FW.

***Ferric reducing/antioxidant power (FRAP) assay*:** The method of Benzie and Strain (20) was used for a FRAP assay with some modification. Stock solutions included a 300 mM acetate buffer (pH 3.6), a 10 mM TPTZ solution acidified using concentrated HCl, and 20 mM $FeCl_3 \cdot 6H_2O$. The fresh working solution was prepared by mixing 10 parts of the acetate buffer, 1 part of the TPTZ solution, and 1 part of the $FeCl_3 \cdot 6H_2O$ solution, and the reaction mixture was incubated in a water bath at 37°C before use. For each assay, 0.15 mL of fruit extract or methanol (for the reagent blank) was reacted with 2.85 mL of the FRAP solution at 37°C for 30 min in the dark. The absorbance of the reaction mixture was determined at 593 nm. Results were expressed as μmol TE/g FW.

Statistical analysis All measurements were performed in triplicate and results were expressed as a mean±SD. The SPSS system (SYSTAT, version 10.0; SPSS, Inc., Chicago, IL, USA) was used for statistical analysis of experimental data. The significance of each variable among different cultivars was determined using an analysis of variance (ANOVA). Differences between means at the 95% (p<0.05) confidence level were considered statistically significant, while differences at the 99% (p<0.01) confidence level were considered highly significant. Pearson correlations were used to determine relationships among phenolic compounds and the antioxidant capacity.

Results and Discussion

Fruit characteristics Sugar, acid, and color values of loquat fruits varied with cultivars (Table 1). The sugar and organic acid contents of a fruit are important factors for development of flavor. The TSS value ranged from 11.8±0.50 to 13.9±1.70%. 'Ninghaibai' had the highest TSS value, and 'Taipingbai' had the lowest. TA values were dramatically different between 6 cultivars (p<0.05). The highest TA value was found in 'Taxiabai' (0.31±0.03%) and the lowest value was in 'Daguotaipingbai' (0.13±0.02%). The TSS and acidity contents of loquat genotypes have been reported to be between 10.87 and 21.38%, and 0.15 and 1.23%, respectively (15,21). The TSS and acidity results of this stuffy were comparable with results from previous studies. The peel color of the 6 cultivars was characterized by the hue angle (H* value, 0° for red-purple and 90° for yellow), and chroma (C* value, indicating the intensity or color saturation) (20). The peel color of tested cultivars tended to be yellow to orange with a hue angle value of 56.0 to 66.9. 'Taxiahong' was deepest in color with the lowest hue angle (H* value), the highest color intensity (C* value), and a relatively low L* value, perhaps due to a high carotenoid content. Furthermore, 'Ninghaibai' had a low C* value and relatively high H* and L* values, corresponding to a lighter and yellower color than the other cultivars.

Total phenolic and flavonoid contents and the DPPH, ABTS, and FRAP antioxidant capacities The total phenolic contents of loquat varieties are shown in Table 2.

Table 1. Total soluble solids, the titratable acidity content, and the color of 6 cultivars of loquat fruits[1)]

Cultivar	TSS (%)	TA (%)	Surface color		
			L*	C*	H*
Ninghaibai	13.9±1.7^{a}	0.18±0.01^{d}	62.2±3.1^{cd}	40.4±2.6^{d}	64.1±3.3^{a}
Taipingbai	11.8±0.5^{b}	0.21±0.01^{c}	61.5±2.4^{d}	46.0±2.5^{b}	59.1±2.4^{b}
Daguotaipingbai	12.1±1.2^{ab}	0.13±0.02^{e}	63.2±2.2^{c}	45.2±3.0^{b}	58.7±3.6^{b}
Taxiabai	13.0±1.5^{a}	0.31±0.03^{a}	66.0±2.0^{a}	42.5±2.0^{c}	66.9±2.2^{a}
Taxiahuang	12.2±1.2^{ab}	0.24±0.02^{b}	64.7±1.9^{b}	46.4±2.1^{b}	59.3±2.5^{b}
Taxiahong	12.2±0.9^{ab}	0.14±0.01^{e}	62.8±1.1^{c}	48.2±2.2^{a}	56.0±2.9^{c}

[1)]Values are expressed as a mean±standard deviation (SD). Mean values within a column followed by different letters are significantly different at p<0.05.

Table 2. Total phenolic and flavonoid contents and antioxidant capacities of 6 cultivars of loquat fruits[1)]

Cultivar	Total phenolics (mg GAE/g FW)	Total flavonoids (mg Rutin/g FW)	DPPH (μmol TE/g FW)	ABTS (μmol TE/g FW)	FRAP (μmol TE/g FW)
Ninghaibai	0.93±0.03[a]	0.15±0.01[b]	4.93±0.26[a]	4.47±0.25[a]	5.09±0.31[b]
Taipingbai	0.66±0.08[c]	0.09±0.01[d]	2.91±0.32[e]	2.73±0.22[e]	3.16±0.34[e]
Daguotaipingbai	0.70±0.05[bc]	0.11±0.02[cd]	3.39±0.30[d]	3.04±0.20[d]	3.58±0.21[d]
Taxiabai	0.77±0.05[b]	0.12±0.02[c]	3.62±0.17[c]	3.15±0.32[c]	3.79±0.18[d]
Taxiahuang	0.89±0.05[a]	0.11±0.02[cd]	4.34±0.13[b]	3.89±0.48[b]	4.75±0.31[c]
Taxiahong	0.96±0.11[a]	0.21±0.02[a]	4.70±0.41[a]	4.55±0.20[a]	5.43±0.22[a]

[1)]Values are expressed as a mean±SD (n=3). Means within a column followed by different letters are significantly different at p<0.05.

The total phenolic content was calculated using a Folin-Ciocalteau reagent assay, which is a convenient, simple, and precise method. Significant varietal differences in total phenolic content were found in the 6 loquat cultivars (p<0.05). Values varied from 0.66±0.08 to 0.96±0.11 mg GAE/g FW. 'Taipingbai' showed the lowest total phenolic value, and 'Taxiahong' showed the highest. The total flavonoid content was similar to the total phenolic content in a range between 0.09±0.01 and 0.21±0.02 mg rutin/g FW. Statistical mean values revealed that 'Taxiahong' had an approximately 2.3 fold higher total flavonoid content than 'Taipingbai'. Many factors are known to affect the phenolic profiles of fruit species, including cultivar/genotype (7-9), growth conditions (22), management techniques (23), and stage of maturity (10,24). Varietal discrepancies observed in this study may be partly attributed to different loquat cultivar genotypes. The total phenolic and flavonoid contents in this study were much higher than in previous reports ('Ninghaibai', 0.45 mg GAE/g FW and 0.05 mg Rutin/g FW, respectively) (9). These previously reported values were even lower than values for the cultivar 'Taipingbai' in this study, which contained the lowest phenolic and flavonoid contents, perhaps partly due to different methods used for extraction and different growth conditions.

Different antioxidant activity assessments are required to take into account different mechanisms of antioxidant action. Antioxidant capacities can be influenced by several factors and cannot be fully described by a single method. Therefore, in this study, antioxidant capacities were measured using DPPH, ABTS, and FRAP assays. FRAP measures the reducing ability of a compound (25), while DPPH and ABTS measure the radical scavenging ability. By the DPPH method, the highest antioxidant activity was observed for 'Ninghaibai' (4.93±0.26 μmol TE/g FW), followed in declining order by 'Taxiahong', 'Taxiahuang', 'Taxiabai', 'Daguotaipingbai', and 'Taipingbai'. ABTS method values ranged from 2.73±0.22 to 4.55±0.20 μmol TE/g FW in an order of increasing antioxidant capacity of 'Taipingbai', 'Daguotaipingbai', 'Taxiabai', 'Taxiahuang', 'Ninghaibai', 'Taxiahong'. The FRAP assay resulted in higher values than the DPPH and ABTS assays for all 6 cultivars. Corresponding values for the FRAP method were 3.16±0.34 to 5.43±0.22 μmol TE/g FW with a ranking of 'Taipingbai', 'Daguotaipingbai', 'Taxiabai', 'Taxiahuang', 'Ninghaibai', 'Taxiahong'. Based on DPPH, ABTS, and FRAP assays results, 'Taxiahong', and 'Ninghaibai' had higher antioxidant levels than the other cultivars, and 'Taipingbai' had the lowest antioxidant levels.

Comparisons with published reports is difficult since just a few studies have been carried out using the same reference standards and extraction solvents. Reports of the antioxidant capacities of loquat pulp are limited. However, the results of this study were comparable with previous work (9,22). Fu *et al*. (26) measured the antioxidant capacities of 62 fruits, and found that different fruits had different antioxidant capacities, with large variations in results. The antioxidant capacity (ABTS assay) of loquat fruits in this study was lower than reported values for olive, pomegranate, sweetsop, guava, persimmon, Chinese date, Chinese wampee, and plum, but higher than values for banana, grape (USA), jackfruit, lemon, peach, pear (honey), and watermelon (26).

The phenolic compound content determined using HPLC In the study, neochlorogenic acid, chlorogenic acid, 4-*O*-caffeoylquinic acid, protocatechuic acid, 4-hydroxybenzoic acid, caffeic acid, ferulic acid, ellagic acid, and *o*-coumaric acid were identified in loquat flesh (Fig. 1). The major phenolic compounds of fruit fractions are shown in Table 3. As a characteristic of the Rosaceae family, chlorogenic acid and neochlorogenic acid are the main phenolic acids in loquat. Chlorogenic acid was the main hydroxycinnamic derivative in mature loquat fruit. A maximal level of chlorogenic acid was observed in 'Taxiahong' (2216.65±272.53 μg/g of FW). In pulp tissue, the concentration of neochlorogenic acid was lower than for chlorogenic acid, and the highest neochlorogenic acid content was found in 'Taxiahuang' and 'Taxiahong' (1125.32±197.03 and 1139.91±181.43 μg/g FW, respectively), in agreement with other reports. Ding *et al*. (10) found that a large rise in the chlorogenic acid concentration appears to

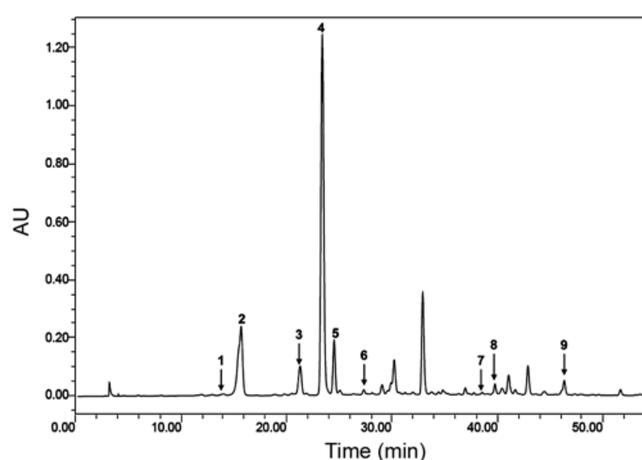

Fig. 1. HPLC chromatograms of phenolic compounds in the flesh of loquat fruit (cv. Ninghaibai), monitored at 280 nm. Peaks are numbered according to the elution sequence. (1), protocatechuic acid; (2), neochlorogenic acid; (3), 4-hydroxybenzoic acid; (4), chlorogenic acid; (5), 4-*O*-caffeoylquinic acid; (6), caffeic acid; (7), ferulic acid; (8), ellagic acid; (9), *o*-coumaric acid.

be a characteristic of loquat fruit ripening. In this work, the 4-*O*-caffeoylquinic acid content was much lower than the chlorogenic acid and neochlorogenic acid contents (Table 3). The concentration ranged from 84.12±6.18 ('Taxiahong') to 245.96±10.12 μg/g FW ('Ninghaibai'). The 4-Hydroxybenzoic acid content varied from 56.27±10.14 ('Taipingbai') to 340.64±67.22 μg/g FW ('Taxiahuang'). The highest protocatechuic acid content was found in 'Taxiahuang' (65.38±6.26 μg/g FW), whereas all other cultivars had lower protocatechuic acid contents. The highest caffeic acid level was found in 'Taxiahuang' (10.29±1.82 μg/g FW) followed by 'Daguotaipingbai' (10.13±1.68 μg/g FW). 'Daguotaipingbai' had the highest ellagic acid content (67.38±12.03 μg/g FW) and the lowest *o*-coumaric acid content (13.43±0.64 μg/g FW). The ferulic acid content was similar in all 6 cultivars. Although there were differences in the phenolic compound contents among the cultivars, in general, 'Taxiahong' showed higher phenolic compound contents than the other cultivars, followed by 'Taxiahuang', 'Ninghaibai', 'Taxiabai', 'Daguotaipingbai' and 'Taipingbai'. The rank order of the sum of phenolic compounds determined using HPLC (SPC) was similar to the order determined using the Folin-Ciocalteu reagent assay (TPC).

The phenolic contents of loquat fruits have been reported with more hydroxycinnamic and benzoic acid derivatives than cyanidine glycoside (5,10,11). Koba *et al.* (5) found significant levels of chlorogenic acid, cyanidine glucoside, and epicatechin in ethanol extracts of loquat flesh using reverse-phase HPLC-ESI EM. Ferreres *et al.* (11) studied the phenolic compositions of 6 loquat cutivars using HPLC-DAD-ESI-MS/MS and identified 6 compounds in loquat flesh with both 3- and 5-caffeoylquinic, and 5-feruloylquinic acid as the major identified compounds. Ding *et al.* (10) showed that 5-caffeoylquinic acid (chlorogenic acid), neochlorogenic acid, hydroxybenzoic acid, 5-*p*-feruloylquinic acid, protocatechuic acid, 4-caffeoylquinic acid, epicatechin, *o*-coumaric acid, ferulic acid, and *p*-coumaric acid phenolic were the main compounds in loquat fruit. Although the phenolic compound content varied by identification method and loquat cultivar, the concentration of chlorogenic acid was predominant in ripe loquat fruit. The sum of the phenolic compounds present determined using HPLC in this study was much higher than previously reported values (5,10,11).

Correlation analysis The results of a correlation study are shown in Table 4. Correlations among the DPPH, ABTS, and FRAP assay results were high and positive, ranging from *r* values of 0.832 to 0.874. The highest correlation was between ABTS and FRAP (r=0.874, p<0.01), and the lowest correlation was between DPPH and ABTS (r=0.832, p<0.01). Therefore the DPPH, ABTS, and FRAP assays produced comparable results for

Table 3. Phenolic compound contents determined using HPLC[1)] (unit: μg/g FW)

Cultivar	Ninghaibai	Taipingbai	Daguotaipingbai	Taxiabai	Taxiahuang	Taxiahong
NCHA	557.08±25.87^{b}	310.09±45.27^{c}	414.293±60.62^{bc}	400.69±39.03^{bc}	1125.32±197.03^{a}	1139.91±181.43^{a}
CHA	1734.99±107.04^{b}	725.90±108.90^{cd}	651.92±93.53^{d}	992.91±139.87^{c}	1147.77±224.50^{c}	2216.65±272.53^{a}
4-CQA	245.96±10.12^{a}	216.48±45.61^{a}	236.04±33.06^{a}	219.55±36.63^{a}	155.31±28.98^{b}	84.12±6.18^{c}
4-HBA	103.27±4.95^{c}	56.27±10.14^{c}	89.26±11.19^{c}	72.29±11.85^{c}	340.64±67.22^{a}	210.82±31.32^{b}
PCA	48.97±1.58^{c}	47.73±1.16^{c}	48.42±1.24^{c}	48.41±0.30^{c}	65.38±6.26^{a}	57.41±5.03^{b}
CFA	6.44±0.52^{b}	9.99±3.10^{a}	10.13±1.68^{a}	7.28±0.74^{ab}	10.29±1.82^{a}	7.84±2.03^{ab}
FLA	5.06±1.14^{a}	5.71±0.30^{a}	6.32±2.55^{a}	4.35±0.33^{a}	4.29±0.82^{a}	5.10±0.51^{a}
EGA	31.76±2.15^{c}	46.35±9.76^{b}	67.38±12.03^{a}	40.37±8.08^{bc}	43.28±7.73^{bc}	60.49±9.44^{ab}
CRA	18.61±0.79^{b}	15.96±0.92^{c}	13.43±0.64^{d}	13.80±0.36^{cd}	23.34±2.95^{a}	21.76±1.17^{a}
SPC	3062.69±162.20^{b}	1620.29±241.70^{c}	1732.66±238.68^{c}	1846.51±151.80^{c}	3315.99±601.69^{b}	4270.42±548.10^{a}

[1)]Values are expressed as a mean±SD (n=3). Means within a row followed by different letters are significantly different at p<0.05; NCHA, neochlorogenic acid; CHA, chlorogenic acid; 4-CQA, 4-*O*-caffeoylquinic acid; 4-HBA, 4-hydroxybenzoic acid; PCA, protocatechuic acid; CFA, caffeic acid; FLA, ferulic acid; EGA, ellagic acid; CRA, *o*-coumaric acid; SPC, sum of phenolic compounds determined using HPLC

Table 4. Pearson's correlation coefficients (*r*) between phenolic compounds and the antioxidant capacities of 6 cultivars of loquat fruits[1)]

	DPPH	ABTS	FRAP	TPC	TFC	NCHA	CHA	4-CQA	4-HBA	PCA	CFA	FLA	EGA	CRA	SPC
DPPH	1	0.832**	0.869**	0.829**	0.810**	0.695**	0.801**	-0.434^{ns}	0.555*	0.463*	-0.368^{ns}	-0.244^{ns}	-0.157^{ns}	0.681**	0.831**
ABTS	-	1	0.874**	0.776**	0.704**	0.739**	0.793**	-0.412^{ns}	0.628**	0.555*	-0.255^{ns}	-0.155^{ns}	-0.238^{ns}	0.819**	0.865**
FRAP	-	-	1	0.808**	0.713**	0.717**	0.862**	-0.407^{ns}	0.575**	0.521*	-0.356^{ns}	-0.291^{ns}	-0.301^{ns}	0.752**	0.872**
TPC	-	-	-	1	0.734**	0.617**	0.751**	-0.408^{ns}	0.474*	0.388^{ns}	-0.364^{ns}	-0.102^{ns}	-0.243^{ns}	0.713**	0.766**
TFC	-	-	-	-	1	0.539*	0.838**	-0.541*	0.241^{ns}	0.180^{ns}	-0.502*	-0.089^{ns}	0.008^{ns}	0.455*	0.746**
NCHA	-	-	-	-	-	1	0.663**	-0.698**	0.922**	0.912**	0.118^{ns}	-0.200^{ns}	0.149^{ns}	0.911**	0.902**
CHA	-	-	-	-	-	-	1	-0.449*	0.380^{ns}	0.381^{ns}	-0.391^{ns}	-0.155^{ns}	-0.099^{ns}	0.679**	0.908**
4-CQA	-	-	-	-	-	-	-	1	-0.579*	-0.541*	0.018^{ns}	0.324^{ns}	-0.078^{ns}	-0.546*	-0.592**
4-HBA	-	-	-	-	-	-	-	-	1	0.973**	0.266^{ns}	-0.239^{ns}	0.075^{ns}	0.873**	0.713**
PCA	-	-	-	-	-	-	-	-	-	1	0.336^{ns}	-0.250^{ns}	0.126^{ns}	0.854**	0.709**
CFA	-	-	-	-	-	-	-	-	-	-	1	0.324^{ns}	0.513*	0.056^{ns}	-0.141^{ns}
FLA	-	-	-	-	-	-	-	-	-	-	-	1	0.500*	-0.208^{ns}	-0.158^{ns}
EGA	-	-	-	-	-	-	-	-	-	-	-	-	1	-0.117^{ns}	0.017^{ns}
CRA	-	-	-	-	-	-	-	-	-	-	-	-	-	1	0.884**
SPC	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1

[1)]TPC, total phenolic content; TFC, total flavonoid content; NCHA, neochlorogenic acid; CHA, chlorogenic acid; 4-CQA, 4-*O*-caffeoylquinic acid; 4-HBA, 4-hydroxybenzoic acid; PCA, protocatechuic acid; CFA, caffeic acid; FLA, ferulic acid; EGA, ellagic acid; CRA, *o*-coumaric acid; SPC, sum of phenolic compounds determined using HPLC; ^{ns}not significant; *significant at $p<0.05$; **significant at $p<0.01$

antioxidant activities measured in loquat fruit extracts. The high correlation between antioxidant capacities measured by these 3 different methods has also been investigated in other plants. Thaipong *et al*. (25) found linear correlations between antioxidant capacities obtained using FRAP, DPPH, ABTS, and oxygen radical absorbance capacity (ORAC) assays.

Positive and significant correlations of total phenolics with flavonoids (r=0.734, p<0.01), DPPH (r=0.829, p<0.05), ABTS (r=0.776, p<0.01), and FRAP (r=0.808, p<0.01) were observed in this study. There were also positive and significant correlations (p<0.01) between the flavonoid content with DPPH (r=0.810), ABTS (r=0.704), and FRAP (r=0.713). Similar results were observed in other studies. Andarwulan *et al*. (27) found that values of total phenols were highly correlated with DPPH, ABTS, and ferric cyanide reducing power antioxidant assay results with r values of 0.77, 0.79, and 0.85, respectively, for extracts from 11 vegetables of Indonesian origin. Also, a high correlation between TPC and the antioxidant activity, determined using DPPH, ABTS, FRAP, and ORAC assays, was reported using methanol extracts of guava fruit (25). Zhou *et al*. (13) measured the antioxidant capacity in methanol extracts of loquat flowers using DPPH, ABTS, and FRAP methods, and found that correlations between flavonoid and phenolic contents and antioxidant capacities obtained using the 3 different assays were high and positive.

TPC was significantly correlated with neochlorogenic acid (r=0.617, p<0.01), chlorogenic acid (r=0.751, p<0.01), 4-hydroxybenzoic acid (r=0.474, p<0.05), and *o*-coumaric acid (r=0.713, p<0.01) contents, and with the sum of phenolic compounds determined using HPLC (SPC) (r=0.766, p<0.01). The total flavonoid content (TFC) was significantly correlated with neochlorogenic acid (r=0.539, p<0.01), chlorogenic acid (r=0.838, p<0.05), 4-*O*-caffeoylquinic acid (r=−0.541, p<0.05), caffeic acid (r=−0.502, p<0.05), and *o*-coumaric acid (r=0.455, p<0.01) contents, and with the total amount of phenolics determined using HPLC (r=0.746, p<0.01).

Phenolic compounds have different antioxidant capacities depending on molecular structure, number of hydroxyl groups, and the hydroxyl group distribution in the structure (28). In this study, the protocatechuic acid, neochlorogenic acid, 4-hydroxybenzoic acid, chlorogenic acid, and *o*-coumaric acid contents were all significantly correlated with the antioxidant capacities determined using DPPH, ABTS, and FRAP assays. These components may be the main contributors to the antioxidant capacity of loquat fruit. Among these phenolic components, the highest correlation value was found between chlorogenic acid and the antioxidant capacity (r≥0.793, p<0.01). The high antioxidant activity of chlorogenic acid has attracted much research attention. The *in vitro* and *in vivo* hypoglycaemic, antiviral, hepatoprotective, and immunoprotective activities, and pharmacological activities of chlorogenic acid have been reported (29); however, no significant correlation has been reported between 4-*O*-caffeoylquinic acid, caffeic acid, ferulic acid, and ellagic acid and antioxidant capacities, perhaps due to a low content or a weak antioxidant capacity.

In conclusion, analysis of phenolic compound contents and the antioxidant capacities of 6 loquat cultivars from China were carried out. The phenolic compound contents and antioxidant capacities varied greatly among different cultivars. 'Taxiahong' contained the highest amounts of total phenolics and total flavonoids and the highest antioxidant activity of all tested cultivars. The phenolic compounds chlorogenic acid, neochlorogenic acid, 4-hydroxybenzoic acid, protocatechuic acid, and *o*-coumaric acid may be the main contributors to the antioxidant activity as all were markedly correlated with the antioxidant capacities (p<0.01).

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