Radical Scavenging Activity and Phenolic Compounds in Persimmon (*Diospyros kaki* L. cv. Mopan)

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ABSTRACT: The Mopan persimmon (*Diospyros kaki* L. cv. Mopan) is the major cultivar of astringent persimmon in northern China. This study investigates the radical scavenging activity against ABTS and DPPH radical, and the content of total and individual phenolics (catechin, epicatechin, epigallocatechin, chlorogenic acid, caffeic acid, and gallic acid) with apple, grape, and tomato as controls. The radical scavenging activities against ABTS and DPPH radicals of the Mopan persimmon are 23.575 and 22.597 μm trolox eq/g f.w., respectively. These findings suggest that the Mopan persimmon's antioxidant activity is significantly ($P < 0.05$) stronger than that of reference materials. The Mopan persimmon showed the highest content of total phenolics among the 4 materials tested. Significant correlations ($R^2 = 0.993$, $P < 0.05$, ABTS radical; $R^2 = 0.980$, $P < 0.05$, DPPH radical) are found between the total phenolics and the radical scavenging activities. The total content of these 6 kinds of phenolics (catechin, epicatechin, epigallocatechin, chlorogenic acid, caffeic acid, and gallic acid) is significantly correlated ($R^2 = 0.831$, $P < 0.05$, ABTS radical; $R^2 = 0.745$, $P < 0.05$, DPPH radical) with the individual radical scavenging activity of the 4 materials, although the total content of the 6 phenolics accounts for no more than 20% of the total phenolics in the Mopan persimmon. Gallic acid exhibits the strongest antioxidant activity in all 6 kinds of phenolics and its content is the largest in the Mopan persimmon, presumably being responsible for its much higher antioxidant activity as compared to apple, grape, and tomato.

Keywords: persimmon, phenolics, radical scavenging activity

Introduction

Regular consumption of fruit and vegetables containing natural antioxidants is correlated with the decreased risk of diseases such as cancer, cardiovascular diseases, and so on (Michels and others 2000; Temple 2000). The Mopan persimmon is highly nutritious compared with other ordinary fruits. Besides its nutritive value, persimmon has long been used for medicinal purposes since ancient times in China. The Pen Ts'ao Kang Mu, China's most famous materia medica, compiled in 1578 by Li Shi-Zhen (1518–1593) and published in 1597, described how persimmon can be used to improve lung, stomach, spleen, and intestinal conditions. It also describes how persimmon can be used to prevent and treat diseases such as sore throat, aphtha, and insomnia. Additionally, it has the added benefit of relieving alcoholism. Recent studies show that the Mopan persimmon possesses antitumor and multidrug resistance reversal properties (Kawase and others 2003), hypocholesterolemic and antioxidant effects (Gorinstein and others 1998), and antidiabetic effects (Lee and others 2006), and prevents the rise in plasma lipids (Matsumoto and others 2006). These beneficial properties are considered to be related to the various antioxidants, including vitamins, phenolic compounds, and carotenoids, contained in this kind of fruit.

The total production of persimmon in China was 1655000 tons in 2006, which accounted for 70.9% of the world's production. As a result, this ranked China first in the world (Liu and others 2007). The Mopan persimmon (*Diospyros kaki* L. cv. Mopan) is the major cultivar of astringent persimmon in northern China and especially in Beijing. Unlike other persimmons, the Mopan persimmon features a fair color, rich nutrition, and a sweet juicy flesh and takes on a millstone-like shape (Figure 1). A single fruit on average weighs 250 g and can reach up to 610 g. Therefore, many attempts have been made to extend the storage and shelf life (Wang and others 2005; Guo and others 2006; Li and others 2006). However, relatively little research on bioactive activities has been reported, especially research on the bioactive compounds of the astringent persimmon.

The aim of this study was to evaluate the radical scavenging activities of the Mopan persimmon by employing the DPPH and ABTS radical scavenging methods. At the same time, the content of the 6 kinds of phenolics along with the total phenolics in the persimmon were determined to find whether there was a relationship between the antioxidant activity and the total content of the 6 kinds of phenolics, as well as the total content of all phenolics. All the DPPH radical scavenging activities of the 6 kinds of phenolics were determined to find which kinds of phenolics contribute to the antioxidant activity in the Mopan persimmon. Grape, apple, and tomato were used as reference plants to compare the antioxidant activity of persimmon due to their well-documented antioxidant properties (Giovanelli and others 1999; Leong and Shui 2002; Matito and others 2003; Hagen and others 2007; Orak 2007).

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Materials and Methods

Chemicals and reagents

Folin and Ciocalteu's phenol reagent, 6-hydroxy-2,5,7,8-tetramethychroman-2-carboxylic acid (trolox), 1,1-diphenyl-2-picrydrazyl (DPPH), and 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) were purchased from Sigma Chemical Co. (St. Louis, Mo., U.S.A.). Gallic acid monohydrate (GA), (–)-catechin (C), (–)-epicatechin (EC), (–)-epigallocatechin (EGC), chlorogenic acid (CHA), and caffeic acid (CA) were also purchased from Sigma Chemical Co. All chemicals used in this study were of analytical grade or HPLC grade.

Fruit materials

Astringent persimmons (*Diospyros kaki* L. cv. Mopan) were harvested in November 2006 in Beijing (Figure 1) with a growth period of 170 d. The fruit selected for the study had a diameter of about 10 ± 2 cm from all 50 trees. The entire persimmons were immediately frozen and stored at -18 °C in the laboratory for future use after 2-h commute below 12 °C.

Since the antioxidant activities of apples, grapes, and tomatoes were well documented, they were used as controls. Fuji apples (Shandong Province, China), Jufeng grapes (Heibei Province, China), and Jinpeng tomatoes (Beijing) are very popular in China. So these varieties were chosen for the experiments. They were all purchased from a local market and were prepared for ethanol extracts immediately for determining ABTS and DPPH radical scavenging activities.

Sample preparation of ethanol extracts

The 50 persimmon samples were randomly chosen and peeled by knife when they were still in their frozen state. The thickness of peel was between 1 and 1.5 mm. The pulp (100 g) was added with 2.5 volumes of ethanol (250 mL) and then was homogenized ($10000 \times g$ for 2 min) by using a TS25BS4 homogenizer (IKA Labortechnic Co., Wilmington, N.C., U.S.A.), followed by boiling in a water bath for 20 min to inactivate polyphenol oxidases. After 15 min of extracting the homogenate with an ultrasonic machine (KQ-100E, KUNSHAN Ultrasonic Co., China) at room temperature, the homogenate was centrifuged (5634 *g*) for 15 min. The resultant supernatant was then filtered and the filtrate was used as the persimmon extracts for analysis of radical scavenging activities. The persimmon extract was lyophilized and the resultant persimmon powder was used for determination of total phenolics and individual phenolics. The grape, apple, and tomato extracts were prepared using the same procedure as described previously.

Figure 1 — Persimmon (*Diospyros kaki* L. cv. Mopan) (Source: Beijing Fangshan Science & Technology Committee, 2005).

ABTS free radical decolourisation assay

The ABTS assay (Re and others 1999) was employed to measure the antioxidant activity of the fruit and vegetable extracts. These extracts were diluted at certain times with distilled water. The diluted extracts (50 μL) were then added to the ABTS radical solution (150 μL) and were kept at room temperature for 6 min. The absorbance of the resulting solution was measured at 405 nm. Controls without ABTS radical were used to allow for any absorbance of the extracts themselves, and 150 μL of PBS was added to these control samples instead. The assay used trolox as a standard. Radical scavenging activities of these extracts were expressed as μM trolox equivalent/g f.w. (fresh weight).

DPPH free radical scavenging assay

Radical scavenging activities of the extracts were assayed according to a method described by Suda (2000). The extract (100 μL) was mixed with the DPPH solution (200 μM) in the ratio of 1:1 and the mixture was kept at room temperature for 20 min. The absorbance of the resulting solution was measured at 520 nm using a Bio-Rad Model 550 microplate reader (Bio-Rad, Hercules, Calif., U.S.A.) with trolox as a standard. The radical scavenging activity of these extracts was expressed as μM trolox equivalent/g f.w.

Determination of the content of total phenolics

The total phenolic content of each extract was measured as gallic acid equivalents using the Folin–Ciocalteau's phenol reagent (FC reagent) according to Peterson and others (2001). One milliliter of the sample (1 g of dried powder was dissolved in 10 mL methanol), 0.5 mL of FC reagent, and 3.0 mL of Na_2CO_3 solution (200 mg/mL) were mixed in that order. The sample was then mixed on a vortex mixer and the reaction proceeded for 15 min at room temperature. The reaction mixtures were diluted with 10 mL of deionized water. The white precipitate that formed was removed by centrifuging for 5 min at $1250 \times g$. Absorbance of supernatants was measured at 725 nm. Methanol was used as a control in place of the sample. Gallic acid equivalents (mg/100 g d.w.) were determined from a standard concentration curve.

Identification and quantification of the 6 kinds of phenolics using HPLC

EGC, C, EC, GA, CHA, and CA content were identified and quantified by HPLC using a flow rate gradient elution system with water–methanol–phosphoric acid (80:20:0.1) (Nishitani and Yuko 2004). The HPLC system consisted of 2 LC-10AD LC pumps for high-pressure gradient elution and a photo diode array (PDA) detector (Shimadzu, Japan). A Dikma Diamonsil (C_{18}) (250 × 4.6 mm) column (Dima Co. Ltd., Orlando, Fla., U.S.A.) was used for the HPLC analyses and was operated at 40 °C. The flow rate started at 0.3 mL/min, was maintained for 12 min, and then linearly increased to 0.45 mL/min in 1 min. This flow rate was maintained for 6 min followed by a linear increase of flow rate to 1.0 mL/min in 1 min. The final flow rate was held for 20 min.

Statistical analysis

The Spearman correlation coefficients were calculated between antioxidant activity of ABTS, DPPH, and the content of total phenol with SPSS 12.0. The difference was considered significant at the 0.05 level. All treatments were run in triplicate.

Results and Discussion

The ABTS and DPPH radical scavenging activities of ethanol extracts of the Mopan persimmon with grape, apple, and tomato as controls were determined, and the results are shown in Figure 2. Three kinds of fruits and 1 kind of vegetable all show radical scavenging activities. The range of activities against the ABTS radical of the 4 materials extended from 1.139 to 23.512 μm trolox eq/g f.w., and that of the DPPH radical ranged from 1.509 to 22.485 μm trolox eq/g f.w. However, the 4 materials exhibited quite different antioxidative activities. The tomato ethanol extract displayed the lowest activity against the 2 radicals among the 4 materials. The activity of apple extract against the radicals was significantly higher than that of tomato extract, while it was significantly lower than that of grape extract ($P < 0.05$). The Mopan persimmon extract exhibited the strongest inhibiting activities against both ABTS and DPPH radical, which were significantly higher than that of other extracts ($P < 0.05$).

Our present results show that the Mopan persimmon possesses more significant radical scavenging activities than grape, apple, and tomato, which were consumed by most individuals regularly for their well-known and widely documented antioxidant properties. Therefore, the results of this experiment demonstrated that the Mopan persimmon could be used as a bioactive food due to its strong antioxidant activity. This conclusion is parallel with Gorinstein and others' (1998) observation showing that the persimmon exercises a marked antioxidant effect on rats fed diets supplemented with this fruit.

When comparing the results of ABTS and DPPH radical scavenging activities, a good correlation between the two can be observed ($R^2 = 0.975$, $P < 0.01$), suggesting that all extracts have almost identical ability to scavenge both ABTS and DPPH radicals. The result is consistent with the previous results reported on other fruits and vegetables (Leong and Shui 2002). Therefore, the 2 models may be useful tools for evaluating the antioxidant capacity of the Mopan persimmon.

Plant phenolics constitute one of the major groups of compounds acting as primary antioxidants or free radical terminators. It was reasonable to determine their total amounts of phenolics in the Mopan persimmon and the controls. Their total phenolics content of extracts can be found in Figure 3. The contents of total phenolics were determined from the regression equation of calibration curve ($y = 0.0034\ X + 0.0033$, $R^2 = 0.9999$) and expressed in gallic acid equivalents (mg/100 g d.w.). The contents of the total phenolics were 168.15 $\pm$ 0.12 mg/100 g d.w., 93.88 $\pm$ 0.04mg/100 g d.w., 47.26 $\pm$ 0.00 mg/100 g d.w., 21.24 $\pm$ 0.04 mg/100 g d.w. for persimmon, grape, apple, and tomato, respectively. The Mopan persimmon showed the highest content of total phenolics, 8 times higher than that of tomato, which is in good agreement with the stronger antioxidant activity of its ethanol extract. Gorinstein and others (2001) obtained similar results, which show that the content of total phenolics in persimmon is higher than that of apple.

It has been reported that the content of total phenolics may vary in different persimmon varieties and analysis methods used (Gorinstein and others 1998; Suzuki and others 2005). Suzuki and others (2005) reported that the total phenolic content in the methanol extracts of Japanese astringent persimmons is up to 84.6 mg/100 g d.w. In comparison with these Japanese persimmons, the Mopan persimmon used in the present study contained 2 times the amount of total phenolics.

The relationship between the content of total phenolics and the radical scavenging activity of the Mopan persimmon along with controls was investigated. The statistical analysis showed a

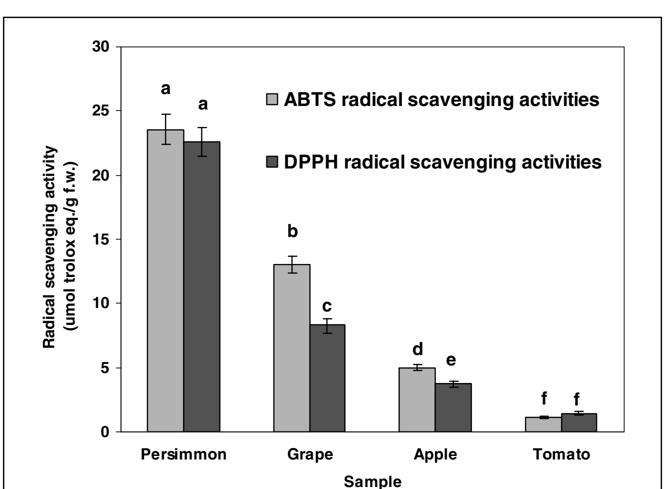

Figure 2—ABTS and DPPH radical scavenging activities of the Mopan persimmon, grape, apple, and tomato. (■), ABTS radical scavenging activity; (□), DPPH radical scavenging activity. Means ± SD (vertical lines). Bars with different letters are statistically significantly different ($P < 0.05$, $n = 3$).

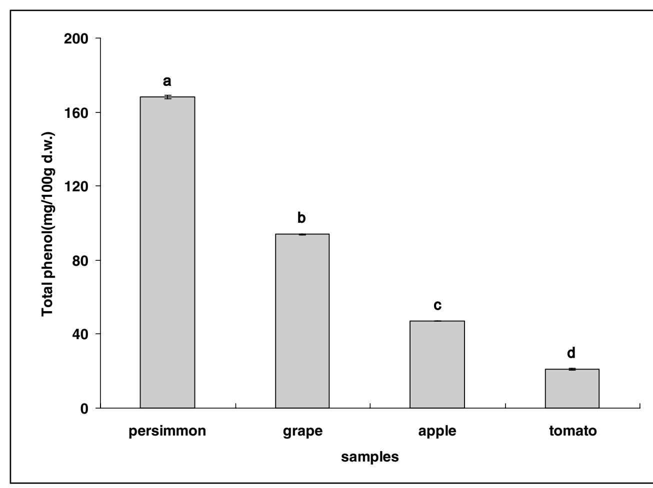

Figure 3—The content of total phenolics in the Mopan persimmon, grape, apple, and tomato. Means ± SD (vertical lines). Bars with different letters are statistically significantly different ($P < 0.05$, $n = 3$).

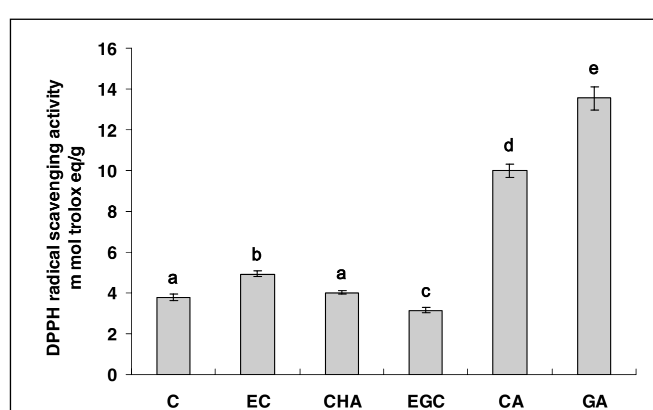

Figure 4—The DPPH radical scavenging activities of the 6 kinds of phenolics. Means ± SD (vertical lines). Bars with different letters are statistically significantly different ($P < 0.05$, $n = 3$).

Table 1—The content of individual phenolics and its individual contribution to the antioxidant activity, which were calculated based on the DPPH radical scavenging activities of the 6 kinds of phenolics (trolox equivalent) in the 4 materials used in this study.

	Persimmon		Grape		Apple		Tomato	
	1	2	1	2	1	2	1	2
C	5.81 ± 0.12	22.021	16.04 ± 0.94	60.795	4.97 ± 0.49	18.837	3.11 ± 0.15	11.788
EC	0.61 ± 0.023	3.009	5.79 ± 0.230	28.56	3.42 ± 0.14	16.87	ND	0
EGC	0.28 ± 0.02	0.883	0.91 ± 0.051	2.869	2.21 ± 0.16	6.967	6.29 ± 0.45	19.828
GA	19.11 ± 0.61	259.033	5.63 ± 0.17	76.314	0.95 ± 0.03	12.877	3.01 ± 0.18	40.8
CHA	3.67 ± 0.07	14.713	1.20 ± 0.02	4.811	14.54 ± 0.71	58.291	1.90 ± 0.06	7.617
CA	2.83 ± 0.07	28.32	ND	0	1.38 ± 0.03	13.81	1.02 ± 0.02	10.207
Total	32.31	327.979	29.57	173.35	27.47	127.651	15.33	90.24

1 = the content of phenolics (mg/100 g d.w.).
2 = the trolox equivalent ($\times 10^{-3}$ mmol/100 g d.w.).
Values are means of 3 replicates ± SD, $n = 3$. ND = not detected.

positive and highly significant relationship between the content of total phenolics and radical scavenging activity against ABTS and DPPH radicals ($R^2 = 0.993$ and 0.980, respectively, $P < 0.01$). Although many other natural compounds, including carotenoids, vitamin E, and vitamin C, may also contribute to the radical scavenging activity of vegetables and fruits, the present results suggest that the total phenolics are mainly responsible for the observed antioxidant activities. Consistent with this idea, previous experiments conducted by Kaur and Kapoor (2002) showed that phenolic compounds might mainly contribute to the radical scavenging activity of these fruit and vegetable extracts.

To obtain information on why there is a big difference in antioxidant activity between the Mopan persimmon and the 3 controls, the DPPH radical scavenging activities of the 6 main kinds of phenolics were determined, respectively (Figure 4). The result shows that GA has the strongest antioxidant activity in the Mopan persimmon. The top 3 radical scavenging activities against DPPH are GA, CA, and EC. Their activities are 13.555 mmol/g, 10.007 mmol/g, and 4.933 mmol/g respectively. Radical scavenging activity against DPPH of CHA and C is almost the same. CHA is slightly higher than C and both are lower than CA, which is consistent with the findings of Valdez and others (2004). EGC shows the lowest antioxidant activity out of these 6 kinds of phenolics.

The contents of 6 phenolics of persimmon and the other fruit and vegetable are shown in Table 1. The individual contributions for antioxidant activities of 6 kinds of phenolics are calculated based on their DPPH radical scavenging activities (trolox equivalent).

Among the Mopan persimmon phenolics, GA showed the highest amount of content, which agrees with the previous results of Japanese persimmon reported by Suzuki and others (2005). Unlike the Japanese astringent persimmon, the Mopan persimmon contained less EC and EGC (Suzuki and others 2005). The results of this study show that GA is the major phenolic, out of these 6 kinds of phenolics, which contributes to the antioxidant activity of the Mopan persimmon. The total content of these 6 kinds of phenolics was only one-fifth of the total phenolics in the Mopan persimmon. However, a correlation was found between the total contents of 6 kinds of phenolics and the radical scavenging activity against ABTS and DPPH radicals ($R^2 = 0.831$ and 0.745, respectively, $P < 0.05$) of the fruits and vegetable used in this study, suggesting that GA is the major substance contributing to the antioxidant activity of the Mopan persimmon. This is different from the Suzuki and others (2005) results, which show that not only GA but also C, EC, and EGC are the major phenolic contributors to the antioxidant activity of persimmon.

The highest content in grape is catechin (16.04 ± 0.94 mg/100 g d.w.), which is lower than the GA content in persimmon. When comparing the antioxidant activity of 6 kinds of phenolics, antioxidant activity of GA is higher than that of catechin. The total equivalent antioxidant activity of the top 3 phenolics, C, EC, and GA, in grape is lower than the antioxidant activity of GA in persimmon. This can further explain why persimmon shows stronger antioxidant activity than grape.

The CHA was shown to have the highest content among all 6 kinds of phenolics determined in apple. This result is parallel to Whang and others (2003) and Wu and others (2007), who have reported that CHA was the dominating phenolic compound in apple. It was also observed that the CHA content in apple is the highest among all fruits and vegetable in the study. The content of CHA in apple reaches $14.54 \pm 0.71 \times 10^{-3}$ mmol/100 g d.w., which is a little lower than the content of GA in persimmon. However, the antioxidant activity of CHA is weaker than GA; therefore, the persimmon shows stronger antioxidant activity in comparison with that of apple.

Both the contents and their equivalent antioxidant activity of the 6 kinds of phenolics in tomato are the lowest, possibly resulting in its lowest antioxidant activity.

The total antioxidant activity of the 6 kinds of phenolics in persimmon, grape, apple, and tomato are 327.979, 173.348, 127.651, and 90.240 ($\times 10^{-3}$ mmol/100 g d.w.), respectively. The total antioxidant activities of the 6 kinds of phenolics in persimmon and controls have parallel trends of DPPH radical scavenging activity of 4 materials. The order is persimmon, grape, apple, and tomato.

Conclusions

The antioxidant activity against ABTS/DPPH radicals and the content of total phenolics of the Mopan persimmon were significantly higher ($P < 0.05$) than that of grape, apple, and tomato. Significant correlations ($R^2 = 0.993$, $P < 0.05$, ABTS radical; $R^2 = 0.980$, $P < 0.05$, DPPH radical) were found to exist between the total phenolics and the radical scavenging activities in all tested fruits and vegetable in this study. Such a correlation also exists between the total content of 6 kinds of phenolics and radical scavenging activity of the fruits and vegetable. GA has the strongest antioxidant activity in comparison to EGC, C, CHA, EC, and CA. The Mopan persimmon has the largest amount of GA, resulting in its strongest antioxidant activity among all tested fruits and vegetables.

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