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COMPARISON ON THE ANTIOXIDANT CAPACITY OF SELECTED FRUITS AND VEGETABLES AND THEIR SEPARATIONS

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Several kinds of fruits, including apple, pear, and grape, and vegetables, including onion and tomato, were studied in this paper. FRAP and DPPH assays were used to determine the antioxidant activity of ethanol extracts and their separations. After being extracted, different phenolic compounds were separated on Sephadex LH-20 column. EC1 and EC50 were used to show the ferric reducing ability and DPPH scavenging activity. The results indicated that the Fe-reducing ability order of different extracts was different with that of DPPH scavenging ability. The mutual effect of the substances in the mixture influenced the antioxidant activity of the separations.

Key words: polyphenols, separations, antioxidant capacity, FRAP, DPPH.

The antioxidant activity of fruits and vegetables differ with varieties and agronomic conditions. The total polyphenols and antioxidant activity of 16 commonly consumed fruits were measured and it was found that there were broad variations in both polyphenol content and *in vitro* antioxidant activity whatever between different cultivars of the same fruit or between different fruits [1]. Hertog studied the content of potential anticarcinogenic flavonoids of 28 vegetables and 9 fruits commonly consumed in the Netherlands [2], but they always used the extracts of the fruits and vegetables to measure the antioxidant activity. It is still unclear which compound in the extract plays an important role in the antioxidant activity. It is necessary to separate the functional compounds into several similar compounds to determine their antioxidant activity.

The functional compounds in fruits and vegetables differ from each other. See the details in Table 1. Sephadex LH-20 column was used to separate the extracts into three complexes, one of which was epicatechin part, another was chlorogenic acid, and the third was procyanidin. The antioxidant activity of epicatechin and chlorogenic acid have been reported [3–7], but these compounds were pure, and the antioxidant capacity of the separations and the extracts of different fruits and vegetables were still unclear. In this paper, these problems were resolved.

The antioxidant activity of fruits and vegetable extracts have been studied in this paper. See details in Fig. 1. It was obvious that the Fe-reducing ability order was grape>apple>pear>tomato>onion, which was in accordance with the results reported by Proteggente [8], while that of DPPH activity was tomato>apple>pear>grape>onion, which was different from their Fe-reducing ability. These two methods were different, and there were other pigments in the separations which also contributed to the total antioxidant activity, so some authors recommended that the antioxidant activity of fruits and vegetables should be evaluated by different methods rather than depending on the results of a single method [9, 10]. The correlations between content of total polyphenols and EC1 (–0.517) and EC50 (–0.029) value were weak. It was not significant and was in accordance with Imeh et al. who have reported that the correlation between the phenolic content of the fruits and the total antioxidant activity was weak ($R^2 = 0.58$) as estimated by FRAP assay [1]. It was possible that the different compounds in the extracts play an important role in antioxidant activity.

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TABLE 1. Different Functional Compositions in Different Fruits and Vegetables (**7, 8, 9**)

Apple	Pear	Grape	Tomato
	Flavonoids (neutral phenols)		
Quercetin Phloretin Phlorezin Epicatechin	Epicatechin Procyanidin	Quercetin Epicatechin Cyanidin	
	Nonflavonoids (acidic phenols)		
p-Coumaric acid Caffeic acid Chlorogenic acid	Chlorogenic acid	*p*-Coumaric acid Cafferic acid	*p*-Coumaric acid Sinapic acid Caffeic acid Chlorogenic acid

Onion: Quercetin and conjugates.

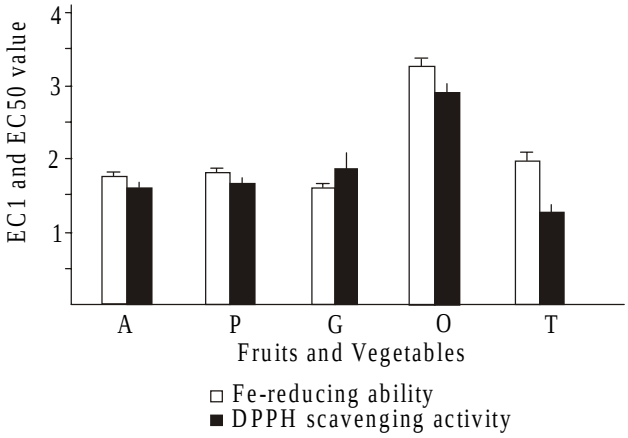

Fig. 1. Comparison of the antioxidant capacity of different fruits and vegetables. A – Apple, P – Pear, G – Grape, O - Onion, T – Tomato.

Vitamin C is also an antioxidant in fruits and vegetables, however the vitamin C contents were lower (< 2 mg/100 g FW in apple and pear, 14 mg/100 g FW in tomato) [8] and the contribution of vitamin C to the total antioxidant activity of fruits was determined to be generally < 15% [11]. It clearly indicated that flavonoids such as quercetin, epicatechin, and procyanidin B_2 rather than vitamin C contributed significantly to the total antioxidant activity of apples [12]. In apple, pear, and grape the functional composition was polyphenols, in onion it was quercetin and its conjugates, and in tomato it was a hydrocarbonlike chemical, lycopene [13]. There are differences in metabolism for the functional compositions with respect to antioxidant activity.

The Fe-reducing ability and DPPH scavenging activity of separations of different fruits and vegetables are listed in Table 2. The antioxidant ability of polyphenols depends on the degree of hydroxylation and the extent of the conjugation [14].

Figure 2 shows the chromatograph of five standards, and according to the retention time and the UV spectrum of the standards we can identify the substance. From Table 2, it was obvious that the antioxidant activity of the epicatechin part was weaker than the standards, for there were two substances in the separation except epicatechin. One absorbed at 254 nm and the other, at 280 nm, and their retention times were different from that of quercetin and epicatechin; these two substances may weaken the total antioxidant activity together with epicatechin. The Fe-reducing ability of the chlorogenic acid part was also by weaker than the standard while its DPPH scavenging activity was stronger than that of the standard, for there was a substance in the mixture that absorbed at 325 nm and the retention time of which was different from chlorogenic acid, which may by acidic phenol and may weaken the Fe-reducing ability of the chlorogenic acid in the mixture. It was reported that caffeic acid was found in apples [15]. So the acid phenol in acid separations may be caffeic acid.

TABLE 2. EC1 and TEAC Value of Different Separations from Different Fruits and Vegetables

Compound	EC1, mg/L		EC50, mg/L	
	sample	standard	sample	standard
		Apple		
Epicatechin	7.5622	3.4839	21.6484	8.5714
Chlorogenic acid	4.9065	4.4502	7.9982	18.0952
Procyanidin	0.5044		1.17395	
		Pear		
Epicatechin	11.1762	3.4839	23.3286	8.5714
Chlorogenic acid	3.7914	4.4502	5.488	18.0952
Procyanidin	0.6342		0.8693	
		Grape		
Epicatechin	19.9574	3.4839	16.1518	8.5714
Chlorogenic acid	32.4776	4.4502	8.9547	18.0952
Procyanidin	0.9315		0.6491	
		Onion		
Quercetin	1.2494	9.5225	13.0913	6.337
		Tomato		
Chlorogenic acid	0.9397	4.4502	10.6796	18.0952

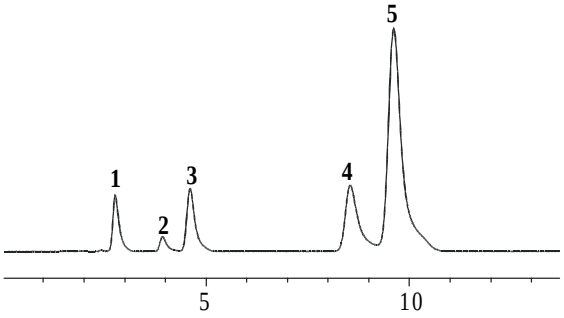

Fig. 2. Chromatograph (280 nm) of five standards. **1** – Epicatechin; **2** – Chlorogenic acid; **3** – Phlorezin; **4** – Quercetin; **5** – Phloretin. Conditions of HPLC: 0.8 mL/min methanol aqueous solution (55:45, v/v), SPD detector.

The antioxidant ability of the chlorogenic acid separation of pear was stronger than the standard, for there was also another substance in the separation. It absorbed at 325 nm and the retention time was different from that in the apple chlorogenic acid separation. It may improve the antioxidant ability of chlorogenic acid separation. The antioxidant ability of epicatechin separation was weaker than that of the standard. In the mixture there were two substances except epicatechin. One absorbed at 254 nm and the retention time was different from that in the apple epicatechin separation, and the other was the same as that in the chlorogenic acid separation at 325 nm. So the substance absorbing at 254 nm may weaken the antioxidant activity of the mixture. Pear extract was also found to contain quercetin-3-glucoside (2.56 mg/100 g FW) [4], which may be the substance in the epicatechin separation.

In the acidic separation of grape, there was no chlorogenic acid which was accordance with the results reported by Brigida. Grape constitutes a particular case since the hydroxy acid that esterifies the cinnamic acid is not quinic but tartaric,

a fairly rare acid in fruits [16]. The Fe-reducing ability of the acidic separation was weaker than that of the standard, while the DPPH scavenging ability of the chlorogenic acid separation was stronger than that of the standard.

The antioxidant capacity of the chlorogenic acid part of tomato was stronger than that of the standard, for the substance existed in the complex, which also absorbed at 325 nm, and the retention time was 8.348 min, the same as that in the pear chlorogenic acid separation.

The Fe-reducing ability of onion separation was stronger than that of the standard while the DPPH activity was weaker than the standard, for there was a substance which also absorbed at 254 nm but had a different retention time from quercetin, and this may improve the Fe-reducing ability of the quercetin separation. Quercetin-3,4′-diglucoside (34.5 mg/ 100 g FW) and quercetin-4′-glucoside (27.5 mg/100 g FW) were the major phenolic compounds found in onion extracts, together with traces of quercetin-3-glucoside (0.8 mg/100 g FW) [8]. So it may be quercetin conjugates.

EXPERIMENTAL

Plant Materials. Pear used in the experiment was obtained from Hebei province, China, and apple, grape, tomato, and onion were got from Shandong province, China.

Standards. The following standards were used: epicatechin, phloretin, phlorezin, quercetin, chlorogenic acid, TPTZ, DPPH all from Sigma Chemical Co. (St. Louis, MO, USA), and ethanol, V_C, $FeSO_4 \cdot 7H_2O$ were from Beijing Chemical Co. and are of analytic quality.

HPLC Equipment. The samples were injected into a Shimadzu VP-ODS C_{18} (4.6 × 150 mm, 5 μm) column at 30°, eluted by methanol aqueous solution (55:45, v/v) with a flow rate of 0.8 mL/min, and detected with a SPD-M10A *vp* detector.

Extraction of Polyphenols. The fruits and vegetables were crashed into a pulp in a blender together with V_C and extracted with ethanol (2 × 100 mL) at room temperature. The complex was centrifuged (3600 g, 5 min) and the supernatants were collected in a volumetric flask and the final volume was made up to 250 mL with ethanol.

Samples. The two columns were preconditioned to a neutral column and an acidic column separately. The neutral one was preconditioned by deionized water (pH 7.0) and the acidic one by 2 mol/L HCl. The extracts were adjusted to pH 7.0 by 1 mol/L NaOH solution and then absorbed on the neutral column. Deionized water (pH 7.0) was used to elute the neutral column and the collection was transferred to the acidic solution by 2 mol/L HCl and finally absorbed on the acidic column. After the neutral column was eluted by deioned water (pH 7.0), ethanol and deionized water were used together in different concentrations (0~100% ethanol solution) to get part 1 – mainly of epicatechin. Then 60% acetone solution was used to elute part 2 – primary of procyanidin. On the acidic column, ethanol was used to elute part 3 – chlorogenic acid part. All these samples were stored at –20° for later analysis.

Ferric Reducing/Antioxidant Power (FRAP) Assay. The antioxidant capacity of each standard (aqueous or methanolic solutions) and sample were estimated according to the procedure described by Benzie and Straim [17] and Pulido [18].

Free Radical Scavenging Activity on DPPH. The free radical scavenging activity of three samples was measured using the method of Sang [19] and Lu [20].

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REFERENCES

1. U. Imeh and S. Khokhar, *J. Agric. Food Chem.*, **50**, 6301 (2002).
2. M. G. L. Hertog, P. C. H. Hollman, and M. B. Katan, *J. Agric. Food Chem.*, **40**, 2379 (1992).
3. M. Ohnishi and H. Morishtta, *Phytochemistry.*, **36**, 3, 579 (1994).
4. F. Nanjo, K. Goto, and R. Seto et al., *Free Radic. Biol. Med.*, **21**, 6, 895 (1996).

5. Y. Kono, K. Kobayashi, S. Tagawa, K. Adachi, A. Ueda, Y. Sawa, and H. Shibata, *Biochim. Biophys. Acta*, **1335**, 335 (1997).
6. H. Shibata, Y. J. Sakanoto, M. Oka, and Y. Kono, *Biosci. Biotechnol. Biochem.*, **63,** 7, 1295 (1999**).**
7. Sh. Sang, Sh. Tian, and H. Wang et al., *Bioorg. Med. Chem.*, **11**, 3371 (2003).
8. A. R. Proteggente, S. P. Ananth, and G Paganga, *Free Radic Res.*, **36,** 2, 217 (2002).
9. K. Schlesier, M. Harwat, V. Bohm, and R. Bitsch, *Free Radic. Res.*, **36**, 2, 177 (2002).
10. K. Charanjit and C. K. Harish, *J. Food Sci. Tech*., **37**, 153 (2002).
11. H. Wang, G. H. Cao, and R. L. Prior, *J. Agric. Food Chem.*, **44**, 701 (1996).
12. K. W. Lee, Y. J. Kim, D. O. Kim, H. J. Lee, and C. Y. Lee, *J. Agric. Food Chem.*, **51**, 6516 (2003).
13. J. H. Weisburger, *Food chem. Toxicol*., **37**, 943 (1999).
14. B. Shi and Y. Di, *Plant Polyphenols*, Science Press, Beijing, 2000, p. 128.
15. H. Leontowicz, S. Gorinstein, A. Lojek, M. Leontowicz, M. Ciz, S. F. Robert, Y. S. Park, S. T. Jung, S. Trakhtenberg, and M. B. Olga, *J. Agric. Food Chem.*, **13**, 603 (2002).
16. B. F. de Simon, P. I. Javier, T. Hernandez, G. C. Carmen, and I. Estrella, *J. Agric. Food Chem.*, **40**, 1531 (1992).
17. I. F. Benzie and J. J. Strain, *Anal Biochem.*, **239**, 70 (1996).
18. R. Pulido, L. Bravo, and F. Saura-Calixto, *J. Agric. Food Chem*., **48**, 3396 (2000).
19. S. M. Sang, Xiaofang, and E. Scheng Ruth, *Bioorg. Med. Chem.*, **10**, 2233 (2002).
20. Y. R. Lu and L. Yeap Foo, *Food Chem.*, **68**, 81 (2000).