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Antioxidant capacities of individual and combined phenolics in a model system

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Abstract

In order to understand how interaction of individual phenolics contributes to the total antioxidant capacity, we quantitatively measured antioxidant capacity of various phenolics in different combinations, using ABTS radical-scavenging ability in a model system. Selected phenolics included in this study were those often found in fruits and vegetables, such as catechin, chlorogenic acid, cyanidin, cyanidin 3-glucoside, cyanidin 3-rutinoside, epicatechin, peonidin, peonidin 3-glucoside, quercetin, quercetin 3-glucoside, quercetin 3-galactoside and quercetin 3-rutinoside. Individual phenolics showed their characteristic antioxidant capacities, while the mixtures, with two or three phenolics combined revealed that the summation of antioxidant capacities of individual phenolics led to total antioxidant capacity. Therefore, it can be concluded that there was no synergistic effect among the phenolics studied. Only an additive effect of antioxidant capacity was observed.

Keywords: ABTS radical; Antioxidant capacity; Additive effect; Phenolics

1. Introduction

Antioxidants can prevent or delay the oxidation process caused by free radicals and reactive oxygen species (ROS) in foods and biological systems. Phenolics, as secondary plant metabolites, are commonly found in various fruits and vegetables and they have been shown to give a defence against oxidative stress from endogenous ROS and free radicals (Choi et al., 2005; Kim & Chung, 2002). Phenolics show various biological properties, such as antioxidative, antiproliferative, antibacterial, antiinflammatory and antiallergic effects (Kim & Kim, 2006; Liu, 2003; Park et al., 2001; Stratil, Klejdus, & Kubáò, 2006). Total antioxidant capacity in fruits, vegetables and their processing products is attributed to three different types of interaction, namely synergistic effect (Cirico & Omaye, 2006; Hsu et al., 2005; Shao et al., 2004; Vattem, Jang, Levin, & Shetty, 2006; Vinson, Su, Zubik, & Bose, 2001; Wei, Zhou, Cai, Yang, & Liu, 2006), negative synergism (Pinelo, Manzocco, Nuñez, & Nicoli, 2004; Wang, Weng, & Cheng, 2000) and additive effect (Philpott, Gould, Lim, & Ferguson, 2004). A synergistic increase of antioxidant capacity was reported between vitamin C and vitamin E (Han, Yi, & Shin, 1991; Moison, Doerga, & van Henegouwen, 2002). Synergism was revealed by the addition of vitamin E to the antioxidants of malt rootlets (Peyrat-Maillard, Bonnely, Rondini, & Berset, 2001) and by the regeneration of vitamin E with flavan-3-ols (Zhou, Wu, Yang, & Liu, 2005; Zhu, Huang, Tsang, & Chen, 1999). Vitamin E is not a major phenolic compound in fruits and vegetables. Vitamin C in fruits contributes less than 15% of total antioxidant capacity (Lee, Kim, Kim, Lee, & Lee, 2003; Wang,

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Cao, & Prior, 1996). Therefore, we can consider phenolics as major contributors to the total antioxidant capacity of fruits.

Phenolics selected in this study were chlorogenic acid, cyanidin, cyanidin 3-glucoside, cyanidin 3-rutinoside, peonidin, peonidin 3-glucoside, quercetin, quercetin 3-glucoside, quercetin 3-galactoside, and quercetin 3-rutinoside, based on the previous reports (Chun, Kim, Moon, Kang, & Lee, 2003; Kim, Chun, Kim, Moon, & Lee, 2003). Catechin and epicatechin were also selected because they are building blocks for the synthesis of condensed tannins (Khanbabaee & van Ree, 2001) and that they have been analyzed in different plum cultivars (de Pascual-Teresa, Santos-Buelga, & Rivas-Gonzalo, 2000; Tomás-Barberán et al., 2001). To evaluate the radical-scavenging capacity of various antioxidants, such as individual phenolics, phenolic extracts, vitamins and synthetic preservatives, ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) radical chromogen has been used as an reliable marker (Choi, Lee, Chun, Lee, & Lee, 2006; Kim et al., 2003; Siddhuraju, 2006; Tarozzi et al., 2006). Chemical explanation of what type of interactions of individual phenolics influences total antioxidant capacity in fruits may provide further understanding of the contribution of individual phenolics to total antioxidant capacity, measured by chemical assay using the ABTS radical in the previous reports (Chun et al., 2003, 2005; Kim et al., 2003).

The objectives in this study were to evaluate the antioxidant capacity, (measured by using ABTS radical), of selected individual phenolics at various concentrations, to quantitatively investigate the effect of a model system, mixed with a few selected phenolics, on total antioxidant capacity, and finally to determine what type of interaction contributes to total antioxidant capacity in the model mixture of phenolics. All of the selected phenolics in this study were monomeric compounds, which might neglect the effect of the steric hindrance from polymerized phenolics, such as tannins, on antioxidant capacity.

2. Materials and methods

2.1. Chemicals

ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) as diammonium salt, (+)-catechin, chlorogenic acid, epicatechin, quercetin and quercetin 3-rutinoside were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Cyanidin, cyanidin 3-glucoside, cyanidin 3-rutinoside, peonidin, peonidin 3-glucoside, quercetin 3-galactoside, and quercetin 3-glucoside were obtained from Extrasynthese (Genay, France). AAPH (2,2′-azobis(2-amidinopropane)dihydrochloride) was obtained from Wako Chemicals USA, Inc. (Richmond, VA, USA). Vitamin C was purchased from Fisher Scientific (Pittsburgh, PA, USA). All other chemicals used were of analytical grade. Anthocyanidins and anthocyanins were dissolved in 50% (v/v) aqueous methanol containing 1.5% (v/v) hydrochloric acid. The other phenolics were dissolved in 50% (v/v) aqueous methanol.

2.2. Antioxidant capacity assay using ABTS radicals

Antioxidant capacity of individual phenolics was evaluated by colorimetric measurement using blue/green ABTS radical chromogens (Kim et al., 2003), in which decolorization takes place due to electron transfer from phenolics as antioxidants to ABTS radicals. Antioxidant capacity of pure phenolics was expressed as mg vitamin C equivalents/l (mg VCE/l). Each chemical was tested with a minimum of 10 replications. As for antioxidant expression, phenolics (100 mg/l) having 100 mg VCE/l contain the same antioxidant capacity as does vitamin C at 100 mg/l. Phenolics (100 mg/l) with antioxidant capacity above 100 mg VCE/l have higher antioxidant capacity than has vitamin C at 100 mg/l. Total antioxidant capacity of a model mixture of two or three individual phenolics was compared with the summation of the antioxidant capacity of individual phenolics, described as "summated antioxidant capacity" in this study. Synergistic and additive effects between tested compounds were measured using CalcuSyn (ver. 2.0) (Biosoft, Cambridge, UK).

3. Results and discussion

3.1. Antioxidant capacity of selected individual phenolics

Antioxidant capacities of selected phenolics (catechin, chlorogenic acid, cyanidin, cyanidin 3-glucoside, cyanidin 3-rutinoside, epicatechin, peonidin, peonidin 3-glucoside, quercetin, quercetin 3-glucoside, quercetin 3-galactoside and quercetin 3-rutinoside) were evaluated at their various concentrations using blue/green ABTS radical chromogen. Our previous reports showed that these phenolics were found in the selected fresh plums grown in NY State, by HPLC analysis, except catechin and epicatechin (Chun et al., 2003; Kim et al., 2003). Two flavan-3-ols (catechin and epicatechin) are used as building blocks for condensed tannins (oligomeric or polymeric proanthocyanidins) in plants (Khanbabaee & van Ree, 2001) and they were found in different plum cultivars (de Pascual-Teresa et al., 2000; Tomás-Barberán et al., 2001). Therefore, flavan-3-ols were also selected to evaluate their effects on antioxidant capacity.

Levels of antioxidant capacity of individual phenolics at 100 mg/l were in wide ranges, e.g. 76.3 in chlorogenic acid to 245 mg vitamin C equivalents/l in epicatechin (Table 1). The antioxidant activity of phenolics is ascribed to the number and position of substituted hydroxyl or methoxyl groups and glycosylation around the flavonoid skeleton (Heim, Tagliaferro, & Bobilya, 2002; Montoro, Braca, Pizza, & De Tommasi, 2005). One methoxyl replacement in the B ring of cyanidin, which caused the absence of a catechol structure, resulted in 55.6% reduction of antioxidant capacity, as in peonidin. Glycosylation

Table 1
Antioxidant capacity of selected individual phenolics (100 mg/l)[a]

Phenolics	Antioxidant capacity
Catechin	216 ± 6.6[b]
Chlorogenic acid	76.3 ± 4.0
Cyanidin	240 ± 6.1
Cyanidin 3-glucoside	144 ± 3.8
Cyanidin 3-rutinoside	114 ± 3.7
Epicatechin	245 ± 6.2
Peonidin	134 ± 2.9
Peonidin 3-glucoside	106 ± 6.5
Quercetin	229 ± 5.1
Quercetin 3-glucoside	112 ± 2.2
Quercetin 3-galactoside	128 ± 3.3
Quercetin 3-rutinoside	88.7 ± 1.6

[a] Antioxidant capacity is expressed as mg vitamin C equivalents/l.
[b] The data are presented as means ± standard deviation ($n \geqslant 10$).

onto flavonoid aglycones led to decrease of antioxidant capacity. Antioxidant capacity of individual phenolics, such as catechin, chlorogenic acid, cyanidin 3-rutinoside and quercetin 3-rutinoside, showed a dose response at various concentrations tested in this study (data not shown).

3.2. Effect of the combination of two phenolics on total antioxidant capacity

First, the simple combination of two phenolics was used to evaluate the effect of phenolic mixtures on total antioxidant capacity. Chlorogenic acid was combined with the other eleven phenolics, with each phenolic at 100 mg/l. Chlorogenic acid was used as a base compound since it was one of the most abundant phenolics in foods such as apple, plum, and coffee (Chun et al., 2003; Clifford, 2000; Kim et al., 2003; Lee et al., 2003). To assess how phenolic interactions contribute to total antioxidant capacity in the model mixture of two phenolics, chlorogenic acid was added to the other phenolics and then antioxidant capacity was analyzed by the ABTS radical. Total concentrations of mixtures with two phenolics were at 200 mg/l of phenolics. Fig. 1 shows the effect of addition of chlorogenic acid to the other eleven phenolics on total antioxidant capacity. The addition of chlorogenic acid to cyanidin, peonidin 3-glucoside, quercetin and quercetin 3-galactoside caused a decrease of antioxidant capacity compared to the summated antioxidant capacity, whilst the other seven phenolics, along with chlorogenic acid, led to increase of the measured total antioxidant capacity. The above results suggested that there was no synergistic effect, by the addition of chlorogenic acid to various (eleven) phenolics, on total antioxidant capacity of the mixture of two phenolics (data not shown).

To further investigate the interaction on antioxidant capacity in two phenolic mixtures, chlorogenic acid had various combinations with catechin, cyanidin 3-rutinoside and quercetin 3-rutinoside at different concentrations. Phenolic combinations in Table 2 led to various concentration ratios of two phenolics in the model mixtures. At 100 mg/l of chlorogenic acid, total antioxidant capacity measured in the combinations with catechin, cyanidin 3-rutinoside and quercetin 3-rutinoside increased from 0.1% (20 mg quercetin 3-rutinoside/l) to 18.6% (5 mg catechin/l), compared to antioxidant capacity on the basis of calculation. At 50 mg/l of chlorogenic acid, the measured total antioxidant

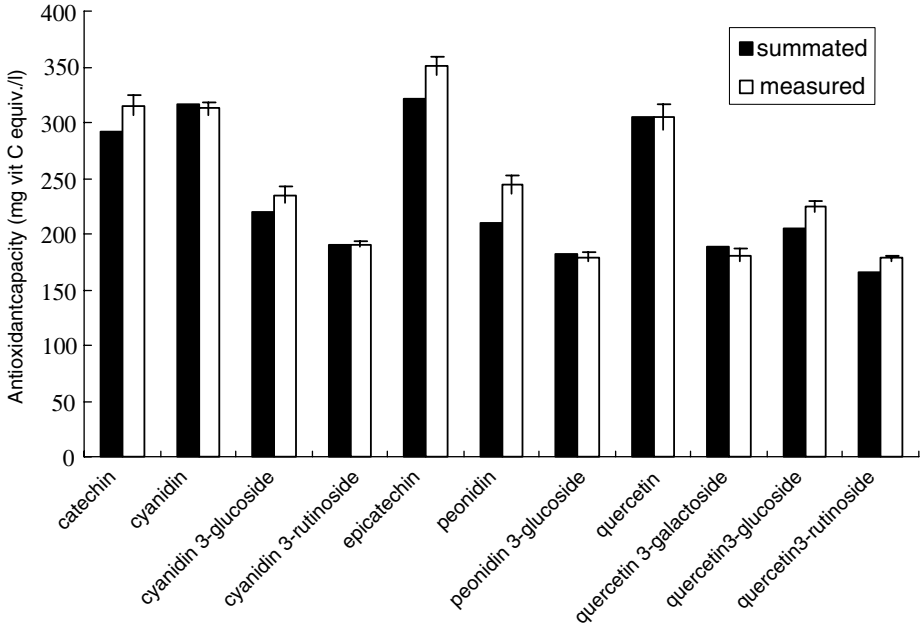

Fig. 1. Effects of addition of the selected phenolics (100 mg/l) to chlorogenic acid (100 mg/l) on antioxidant capacity. Summated antioxidant capacity denoted the sum of antioxidant capacities of individual phenolics in the mixture, which was presented in Table 1. Measured antioxidant capacity represented total antioxidant capacity of the mixture of chlorogenic acid and the other phenolics shown in *x*-axis.

Table 2
Effects of two phenolics, at concentrations with various combinations, on total antioxidant capacity measured[a]

		Chlorogenic acid (mg/l)[b]		
		10	50	100
Catechin (mg/l)[c]	5	19.9 ± 1.5 (20.5)	51.7 ± 1.8 (50.9)	104 ± 4.3 (87.4)
	20	50.9 ± 1.2 (50.0)	87.1 ± 3.1 (80.4)	131 ± 4.4 (117)
	50	117 ± 5.1 (114)	152 ± 5.2 (145)	203 ± 7.6 (181)
	100	217 ± 7.3 (225)	263 ± 13.7 (255)	316 ± 9.5 (292)
Cyanidin 3-rutinoside (mg/l)[d]	5	14.9 ± 0.4 (15.2)	48.3 ± 1.4 (45.6)	89.9 ± 4.9 (82.1)
	20	33.3 ± 2.7 (32.4)	64.4 ± 2.3 (62.8)	103 ± 2.8 (99.3)
	50	66.5 ± 1.1 (61.4)	101 ± 2.2 (91.8)	138 ± 1.3 (128)
	100	116 ± 2.8 (123)	150 ± 3.8 (154)	191 ± 2.5 (190)
Quercetin 3-rutinoside (mg/l)[e]	5	14.9 ± 1.1 (15.4)	46.1 ± 0.8 (45.8)	84.6 ± 4.1 (82.3)
	20	31.6 ± 1.4 (30.3)	68.1 ± 2.6 (60.7)	97.3 ± 7.1 (97.2)
	50	58.2 ± 1.3 (56.5)	92.0 ± 2.5 (86.9)	132 ± 3.0 (123)
	100	97.5 ± 1.8 (98.1)	138 ± 3.0 (129)	179 ± 2.5 (165)

[a] Antioxidant capacity is expressed as mg vitamin C equivalents/l. Data are presented as means ± standard deviation of antioxidant capacity measured in ten replications. The values in parentheses are the summations of antioxidant capacities of individual phenolics at corresponding concentrations.
[b] Antioxidant capacities of chlorogenic acid at 10, 50, and 100 mg/l were 9.4 ± 1.6, 39.8 ± 0.9, and 76.3 ± 4.0, respectively.
[c] Antioxidant capacities of catechin at 5, 20, 50, and 100 mg/l were 11.1 ± 2.8, 40.6 ± 1.6, 105 ± 5.3, and 216 ± 6.6, respectively.
[d] Antioxidant capacities of cyanidin 3-rutinoside at 5, 20, 50, and 100 mg/l were 5.8 ± 0.2, 23.0 ± 1.1, 52.0 ± 0.9, and 114 ± 3.7, respectively.
[e] Antioxidant capacities of quercetin 3-rutinoside at 5, 20, 50, and 100 mg/l were 6.0 ± 1.1, 20.9 ± 2.3, 47.1 ± 4.4, and 88.7 ± 1.6, respectively.

capacity, except for the combination with cyanidin 3-rutinoside at 100 mg/l, was slightly higher than the summated antioxidant capacity. At 10 mg/l of chlorogenic acid, six combinations showed 1.8–8.3% increase of antioxidant capacity, whereas the other six combinations showed 0.6–5.5% of antioxidant capacity reduction. The above results indicated that the measured total antioxidant capacity of two phenolic mixtures with various concentration ratios resulted from the additive effect of individual phenolics, like the model mixtures at a total 200 mg/l of phenolics shown in Fig. 1.

3.3. Effect of the combination of three phenolics on total antioxidant capacity

Total antioxidant capacity in different combinations was measured in a model mixture with three selected phenolics, including chlorogenic acid, cyanidin 3-rutinoside and quercetin 3-rutinoside (Table 3), in order to understand how phenolic interaction influences the total antioxidant capacity. These three phenolics were the principal compounds representing each phenolic subclass in plums (Chun et al., 2003; Kim et al., 2003). The concentrations of combined

Table 3
Effects of the mixtures of chlorogenic acid, cyanidin 3-rutinoside, and quercetin 3-rutioside, at concentrations with various combinations, on total antioxidant capacity measured[a]

Cyanidin 3-rutinoside (mg/l)[b]	Quercetin 3-rutinoside (mg/l)[c]	Chlorogenic acid (mg/l)[d]		
		10	50	100
5	5	19.1 ± 0.6 (21.2)	51.1 ± 0.8 (51.6)	90.7 ± 1.6 (88.1)
20	5		71.3 ± 1.0 (68.8)	109 ± 1.6 (105)
	20		83.5 ± 1.3 (83.7)	122 ± 2.2 (120)
50	5		103 ± 1.4 (97.8)	142 ± 2.4 (134)
	20		115 ± 1.5 (113)	150 ± 4.7 (149)
	50		135 ± 2.0 (139)	181 ± 5.0 (175)
100	5			202 ± 5.4 (196)
	20			210 ± 6.2 (211)
	50			246 ± 3.8 (237)
	100			289 ± 5.6 (279)

[a] Antioxidant capacity is expressed as mg vitamin C equivalents/l. Data are presented as means ± standard deviation of antioxidant capacity measured in ten replications. The values in parentheses are the summation of antioxidant capacities of individual phenolics at corresponding concentrations.
[b] Antioxidant capacities of cyanidin 3-rutinoside at 5, 20, 50, and 100 mg/l were 5.8 ± 0.2, 23.0 ± 1.1, 52.0 ± 0.9, and 114 ± 3.7, respectively.
[c] Antioxidant capacities of quercetin 3-rutinoside at 5, 20, 50, and 100 mg/l were 6.0 ± 1.1, 20.9 ± 2.3, 47.1 ± 4.4, and 88.7 ± 1.6, respectively.
[d] Antioxidant capacities of chlorogenic acid at 10, 50, and 100 mg/l were 9.4 ± 1.6, 39.8 ± 0.9, and 76.3 ± 4.0, respectively.

mixtures of three phenolics were in wide ranges, 20–300 mg/l, covering various concentration ratios in the mixtures. The measured total antioxidant capacity of mixtures with three phenolics was shown to be decreased by 9.9% (5 mg cyanidin 3-rutinoside, 5 mg quercetin 3-rutinoside, and 10 mg chlorogenic acid) and increased by 5.5% (50 mg cyanidin 3-rutinoside, 5 mg quercetin 3-rutinoside and 100 mg chlorogenic acid) on the basis of the summation of individual antioxidant capacity of three phenolics. Like the combined mixtures of two phenolics, there was no difference between total antioxidant capacity measured in a mixture and the summation of antioxidant capacities of its individual phenolics. This suggested that total antioxidant capacity was equal to the summation of individual antioxidant capacity of phenolics.

Fig. 2 shows correlation between the summation of each antioxidant capacity of phenolics and the total antioxidant capacity measured on the various mixtures of two or three phenolics. The first order of regression analysis between the total antioxidant capacity measured of phenolic mixtures and the summation of antioxidant capacity of individual phenolics had a slope of 1.037, a y-intercept of 0.203, and correlation coefficient (r^2) of 0.991, the values of which were obtained from sixty one discrete data points from Tables 2, 3 and Fig. 1. Although total antioxidant capacity, measured on various mixtures, was slightly higher (3.7%), based on the slope (1.037), than the summation of antioxidant capacity of individual phenolics at the known concentration, it seems that the total antioxidant capacity can be estimated without antioxidant capacity assay using ABTS radical, if the concentration of each phenolic could be estimated in complex systems (i.e., phenolic extracts of fruits and vegetables). Total antioxidant capacity in the phenolic mixture was almost equal to the sum of antioxidant capacity of its individual phenolics. Indeed, anthocyanins in sweet potato had an additive effect with hydroxycinnamic acids on antioxidant capacity (Philpott et al., 2004).

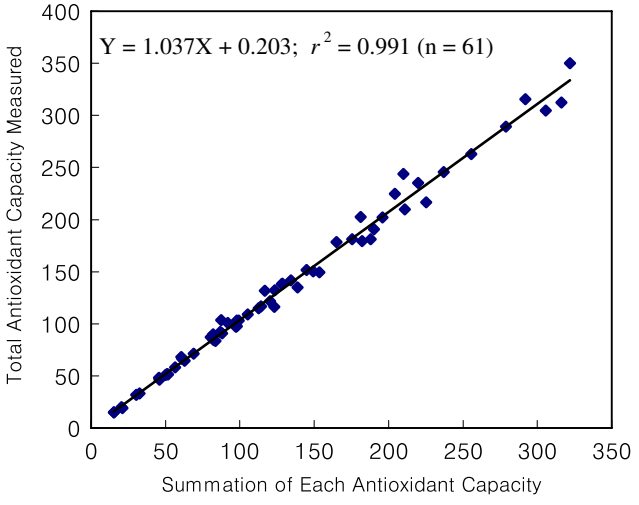

Fig. 2. Relationship between the summation of antioxidant capacities of individual phenolics and the total antioxidant capacity measured on mixtures of two or three phenolics. (Antioxidant capacity is expressed as mg vitamin C equivalents/l.)

In conclusion, individual phenolics showed characteristic antioxidant capacities. To investigate how phenolic interactions contribute to the total antioxidant capacity, we quantitatively measured the level of antioxidant capacity of various phenolics in different combinations using blue/green ABTS radical-scavenging ability in a model system. Total antioxidant capacities measured, of the phenolic mixture at known concentrations, were equal to the summation of antioxidant capacities of individual phenolics, indicating that there was no synergistic effect among the phenolics we studied, but only an additive effect of antioxidant capacity was observed. Also, this feature of antioxidant capacity assay, using ABTS radicals, suggested that total antioxidant capacity in complex extracts of phenolics from fruits and vegetables could be estimated if the concentrations of individual phenolics and their own antioxidant capacities analyzed by using ABTS radicals were known.

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