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Contribution of Individual Polyphenolics to Total Antioxidant Capacity of Plums

OCK KYOUNG CHUN, DAE-OK KIM, HAE YEON MOON,† HEE GON KANG,‡ AND CHANG YONG LEE*

Department of Food Science and Technology, Cornell University, Geneva, New York 14456

To study the effect of polyphenolics on antioxidant capacities of plums, the amounts of total phenolics, total flavonoids and individual phenolic compounds, and vitamin C equivalent antioxidant capacity (VCEAC) of eleven plum cultivars was determined. There was a good linear relationship between the amount of total phenolics and total antioxidant capacity ($r^2 = 0.9887$). The amount of total flavonoids and total antioxidant capacity also showed a good correlation ($r^2 = 0.9653$). Although the summation of individual antioxidant capacity was lower than the total antioxidant capacity of plum samples, there was a positive correlation ($r^2 = 0.9299$) of total antioxidant capacity of plum samples with the sum of the VCEACs calculated from individual phenolics. Chlorogenic acids and glycosides of cyanidin, peonidin, and quercetin were major phenolics among eleven plum cultivars. The antioxidant capacity of chlorogenic acids and anthocyanins showed higher correlation (r^2) of 0.7751 and 0.6616 to total VCEAC, respectively, than that of quercetin glycosides ($r^2 = 0.0279$). Chlorogenic acids were a major source of antioxidant activity in plums, and the consumption of one serving (100 g) of plums can provide antioxidants equivalent to 144.4−889.6 mg of vitamin C.

KEYWORDS: Plums; phenolics; flavonoids; HPLC; vitamin C equivalent antioxidant capacity (VCEAC); ABTS•-; chlorogenic acid

INTRODUCTION

There is strong evidence to indicate that free radicals cause oxidative damage to lipids, proteins, and nucleic acids, and that high intake of fruits and vegetables has been associated with lower incidences of chronic diseases such as cancer and heart disease (*1*−*5*). In addition to the vitamins and minerals in fruits and vegetables, phytochemicals such as flavonoids and other phenolics may contribute to this protective effect. Many of these phytochemicals have antioxidant activity and may help protect cells against the oxidative damage caused by free radicals (*6*). Plant polyphenolics are known to have multifunctional properties such as reducing agents, hydrogen donating antioxidants, and singlet oxygen quenchers. Flavonoids and their derivatives are the largest and most important group of plant polyphenolics (*7*), which have shown various biological effects including inhibition of low-density lipoprotein (LDL) oxidation, inhibition of human immunodeficiency virus type 1 protease, and antimicrobial and anticarcinogenic activities (*8*−*11*).

Plums are the fruits of the genus *Prunus* in the Rosaceae family and have been proven to have effective antioxidant activity that resulted from polyphenolics (*12*−*17*). It was reported that neochlorogenic acid (3-*O*-caffeoylquinic acid, 3-CQA) was a major hydroxycinnamate in the fresh or dried plum (*18*), and chlorogenic acid (5-*O*-caffeoylquinic acid, 5-CQA) and cryptochlorogenic acid (4-*O*-caffeoylquinic acid, 4-CQA) were often found at lower concentration (*15*, *18*, *19*).

Chlorogenic acid is widely recognized as an antioxidant for human LDL (*20*, *21*). It is also known as a scavenger for reactive oxygen and nitrogen species (*22*) and an inhibitor against formation of conjugated diene from linoleic acid oxidation (*23*). It was reported that each chlorogenic acid isomer showed almost the same antioxidant activities (*15*). Antioxidant activities of various phenolics, vitamins, and food additives in relation to their specific chemical structures have been reported (*21*, *24*, *25*). Although plums are known to contain various kinds of phenolic compounds, including hydroxycinnamic acids, flavonols, and anthocyanins, the specific information on their contribution to total antioxidant capacity of plums is limited.

The objectives of this study were to quantify individual phenolics in various plum cultivars and to determine the contribution of individual polyphenolics on total antioxidant capacity of plums by using ABTS radical anion scavenging activity assay.

MATERIALS AND METHODS

Chemicals. 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) as diammonium salt, ammonium hydroxide, (+)-catechin, chlorogenic acid, Folin-Ciocalteu's phenol reagent, gallic acid, quer-

* To whom correspondence should be addressed. Tel.: +1-315-787-2271. Fax: +1-315-787-2284. E-mail: cyl1@cornell.edu.
† On leave from Division of Food, Biological and Chemical Engineering, Daegu University, Gyeongbuk 712-714, Korea.
‡ Current address: Seoul Health and Environment Research Institute, Seoul 137-130, Korea.

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cetin, quercetin-3-galactoside (hyperoside), and quercetin 3-rutinoside (rutin) were purchased from Sigma Chemical Co. (St. Louis, MO). Vitamin C was obtained from Fisher Scientific (Fair Lawn, NJ). Ammonium phosphate monobasic was purchased from Mallinckrodt Baker, Inc. (Phillipsburg, NJ). 2,2-Azobis(2-amidinopropane)dihydrochloride (AAPH) was obtained from Wako Chemicals USA, Inc. (Richmond, VA). Cyanidin, cyanidin 3-glucoside (kuromanin), cyanidin 3-rutinoside (keracyanin), peonidin, peonidin 3-glucoside, and quercetin 3-glucoside were obtained from Extrasynthese (Genay, France). All other chemicals used were analytical grade (Fisher, Springfield, NJ).

Fruits. Eleven plum cultivars (*Prunus domestica* L.: Beltsville Elite B78197, Cacaks Best, Castleton, Early Magic, Empress, Longjohn, Mirabellier, NY 101, NY 6, NY 9, Stanley) used in this study were obtained at commercial maturity between late July and early September 2002 from the New York State Agricultural Experiment Station orchard in Geneva, New York. These cultivars can be categorized into three classes based on their skin colors. Early Magic is red plum, Mirabellier and NY 101 are yellow plums, and the others are dark blue or purple plum (*26*). Immediately after harvest, the plums (water content 67.0−84.6%) were stored in a 2−5 °C refrigerator. Plums were halved, and seeds were removed by hand with care. Plums, cut into several pieces, were frozen and freeze-dried. Samples were ground to powder and then stored at −20 °C until analyzed.

Extraction of Phenolics. The phenolics in plums were extracted from 10 g of ground freeze-dried samples using 80% aqueous methanol in a 250-mL round-bottomed flask by the ultrasound-assisted method (*27*). The mixture of plum powder and aqueous methanol was sonicated for 20 min with continuous nitrogen gas purging. The mixture was filtered and evaporated using a rotary evaporator under reduced pressure at 40 °C. The phenolic concentrate was dissolved in 50 mL of 100% methanol and then made to the final volume of 100 mL with distilled deionized water (ddH_2O) obtained with a NANOpure water system (Barnstead, Dubuque, IA). The solution was then centrifuged in a Sorvall RC-5B refrigerated superspeed centrifuge (Du Pont Company, Biomedical Products Department, Wilmington, DE) at 12000*g* using a GSA rotor for 20 min. The final extract solution was stored at −20 °C until analyzed. Extraction was done in duplicate.

Total Phenolics and Total Flavonoids. Total polyphenolics were determined by the spectrophotometric method (*14*). One mL of appropriately diluted extracts was added to a 25-mL volumetric flask filled with 9 mL of ddH_2O. A reagent blank using ddH_2O instead of sample was prepared. One mL of Folin and Ciocalteu's phenol reagent was added to the mixture and mixed. After 5 min, 10 mL of 7% Na_2CO_3 solution was added with mixing. The solution was diluted to the volume (25 mL) with ddH_2O then allowed to stand for 90 min, and the absorbance was measured at 750 nm versus the prepared blank. Total phenolics of plums were expressed on a fresh weight basis as mg of gallic acid equiv (GAE)/100 g. Samples of each extraction were analyzed in triplicate.

Total flavonoids were measured using a colorimetric assay developed by Zhishen et al. (*28*). One mL of appropriately diluted extracts was added to a 10-mL volumetric flask filled with 4 mL of ddH_2O. A reagent blank using ddH_2O instead of sample was prepared. Five min from the starting time, 0.3 mL of 5% $NaNO_2$ was added to the flask, followed by 0.3 mL of 10% $AlCl_3$. At 6 min, 2 mL of 1 M NaOH was added and diluted to volume (10 mL) with ddH_2O then thoroughly mixed. The absorbance of the pink mixture was measured at 510 nm versus the prepared blank. Total flavonoids of plums were expressed on a fresh weight basis as mg of catechin equiv (CE)/100 g. Samples were analyzed in triplicate.

Identification of Polyphenolics Using HPLC and Acid Hydrolysis. HPLC analysis was performed according to the method of Kim and Lee (*29*). Extracted sample was analyzed using an HPLC system (Hewlett-Packard Model 1100) equipped with quaternary pump and photodiode array detector. The injection volume was 20 μL, and a C18 reverse-phase Symmetry Analytical column (5 μm × 250 mm × 4.6 mm, Waters Co., MA) was used. To separate pH-dependent phenolics, three mobile phases were used: solvent A, 50 mM ammonium phosphate monobasic ($NH_4H_2PO_4$), adjusted to pH 2.6 with *o*-phosphoric acid; solvent B, 80:20 (v/v) acetonitrile/solvent A; and solvent C, 0.2 M *o*-phosphoric acid (H_3PO_4) adjusted with ammonium hydroxide to pH 1.5. The flow rate was set to 1.0 mL/min at 23 °C. The total run time was 60 min, and the pumps were operated with linear gradients as follows: 0.0 min at %A = 100.0; 4.0 min at %A = 92.0 and %B = 8.0; 10.0 min at %B = 14.0 and %C = 86.0; 22.5 min at %B = 16.5 and %C = 83.5; 27.5 min at %B = 25.0 and %C = 75.0; 50.0 min at %B = 80.0 and %C =20.0; 55.0 min at %A = 100.0; 60.0 min at %A = 100.0. The detector was set at 320 nm for hydroxycinnamic acids, 370 nm for flavonols and 520 nm for anthocyanins, and the standards were prepared at concentrations of 50, 100, 200, and 400 ng/20 μL to obtain the calibration curves. Each peak of plum extract was identified by comparing retention time and UV−vis spectra given by the photodiode array detector with the standards and by spiking the extract with polyphenolic standards. Due to the unavailability of authentic commercial neochlorogenic acid standard, its identification was accomplished with the comparison of previously reported reversed-phase HPLC separation results (*12*, *15*, *30*) and of its UV−vis spectrum with chlorogenic acid. Chlorogenic acid was identified with the comparison of its retention time, UV−vis spectrum, and with the spiked input of its commercial standard. The amount of neochlorogenic acid was expressed as chlorogenic acid equivalent (CAE) (*12*). Quercetin derivatives and a peonidin derivative were used to express the amount of some derivatives of quercetin and peonidin that could not be identified by their commercial standards but could be confirmed by their aglycones after acid hydrolysis.

Acid hydrolysis was used to identify aglycones of unknown flavonols and anthocyanins (*31*). The peak that showed the same UV−vis spectrum with an agycone but had different retention time in HPLC analysis was collected repeatedly from analytical column, added with 1.2 N HCl, and refluxed in a 90 °C water bath for 2 h. The resulting acid hydrolyzate was analyzed by HPLC to identify the aglycone of the unknown peak.

Total Antioxidant Capacity Expressed by VCEAC. The method developed by Kim et al. (*32*) was used in this study. One mM AAPH was mixed with 2.5 mM ABTS as diammonium salt in phosphate buffered saline (PBS) solution (100 mM potassium phosphate buffer (pH 7.4) containing 150 mM NaCl). After the mixture was heated in a water bath at 68 °C for 13 min, the blue-green ABTS•- solution was adjusted with fresh PBS solution to an absorbance of 0.650 ± 0.020 at 734 nm. Twenty μL of the sample solution added to 980 μL of the ABTS radical solution was incubated in a water bath at 37 °C for 10 min. The decrease of absorbance at 734 nm was measured at 10 min. A control consisted of 20 μL of 50% methanol and 980 μL of ABTS•- solution. The ABTS radical scavenging capacities of plum extracts were expressed on the fresh weight basis as mg of vitamin C equivalent antioxidant capacity/100 g (VCEAC). Samples of each extraction were analyzed in triplicate.

Vitamin C standard curves, 2.5, 5, 10, 20, 40, 60, 80, and 100 mg/L concentrations of L-ascorbic acid, were obtained using the ABTS. The absorbance reduction at 734 nm of ABTS•- solution by selected pure polyphenolics (chlorogenic acid, quercetin, quercetin 3-galactoside, quercetin 3-glucoside, quercetin 3-rutinoside, cyanidin, cyanidin 3-glucoside, cyanidin 3-rutinoside, peonidin, and peonidin 3-glucoside) was measured at concentrations of 2.5, 5, 10, and 20 mg/L and expressed as VCEAC using the vitamin C standard curve.

RESULTS AND DISCUSSION

Total Phenolics and Total Flavonoids. Table 1 shows the amount of total phenolics in eleven plum cultivars expressed as gallic acid equivalent (GAE) on a fresh weight basis. Beltsville Elite B78197 exhibited the highest content of total phenolics of 684.5 ± 2.6 mg/100 g of fresh sample, which was followed by Cacaks Best, Longjohn, Empress, Castleton, Stanley, Mirabellier, NY 101, Early Magic, NY 6, and NY 9. The average content of total phenolics was 311.8 mg/100 g of fresh weight. This result was much higher than that of Gil et al. (*13*) on several plums cultivated in California (42.0−109.2 mg/100 g) and a little higher than that of Kim et al. (*14*) on six plum cultivars in 2000 from New York (173.9−374.6 mg/100 g).

Table 1. Total Phenolics, Total Flavonoids, and VCEACs of Plum Cultivars on the Fresh Sample Basis[a]

cultivars[b]	total phenolics (mg GAE[c]/100 g)	total flavonoids (mg CE[d]/100 g)	VCEAC (mg VCE[e]/100 g)
BE	684.5 ± 2.6	366.0 ± 10.0	889.6 ± 3.2
CB	571.7 ± 7.6	341.6 ± 9.0	805.3 ± 5.0
CT	250.5 ± 1.6	152.1 ± 6.5	337.8 ± 13.2
EM	192.1 ± 5.6	100.9 ± 6.4	251.2 ± 6.5
EP	398.7 ± 8.8	227.4 ± 4.9	524.8 ± 3.9
LJ	398.9 ± 8.4	226.5 ± 5.5	550.4 ± 11.1
MB	215.7 ± 2.9	95.4 ± 8.0	171.1 ± 6.6
NY 101	196.1 ± 1.5	98.5 ± 6.5	216.2 ± 3.2
NY 6	146.6 ± 1.0	71.4 ± 3.1	154.9 ± 6.6
NY 9	138.1 ± 2.9	59.3 ± 4.6	144.4 ± 11.2
ST	236.7 ± 4.5	140.9 ± 9.3	308.0 ± 5.3

[a] Each value is the mean ± SD ($n = 6$). [b] BE stands for Beltsville Elite B78197; CB, Cacaks Best; CT, Castleton; EM, Early Magic; EP, Empress; LJ, Longjohn; MB, Mirabellier; ST, Stanley. [c] GAE stands for gallic acid equiv. [d] CE catechin equiv. [e] VCE vitamin C equiv.

The total flavonoid contents in plums showed the similar tendency with the total phenolics in ranking: Beltsville Elite B78197 of 366.0 ± 10.0 mg catechin equiv (CE)/100 g of fresh sample followed by Cacaks Best, Empress, Longjohn, Castleton, Stanley, Early Magic, NY 101, Mirabellier, NY 6, and NY 9. The average content of the total flavonoids was 170.9 mg CE/100 g of fresh sample. Kim et al. (*14*) reported that the average of the total flavonoid content in six plum cultivars from the 2000 crop was 121.4 mg CE/100 g fresh weight. As many other constituents, the amounts of total phenolics and total flavonoids in fruits and vegetables may be influenced by cultivars, geographic origin, growing seasons, agricultural practices, and analytical methods (*13*, *33*, *34*). According to the weather data of Northeast Weather Association, New York State Integrated Pest Management Program, the later part of the 2002 growing season in Geneva, NY was exceptionally dry and warmer than usual (*35*).

Identification of Polyphenolics. The major polyphenolics identified in eleven plum cultivars by HPLC analysis were shown in **Table 2**. Neochlorogenic acid was identified by the comparison of previously reported reversed-phase HPLC separation results (*12*, *15*, *30*) and of its UV−vis spectrum with chlorogenic acid. Neochlorogenic acid was eluted prior to chlorogenic acid (data not shown). The UV−vis spectrum of neochlorogenic acid almost totally overlapped with that of authentic chlorogenic acid. The amount of neochlorogenic acid was expressed as chlorogenic acid equivalent (CAE) (*12*). Four quercetin derivatives and a peonidin derivative were used to express the amount of some derivatives from quercetin and peonidin that could not be identified by their aglycones but could be confirmed by acid hydrolysis. **Figure 1** shows chromatograms of anthocyanins in Empress detected at 520 nm before and after acid hydrolysis.

As seen **Table 2**, neochlorogenic acid was the major polyphenol in plum cultivars, and the content was broadly ranged from 19.1 mg/100 g (Early Magic) to 184.6 mg/100 g (Beltsville Elite B78197) on the fresh weight basis. The average content of chlorogenic acids (the summation of chlorogenic acid and neochlorogenic acid) was 71.4% of total phenolics, and that of cyanidin 3-rutinoside was the second highest at 16.9% (data not shown).

In all samples, the amount of neochlorogenic acid was higher than that of chlorogenic acids, as reported in previous studies (*12*, *14*, *15*). In addition, quercetin 3-rutinoside existed in all the plums at relatively low levels (1.0−8.3 mg/100 g). Even though the amount of quercetin 3-rutinoside was relatively low, it was the major flavonol in plums.

All the eleven plum cultivars except yellow plums (Mirabellier and NY 101) commonly contained anthocyanins such as cyanidin 3-glucoside, cyanidin 3-rutinoside, peonidin 3-glucoside and peonidin derivative. This result was in good agreement with that of Tomás-Barberán et al. (*30*). Cyanidin 3-rutinoside was a predominant anthocyanin found in red and blue-purple plums. Cacaks Best had the highest amount of cyanidin 3-rutinoside, which was six times higher than that of NY 6. The amount of peonidin derivative, which was identified by acid hydrolysis and HPLC, was higher than that of peonidin 3-glucoside. The sum of individual flavonoids measured by HPLC followed similar trends of total phenolics and total flavonoids measured by spectrophotometric methods.

Total Antioxidant Capacity Expressed by VCEAC. The total antioxidant capacity of the plum cultivars determined by ABTS radical scavenging activity is shown in **Table 1**. Similarly to the result of total phenolics and total flavonoids, Beltsville Elite B78197 showed the highest antioxidant capacity with 889.6 ± 3.2 mg of vitamin C equivalent (VCE), followed by Cacaks Best, Longjohn, Empress, Stanley, Castleton, Early Magic, NY

Table 2. The Content of Polyphenolics (mg) in Plum Cultivars on the 100-g Fresh Sample Basis[a]

	cultivars[b]										
polyphenolics[c]	BE	CB	CT	EM	EP	LJ	MB	NY 101	NY 6	NY 9	ST
3CQA	184.6 ± 12.0	133.0 ± 7.3	66.8 ± 2.2	19.1 ± 0.1	155.7 ± 13.3	121.8 ± 4.4	44.1 ± 2.4	78.8 ± 6.1	35.5 ± 2.2	28.1 ± 1.7	108.6 ± 4.3
5CQA	10.4 ± 0.7	20.4 ± 1.3	8.0 ± 0.3	3.0 ± 0.0	7.3 ± 0.7	11.5 ± 0.4	15.9 ± 0.9	5.8 ± 0.4	4.3 ± 0.3	3.3 ± 0.3	9.2 ± 0.3
CGL	7.4 ± 0.4	9.8 ± 0.6	14.2 ± 0.3	4.1 ± 0.1	1.4 ± 0.4	7.2 ± 0.2	ND[d]	ND	3.8 ± 0.7	2.6 ± 0.2	3.4 ± 0.1
CRT	33.0 ± 1.9	60.5 ± 3.2	22.1 ± 0.7	23.4 ± 0.5	22.4 ± 2.8	41.1 ± 0.6	ND	ND	8.9 ± 0.5	17.4 ± 1.1	25.5 ± 0.9
PGL	1.5 ± 0.2	0.4 ± 0.0	2.3 ± 0.2	0.3 ± 0.0	ND	0.7 ± 0.0	ND	ND	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.1
PDR	16.2 ± 1.0	7.6 ± 0.4	6.9 ± 0.4	3.1 ± 0.1	3.1 ± 0.4	9.1 ± 0.2	ND	ND	2.1 ± 0.1	7.1 ± 0.4	6.1 ± 0.4
QGA	ND	ND	ND	1.2 ± 0.0	ND	0.7 ± 0.0	0.6 ± 0.0	ND	ND	ND	ND
QGL	ND	ND	0.8 ± 0.0	1.5 ± 0.0	ND	0.4 ± 0.0	0.5 ± 0.0	ND	ND	ND	ND
QRT	6.1 ± 0.4	3.9 ± 0.2	3.8 ± 0.2	8.3 ± 0.1	3.7 ± 0.5	6.2 ± 0.3	7.2 ± 0.4	1.0 ± 0.1	1.4 ± 0.1	2.7 ± 0.2	3.3 ± 0.1
QDR1	ND	ND	ND	ND	ND	0.3 ± 0.0	ND	ND	ND	ND	ND
QDR2	0.4 ± 0.0	0.3 ± 0.0	ND	ND	ND	0.3 ± 0.0	ND	ND	ND	ND	ND
QDR3	ND	ND	ND	1.4 ± 0.0	ND	ND	ND	ND	ND	ND	ND
QDR4	ND	ND	ND	0.4 ± 0.0	ND	ND	ND	ND	ND	ND	ND
total	259.5	235.9	125.0	65.7	193.6	199.3	68.4	85.7	56.3	61.5	156.5

[a] Each value is the mean ± SD ($n = 4$). [b] BE stands for Beltsville Elite B78197; CB, Cacaks Best; CT, Castleton; EM, Early Magic; EP, Empress; LJ, Longjohn; MB, Mirabellier; ST, Stanley. [c] 3CQA stands for 3-*O*-caffeoylquinic acid; 5CQA, 5-*O*-caffeoylquinic acid; CGL, cyanidin 3-glucoside; CRT, cyanidin 3-rutinoside; PGL, peonidin 3-glucoside; PDR, peonidin derivative; QGA, quercetin 3-galactoside; QGL, quercetin 3-glucoside; QRT, quercetin 3-rutinoside; QDR1, quercetin derivative 1; QDR2, quercetin derivative 2; QDR3, quercetin derivative 3; QDR4, quercetin derivative 4. [d] ND stands for not detected.

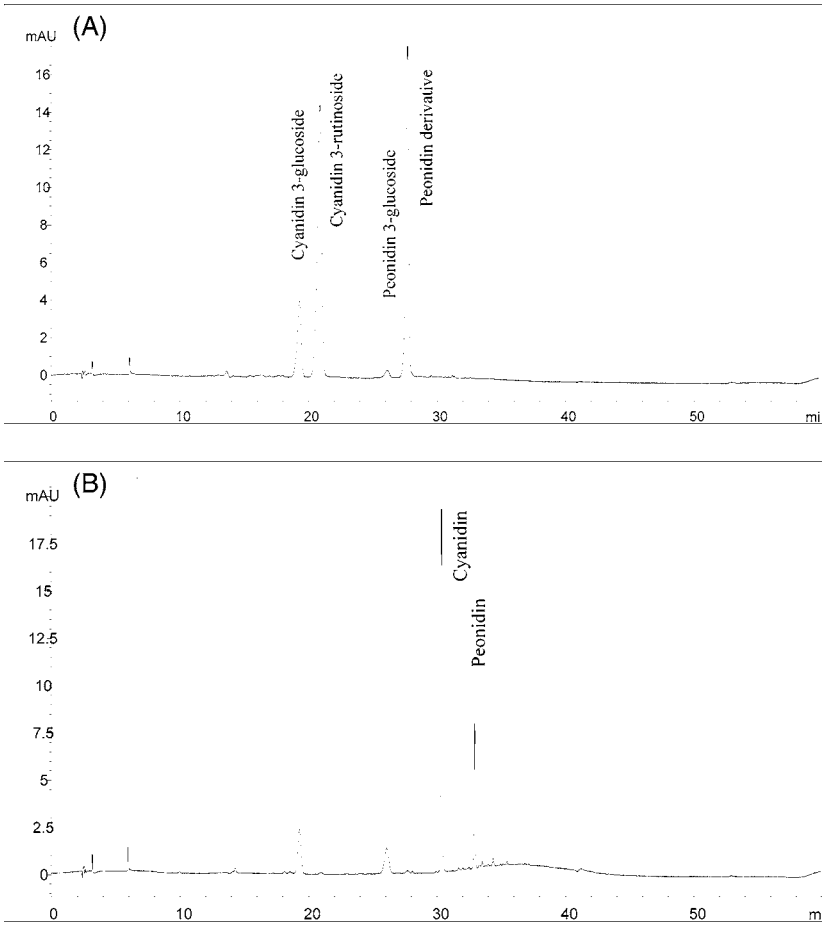

Figure 1. Anthocyanins in Empress detected at 520 nm by HPLC analysis. (A) chromatogram of anthocyanins before acid hydrolysis and (B) chromatogram of anthocyanidins after acid hydrolysis.

101, Mirabellier, NY 6, and NY 9. The average amount of the antioxidant capacity in eleven plum cultivars was 409.4 mg VCE, which is higher than that of Leong and Shui's 312 mg/100 g (*36*). There was a good linear relationship between the amount of total phenolics and total antioxidant capacity ($r^2 = 0.9887$). The amount of total flavonoids and antioxidant capacity also showed a good correlation ($r^2 = 0.9653$) (data not shown). Therefore, polyphenolics may play a major role in the antioxidant capacity in plum.

Polyphenolics were present in plums at various concentrations, so it was necessary to confirm how the antioxidant capacity of each polyphenol changed with concentration. First order of linear regression in relation to the VCEAC versus four concentration levels of 10 phenolic compounds (chlorogenic acid, quercetin, quercetin 3-galactoside, quercetin 3-glucoside, quercetin 3-rutinoside, cyanidin, cyanidin 3-glucoside, cyanidin 3-rutinoside, peonidin, and peonidin 3-glucoside) was attained; the correlation coefficient (r^2) of which was found to be higher than 0.990.

Glycoside forms of anthocyanidins and flavonols, such as cyanidin 3-glucoside, cyanidin 3-rutinoside, peonidin 3-glucoside, quercetin 3-glucoside and quercetin 3-rutinoside, showed a wide range of antioxidant capacity according to their own structural characteristics (*21*, *24*, *25*, *37*). The VCEAC values for each polyphenolic at the various concentration levels are shown in **Figure 2**. Chlorogenic acid showed the lowest antioxidant activity among 10 polyphenolics, whereas cyanidin showed the highest antioxidant activity. Chlorogenic acid is the esterified form of caffeic acid with quinic acid on the carboxyl group, and this esterification resulted in decreased antioxidant

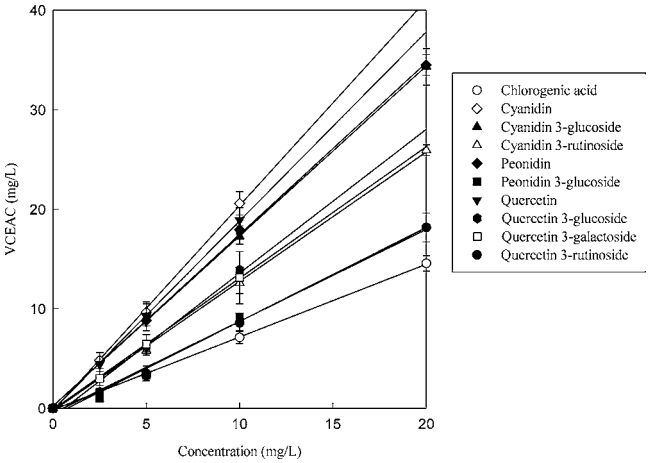

Figure 2. VCEAC by ABTS of 10 polyphenolics identified in eleven plum cultivars.

activity (*38*). Among cyanidins, cyanidin 3-glucoside and cyanidin 3-rutinoside showed decreased antioxidant activity by glycosylation. Peonidin and peonidin 3-glucoside, quercetin and quercetin 3-glucoside, quercetin 3-galactoside, and quercetin 3-rutinoside also showed the same tendency due to their glycosylation. This agreed with the previous studies that reported that the substitution of sugar moieties onto the generic structure of flavonoid generally resulted in the decrease of antioxidant capacity compared to the corresponding aglycone, and the diglycosylation into flavonoid aglycone decreased antioxidant activity more than monoglycosylation (*37*, *39*). This decreased antioxidant capacity may be due to steric hindrance of sugars

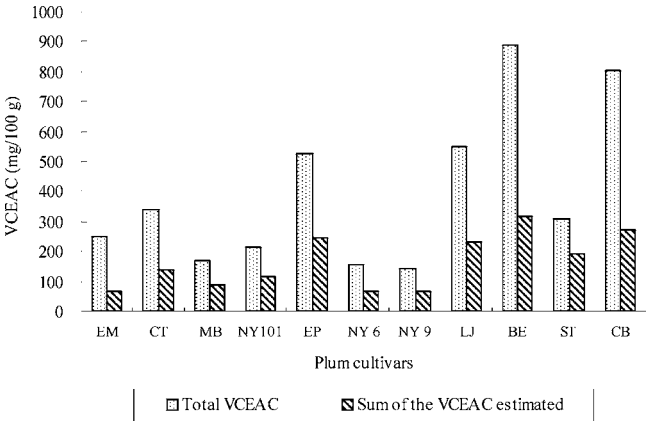

Figure 3. Comparison of total VCEAC (by ABTS) with the sum of the VCEACs calculated from phenolics identified by HPLC analysis. BE stands for Beltsville Elite B78197; CB, Cacaks Best; CT, Castleton; EM, Early Magic; EP, Empress; LJ, Longjohn; MB, Mirabellier; ST, Stanley.

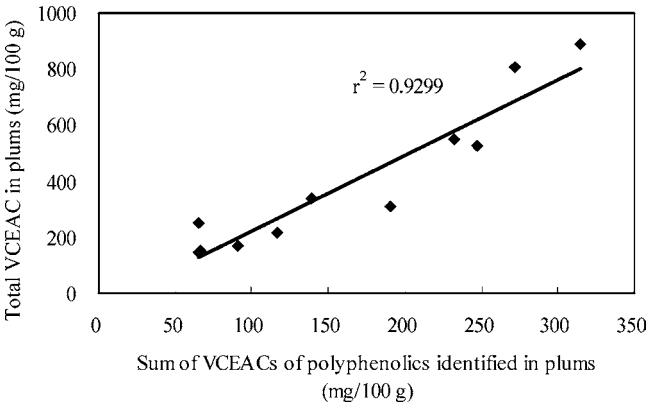

Figure 4. Correlation of total VCEAC by ABTS with the sum of the VCEACs calculated from phenolics identified by HPLC analysis.

(*37*). Over all, anthocyanins showed higher antioxidant activity, followed by quercetins and then chlorogenic acids.

Figure 3 shows the differences between the total VCEAC of plum samples and the sum of the VCEACs calculated from individual phenolics identified by HPLC analysis. The difference in the two values of VCEACs in each plum might be influenced by the difference of matrix where the assay was carried out (*40*), or in part, it might be attributed to other antioxidants in plums, such as carotenoids and vitamin C, although they were minor (*13*). However, there was a good correlation ($r^2 = 0.9299$) between total VCEAC of plum samples and the sum of the VCEACs calculated from phenolics (**Figure 4**). It can be concluded, therefore, that total antioxidant capacity of plums is directly contributed by individual phenolics.

To evaluate which compounds are mainly responsible for total antioxidant capacity of plums, the amount of polyphenolics determined in plums was converted to the antioxidant capacity based on their VCEAC values, and the results were summed into major polyphenolic categories. The relationship of total VCEAC with the antioxidant capacity of chlorogenic acids ($r^2 = 0.7751$) and anthocyanins ($r^2 = 0.6616$) showed higher correlation than that of quercetin glycosides ($r^2 = 0.0279$). Although chlorogenic acids themselves exhibited lower antioxidant capacity than other phenolics (as shown in **Figure 2**), they showed the major effect on the total antioxidant capacity of plums owing to their high concentrations in plums.

Over all, antioxidant capacity of plums varied greatly among the different cultivars used in this study, but it was highly correlated with the content of polyphenolics. The total antioxidant capacity of phenolics, which was the summation of each antioxidant capacity estimated by its concentration in plum, showed very good correlation with total antioxidant capacity of plum samples by ABTS method. Chlorogenic acids were the major sources of antioxidant capacity in plums. It can also be suggested that, in daily food consumption, a serving (100 g) of plums can provide a significant amount of antioxidant capacity at 144.4−89.6 mg of VCEAC.

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ABBREVIATIONS USED

VCEAC, vitamin C equivalent antioxidant capacity; 3-CQA, 3-*O*-caffeoylquinic acid, neochlorogenic acid; 5-CQA, 5-*O*-caffeoylquinic acid, chlorogenic acid; 4-CQA, 4-*O*-caffeoylquinic acid, cryptochlorogenic acid; CGL, cyanidin 3-glucoside; CRT, cyanidin 3-rutinoside; PGL, peonidin 3-glucoside; PDR, peonidin derivative; QGA, quercetin 3-galactoside; QGL, quercetin 3-glucoside; QRT, quercetin 3-rutinoside; QDR1, quercetin derivative 1; QDR2, quercetin derivative 2; QDR3, quercetin derivative 3; QDR4, quercetin derivative 4; LDL, low-density lipoprotein; ABTS, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid; AAPH, 2,2-azobis(2-amidinopropane)dihydrochloride; GAE, gallic acid equivalent; CE, catechin equivalent; VCE, vitamin C equivalent; BE, Beltsville Elite B78197; CB, Cacaks Best; CT, Castleton; EM, Early Magic; EP, Empress; LJ, Longjohn; MB, Mirabellier; ST, Stanley.

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