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Quantification of Polyphenolics and Their Antioxidant Capacity in Fresh Plums

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Total phenolics, total flavonoids, and antioxidant capacity of 11 cultivars of fresh plums were determined using spectrophotometric methods. Identification and quantification of individual polyphenolics were performed using reversed-phase high-performance liquid chromatography equipped with a diode array detector. The total phenolic contents of various cultivars widely varied from 125.0 to 372.6 mg/100 g expressed as gallic acid equivalents. The level of total flavonoids in fresh plums ranged between 64.8 and 257.5 mg/100 g expressed as catechin equivalents. Antioxidant capacity, expressed as vitamin C equivalent antioxidant capacity (VCEAC), ranged from 204.9 to 567.0 mg/100 g with an average of 290.9 mg/100 g of fresh weight. Cv. Beltsville Elite B70197 showed the highest amounts of total phenolics and total flavonoids and the highest VCEAC. A positive relationship (correlation coefficient $r^2 = 0.977$) was presented between total phenolics and VCEAC, suggesting polyphenolics would play an important role in free radical scavenging. The level of IC_{50} value of superoxide radical anion scavenging activity of the plum cultivars ranged from 13.4 to 45.7 mg of VCEAC/100 g. Neochlorogenic acid was the predominant polyphenolic among fresh plums tested. Flavonols found in plum were commonly quercetin derivatives. Rutin was the most predominant flavonol in plums. Various anthocyanins containing cyanidin aglycon and peonidin aglycon were commonly found in all plums except for cv. Mirabellier and NY 101.

KEYWORDS: Free radicals; plums; polyphenolics; reversed-phase HPLC; superoxide radical anion; vitamin C equivalent antioxidant capacity (VCEAC)

INTRODUCTION

Polyphenolics are a diverse group of compounds that are composed of an aromatic benzene ring substituted with hydroxyl groups, including their functional derivatives. Polyphenolics are ubiquitous secondary metabolites of the plant kingdom. Sensory qualities such as taste, color, and flavor in fruits, vegetables, and beverages, including wine, are influenced by their polyphenolics. Among polyphenolics, flavonoids frequently occur as glycoside forms, whereas hydroxycinnamic acids generally exist as ester forms. Some polyphenolics are known to be free radical scavengers or antioxidants that have beneficial health-promoting effects in such chronic and degenerative diseases (*1*).

Foods for human consumption contain various molecules such as proteins, lipids, vitamins, and carbohydrates, which are vulnerable to attack by reactive oxygen species (ROS) (*2*). ROS embrace superoxide radical anion, hydrogen peroxide, hydroxyl radical, and singlet oxygen, which are normally generated during physiological metabolic activities (*3*, *4*). ROS have been considered to be linked with aging and many degenerative diseases such as cancers, inflammation, immune system decline, cardiovascular diseases, neurological diseases, and atherosclerosis (*3*).

Antioxidants retard or inhibit the oxidation possibly by reactive radicals including ROS in a biological system. A battery of polyphenolic phytochemicals such as flavonoids and phenolic acids, which are routinely consumed in our diet, have shown antioxidant properties by chelating metal ions (*5*), inhibiting lipid oxidation (*6*−*8*), inhibiting radical-forming enzymes (*9*, *10*), or quenching free radicals (*11*, *12*). Active research has been driven in recent years on fruits and vegetables due to their biologically beneficial effects emanating from antioxidant activities of phenolic phytochemicals.

Plums may be good sources of natural antioxidants. Recently, the high level of polyphenolic compounds and antioxidant activity in plums has been reported (*13*−*15*). The predominant phenolic compounds in plums were identified as hydroxycinnamic acid derivatives such as chlorogenic acid and neochlorogenic acid (*16*−*18*). The hydroxycinnamates were found only

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in their esterified forms in fresh plums (*17*). Anthocyanins were found in fresh plums predominantly as rutinoside derivatives, such as cyanidin 3-rutinoside and peonidin 3-rutinoside (*16*). Rutin (quercetin 3-rutinoside) was the principal polyphenol among the flavonol glycosides found in plum (*16*, *17*). However, there is limited information on total phenolics, total flavonoids, antioxidant activity, and quantification of individual polyphenolic constituents in plums. Because polyphenolic distributions in fruits and vegetables vary greatly due to the different cultivars, cultural practices, climatic conditions, storage conditions, and industrial processing (*15*, *17*, *19*, *20*), information on polyphenolic compositions and antioxidant activity among different cultivars of plums is needed.

The purposes of this study were to evaluate total phenolics and total flavonoids, to determine antioxidant capacity, and to quantify the individual polyphenolics among different cultivars of fresh plums. Antioxidant capacity in various plum cultivars was expressed on the basis of vitamin C equivalents using two different free radical species, the 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical and the superoxide radical anion. The removal of the superoxide radical anion by polyphenolics was evaluated because of its role as the initiator of a ROS generation system (*21*, *22*). Individual polyphenolic compounds from each plum cultivar were determined using reversed-phase high-performance liquid chromatography (HPLC).

MATERIALS AND METHODS

Fruits. Eleven cultivars of plums (Autumn Sweet, Beltsville Elite B70197, Castleton, Early Magic, Empress, Longjohn, Mirabellier, NY 6, NY 9, NY 101, and Stanley) were picked at commercial maturity during the 2001 harvest season at the New York State Agricultural Experiment Station orchard in Geneva, NY. Three cultivars of plums, Beltsville Elite B70197, Longjohn, and Stanley, previously determined in the 2000 harvest season were included in this study (*15*). Most of them are among the recommended European and Japanese type plum cultivars for planting in New York state (*23*). These plum cultivars are categorized by skin color as follows: red plums (Early Magic), yellow plums (Mirabellier and NY 101), dark blue or purple plums (Autumn Sweet, Beltsville Elite B70197, Castleton, Empress, Longjohn, NY 6, NY 9, and Stanley). Immediately upon arrival in the laboratory after harvest, plums were stored in a 2−5 °C cold room. Plums were carefully cut in half and the pits removed. Pitted plums was frozen and lyophilized, and then dried samples were ground to powder and stored at −20 °C until analyzed.

Chemicals. Ammonium hydroxide, ABTS as diammonium salt, catechin, chlorogenic acid, Folin−Ciocalteu's phenol reagent, gallic acid, nitro blue tetrazolium (NBT), quercetin, quercetin 3-galactoside, rutin, xanthine, and xanthine oxidase were obtained from Sigma Chemical Co. (St. Louis, MO). 2,2′-Azobis(2-amidinopropane) dihydrochloride (AAPH) was obtained from Wako Chemicals USA, Inc. (Richmond, VA). Ammonium phosphate monobasic was purchased from Mallinckrodt Baker, Inc. (Phillipsburg, NJ). Cyanidin, peonidin, cyanidin 3-glucoside, cyanidin 3-rutinoside, peonidin 3-glucoside, and quercetin 3-glucoside were obtained from Extrasynthese (Genay, France). Ascorbic acid and hydrochloric acid were purchased from Fisher Scientific (Pittsburgh, PA). All other chemicals used were of either analytical grade or HPLC grade.

Extraction of Phenolics. The polyphenolics in plums were extracted by the ultrasound-assisted method (*24*). The mixture of 10 g of freeze-dried powder and 100 mL of 80% aqueous methanol was sonicated for 20 min with a continual stream of nitrogen gas purging to prevent possible oxidative degradation of polyphenolics. The mixture was filtered through Whatman no. 2 filter paper (Whatman International Limited, Kent, U.K.) using a chilled Büchner funnel and rinsing with 50 mL of 100% methanol. Extraction of the residue was repeated using the same conditions. The two filtrates were combined and transferred into a 1 L evaporating flask with an additional 50 mL of 80% aqueous methanol. The solvent was removed using a rotary evaporator at 40 °C. The remaining phenolic concentrate was first dissolved in 50 mL of 100% methanol and diluted to a final volume of 100 mL using distilled deionized water (ddH_2O). The mixture was centrifuged at refrigerated temperatures at 12000*g* for 20 min and stored at −20 °C until analyses. Extraction for plums was done in duplicate.

Determination of Total Phenolics. Total phenolics were evaluated using the spectrophotometric analysis with Folin−Ciocalteu's phenol reagent (*15*). In brief, an aliquot (1 mL) of appropriately diluted extracts was added to a 25 mL volumetric flask containing 9 mL of ddH_2O. A reagent blank using ddH_2O was prepared. One milliliter of Folin−Ciocalteu's phenol reagent was added to the mixture and then shaken. After 5 min, 10 mL of a 7% Na_2CO_3 solution was added with mixing. The solution was then immediately diluted to volume (25 mL) with ddH_2O and mixed thoroughly. After 90 min at 23 °C, the absorbance was read against the prepared blank at 750 nm. The standard curve for total phenolics was made using gallic acid standard solution (0−100 mg/L) under the same procedure as above. Total phenolics in plums were expressed as milligrams of gallic acid equivalents (GAE) per 100 g of fresh sample. All samples were analyzed in six replications.

Determination of Total Flavonoids. Total flavonoids were measured according to a colorimetric assay (*25*). A 1 mL aliquot of appropriately diluted sample was added to a 10 mL volumetric flask containing 4 mL of ddH_2O. At zero time, 0.3 mL of 5% $NaNO_2$ was added to the flask. After 5 min, 0.3 mL of 10% $AlCl_3$ was added. At 6 min, 2 mL of 1 M NaOH was added to the mixture. Immediately, the contents of the reaction flask were diluted to volume with the addition of 2.4 mL of ddH_2O and thoroughly mixed. Absorbance of the mixture was determined at 510 nm versus a prepared water blank. Catechin was used as standard compound for the quantification of total flavonoids, the amount of which in plums was calculated on a fresh weight basis as milligrams per 100 g of catechin equivalents (CE). All samples were analyzed in six replications.

Vitamin C Equivalent Antioxidant Capacity (VCEAC) Assay Using ABTS Radical. Blue-green ABTS radicals were used to evaluate the antioxidant capacity of various plum cultivars (*26*). A radical initiator, 1.0 mM AAPH, was added to 2.5 mM ABTS in phosphate-buffered saline (PBS; pH 7.4; 0.1 M K_2HPO_4/KH_2PO_4 buffer; 150 mM NaCl). The mixed solution was heated in a water bath at 68 °C. The resulting blue-green ABTS radical solution was adjusted to an absorbance of 0.650 ± 0.020 at 734 nm with additional PBS. Twenty microliters of sample was added to 980 μL of the ABTS radical solution. The mixture was incubated in a 37 °C water bath under restricted light for 10 min. A control (20 μL of 50% methanol and 980 μL of ABTS radical solution) was run with each series of samples. The reduction of absorbance at 734 nm was measured 10 min later. The ABTS radical, showing characteristic blue-green color in its odd-electron state, loses color when its unpaired electron is paired by the electron from antioxidants in plums. The radical stock solution was freshly prepared daily. The antioxidant capacity of plums was expressed on a fresh weight basis as milligrams per 100 g of vitamin C equivalents (VCEAC). All tested samples were replicated six times.

Superoxide Radical Anion Scavenging Activity Assay. This assay is based on the removal of superoxide radical anion ($O_2^{\bullet -}$) generated by the xanthine/xanthine oxidase system by measuring the reduction of NBT. Xanthine oxidase catalyzes the oxidation of xanthine to uric acid, during which the production of superoxide radical anion takes place. Superoxide radical anions reduce the tetrazolium blue into formasan blue, but after the addition of some radical scavengers (i.e., phenolic phytochemicals), the formation of formasan blue is restricted and the absorbance at 560 nm decreases (*27*). Absorption at 560 nm is proportional to the amount of residual superoxide radical anions (*27*). The quenching of superoxide radical anion was evaluated according to a minor modification of the method of Kweon et al. (*28*). Tetrazolium blue solution, the mixture of 0.1 mM xanthine and 0.1 mM NBT, was prepared in 50 mM potassium phosphate buffer (pH 7.4) containing 0.05 mM EDTA (PBE). An aliquot (0.9 mL) of tetrazolium blue solution was added to 0.1 mL of polyphenolics sample properly diluted in 50% aqueous methanol. The reaction was initiated by the addition of 1 mL of xanthine oxidase solution (0.05 unit/mL) in PBE. The resulting mixture was incubated in a water bath at 37 °C for 20 min. The reaction was terminated by the addition of 2 mL of 2.0 N HCl to

Table 1. Levels of Total Phenolics and Total Flavonoids, Vitamin C Equivalent Antioxidant Capacity (VCEAC), and IC_{50} from 11 Cultivars of Plums[a]

cultivar	total phenolics (mg of GAE/100 g)	total flavonoids (mg of CE/100 g)	VCEAC (mg of VCE/100 g)	IC_{50} (mg of VCE/100 g)
Autumn Sweet	229.5 ± 3.9 B	92.3 ± 4.0 E	351.2 ± 22.8 B	20.7
Beltsville Elite B70197	372.6 ± 7.5 A	257.5 ± 16.1 A	567.0 ± 17.6 A	31.0
Castleton	176.4 ± 1.8 F	108.7 ± 3.6 D	264.3 ± 10.3 C	13.4
Early Magic	143.1 ± 2.0 H	67.9 ± 1.9 F	204.9 ± 16.7 E	15.5
Empress	187.0 ± 2.1 D	133.4 ± 4.5 C	269.8 ± 11.8 C	18.4
Longjohn	216.2 ± 1.8 C	150.3 ± 4.4 B	289.8 ± 38.4 C	17.0
Mirabellier	136.8 ± 2.3 I	95.8 ± 3.4 E	211.6 ± 20.2 E	31.7
NY 6	162.8 ± 1.6 G	109.3 ± 3.5 D	262.3 ± 23.7 CD	24.1
NY 9	125.0 ± 2.3 J	64.8 ± 1.5 G	239.1 ± 21.2 CD	45.7
NY 101	181.9 ± 2.2 E	145.2 ± 6.6 B	289.6 ± 17.9 C	28.2
Stanley	181.3 ± 4.6 DE	110.0 ± 2.3 D	249.9 ± 12.5 CD	19.2

[a] The data except for IC_{50} are presented with mean ± standard deviation of six replications. GAE, gallic acid equivalent; CE, catechin equivalent; VCE, vitamin C equivalent. Values with the same letter in each column are not significantly different at the level of $p < 0.01$.

the reaction mixtures. The coloration of NBT was measured at 560 nm against a blank that was similarly prepared but with the enzyme solution added to the assay mixture after the addition of 2 N HCl. The enzyme solution and tetrazolium blue solution were freshly prepared daily. All tested extracts were analyzed six times. The superoxide radical anion scavenging activity assay was expressed as percent superoxide quenching according to the equation

$$\%\ \text{superoxide quenching} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

where A_{control} is the absorbance of the control at 20 min and A_{sample} is the absorbance of the sample at 20 min.

Evaluation of the antioxidant activity in various plum cultivars was based on IC_{50}. The IC_{50} index value was defined as the amount of antioxidant necessary to reduce the generation of superoxide radical anions by 50% at 560 nm at 20 min and 37 °C. A low IC_{50} value represents a high antioxidant activity. The IC_{50} values were expressed as milligrams per 100 g of fresh weight based on vitamin C equivalent.

Identification of Polyphenolics Using HPLC. HPLC analysis was performed according to the method of Kim and Lee (*29*). Extracted sample was filtered through a 0.45 μm poly(tetrafluoroethylene) syringe-tip filter. Using a 20 μL sample loop, the sample was analyzed using a reversed-phase HPLC system (Hewlett-Packard model 1100) equipped with a photodiode array detector, a quaternary pump, and a vacuum degasser. A C18 reversed-phase Symmetry Analytical column (5 μm × 250 mm × 4.6 mm) was used with a Symmetry Sentry guard column of the same packing material as the analytical column (Waters Corp., Milford, MA). Three mobile phases were used in the current study: solvent A, 50 mM ammonium phosphate monobasic ($NH_4H_2PO_4$), pH 2.6 (pH adjusted with phosphoric acid); solvent B, 80:20 (v/v) acetonitrile/50 mM $NH_4H_2PO_4$, pH 2.6; solvent C, 200 mM phosphoric acid (H_3PO_4), pH 1.5 (pH adjusted with ammonium hydroxide). The gradient for HPLC analysis was linearly changed as follows (total 60 min): 100% A at zero min, 92% A/8% B at 4 min, 14% B/86% C at 10 min, 16.5% B/83.5% C at 22.5 min, 25% B/75% C at 27.5 min, 80% B/20% C at 50 min, 100% A at 55 min, 100% A at 60 min. Flow rate was set to 1.0 mL/min at constant room temperature (23 °C). Detector was set at 320 nm for hydroxycinnamic acids, 370 nm for flavonols, and 520 nm for anthocyanins. Polyphenolic standards were used to generate characteristic UV−vis spectra and calibration curves. Individual polyphenolics in the sample were tentatively identified by comparison of their UV−vis spectra and retention times with spiked input of polyphenolic standard. Six replicated HPLC analyses were performed for each cultivar of plum. The amount of neochlorogenic acid was expressed as chlorogenic acid equivalents (CAE). The amount of quercetin derivatives was described by quercetin equivalents (QE). The concentration of anthocyanin derivatives having cyanidin aglycon or peonidin aglycon was expressed by cyanidin equivalents or peonidin equivalents, respectively.

Acid Hydrolysis. Acid hydrolysis was used for the identification of aglycons of unknown flavonols and anthocyanins (*30*). Individual peaks that needed to be identified in HPLC analysis were collected, and the sample containing 1.2 N HCl was hydrolyzed in a water bath at 90 °C for 2 h. The resulting acid hydrolysate was injected into the HPLC system for the aglycon identification of an unknown peak. Tentative confirmation of aglycon was made on the basis of the retention time, the characteristic UV−vis spectrum, and the spiked input of the commercial standard.

Statistical Analysis. Results are presented as mean ± standard deviation. Statistical analysis of experimental results was based on analysis of variance. Significant difference was statistically considered at the level of $p < 0.01$.

RESULTS AND DISCUSSION

Total Phenolics and Total Flavonoids. Total phenolics and total flavonoids in 11 plum cultivars are shown in **Table 1**. The total phenolic contents of various cultivars of plums were in a wide range from 125.0 to 372.6 mg of GAE/100 g with an average of 192.1 mg of GAE/100 g. There was a significant difference at a level of $p < 0.01$ in average content of total phenolics among plum cultivars except Empress, NY 101, and Stanley. Beltsville Elite B70197 was found to have the highest total phenolics among the 11 plum cultivars, whereas NY 9 had the lowest. The level of total phenolics in Beltsville Elite B70197 was ~3.0-fold higher than in NY 9, showing the wide variance of total phenolic concentrations in plums. Total phenolic content of red plum cultivars was reported to be at the level of 320 mg of GAE/100 g (*14*). Autumn Sweet, a red plum cultivar among the tested plums, showed 229.5 mg of GAE/100 g of fresh weight. Kim et al. (*15*) reported that the total phenolic content was in a range of 173.9−374.6 mg among six cultivars of plum tested, Beltsville Elite B70197 being among those with high total phenolic content. Total phenolics were 42−109 mg/100 g of fresh weight in various plum cultivars (Wickson, Black Beaut, Red Beaut, Santa Rosa, and Angeleno) from California (*31*). The various levels of total phenolics may possibly result from cultivars, geographic origin, growing seasons, other agricultural practices, and differences in analytical methods.

The range of total flavonoids in plums varied between 64.8 and 257.5 mg of CE/100 g of fresh weight. The mean content of total flavonoids among the 11 cultivars of plums was 121.4 mg of CE/100 g of fresh weight. Beltsville Elite B70197 had the highest total flavonoid content, whereas NY 9 had the lowest. The total flavonoid content of Beltsville Elite B70197 was ~4 times higher than that of NY 9. Total flavonoid concentrations of six various cultivars of plums harvested in 2000 (Beltsville Elite B70197, Cacak Best, French Damson, Longjohn, Stanley, and Yugoslavian Elite T101) ranged from 118.0 to 237.0 mg

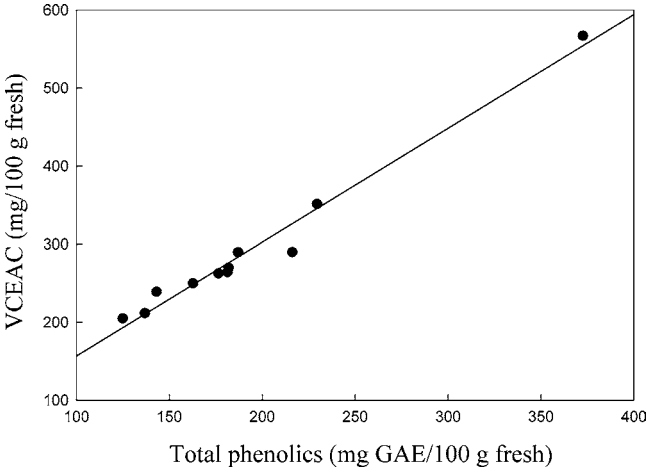

Figure 1. Relationship between total phenolics and vitamin C equivalent antioxidant capacity (VCEAC).

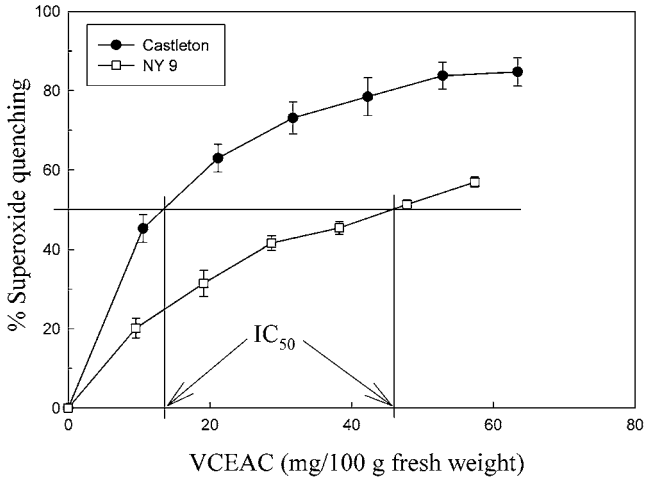

Figure 2. Effects of Castleton and NY 9 on scavenging of superoxide radical anions. Each point represents the average of six replications of experiments. Vertical bars indicate standard deviations.

of CE/100 g of fresh weight (*15*), which is consistent with the results for various cultivars of plums picked during the 2001 harvest season. Like total phenolics in plums tested, the total flavonoids among the plum cultivars resulted in significant difference at a significance level of $p < 0.01$.

VCEAC and Superoxide Radical Anion Scavenging Activity. The total antioxidant capacities of the plum cultivars by VCEAC assay using free ABTS radicals are given in **Table 1**. Fresh plums possessed total antioxidant capacities broadly ranging from 204.9 to 567.0 mg/100 g of VCEAC. Beltsville Elite B70197, highest in VCEAC among the tested cultivars of plums, carried a 2.8 times higher antioxidant capacity than Early Magic, which had the lowest VCEAC. These 11 plum cultivars averaged 290.9 mg/100 g in VCEAC.

Using the blue-green ABTS radical, Leong and Shui (*32*) reported that plums (unknown cultivars) had an antioxidant capacity of 312 mg/100 g of vitamin C equivalents. The previous report showed that the total antioxidant capacities of plums, expressed as VCEAC, ranged from 265.8 to 559.1 mg/100 g of fresh weight (*15*). Gil et al. (*31*) reported that antioxidant capacity of plums from California ranged between 27.4 and 61.1 mg/100 g of vitamin C equivalent, on the basis of the free DPPH radical scavenging capacity. The underestimation of antioxidant capacity by the DPPH method may be caused by the interference of other absorbing compounds at the wavelength detecting the DPPH radical (*26*).

A positive linear relationship with a correlation coefficient of $r^2 = 0.977$ was displayed between total phenolics and antioxidant capacity (VCEAC) in the 11 plum cultivars, indicating polyphenolics may play an important role in free radical scavenging (**Figure 1**). However, antioxidant capacity and total flavonoids showed a relatively weak relationship ($r^2 = 0.762$; data not shown). A good correlation between total phenolics and antioxidant activity in fresh plums was previously observed (*31*).

On the basis of the results reported in this study, plums may be used as a good source of natural antioxidant. The utilization of plums (or dried plums) in commercial food processing may efficiently prevent lipid oxidation that results in the prevention of various off-flavors and undesirable byproducts, thus increasing the shelf life of foods (*33*). Furthermore, the use of synthetic antioxidants such as propyl gallate and butylated hydroxytoluene may be reduced or replaced by plums in food processing.

Antioxidant activities (IC_{50}), based on the scavenging of superoxide radical anion, are shown in **Table 1**. Castleton had a 3.4 times higher antioxidant activity than NY 9. It was reported that the generated reactive superoxide radical anions were quenched by a wide range of polyphenolics including flavonoids (*34*−*36*). The effects of Castleton with the lowest IC_{50} value and NY 9 with the highest IC_{50} value on the scavenging of superoxide radical anions is depicted, IC_{50} values of which were graphically calculated on the basis of VCEAC (**Figure 2**). The dose−response relationship is displayed between VCEAC and percent superoxide quenching.

Variable levels of IC_{50} values among different plum extracts may indicate that various polyphenolic phytochemicals may react with free superoxide radical anions in different manners and therefore lead to different kinetic behaviors. Actually, some polyphenolics are known to play a role in either scavenging the generated superoxide radical anions or inhibiting the xanthine oxidase activity (*9*) or sometimes both (*11*, *36*). However, a net effect of polyphenolics manifesting this dual action on xanthine oxidase further complicates the matter (*34*). The mechanism proposed for xanthine oxidase inhibition is that some flavonoids may take the place of the active site of this enzyme (*11*). The planar flavones (e.g., chrysin and luteolin) and flavonols (e.g., kaempferol, quercetin, and myricetin) with a hydroxy group at the 7-position inhibited xanthine oxidase activity at low concentrations, whereas the nonplanar flavonoids and isoflavones were less inhibitory to xanthine oxidase (*9*). Another reason for different IC_{50} values among various cultivars of plums is that the diversity of phenolics present in each cultivar may lead to different influences on quenching of superoxide radical anions, some polyphenolics of which exhibit higher bioactive properties against superoxide radical anions and others lower.

Because some polyphenolics quench the superoxide radical anion that is related to the production of various reactive compounds such as peroxynitrite and hydroxyl radical (*2*), the consumption of plums rich in polyphenolics may reduce or quench the ROS and improve human health by the prevention of chronic diseases.

Content of Polyphenolics. Due to the unavailability of authentic commercial neochlorogenic acid (3-caffeoylquinic acid) standard, its identification was accomplished with the comparison of a previously reported HPLC separation pattern and of its UV−vis spectrum with chlorogenic acid (5-caffeoylquinic acid) (*17*, *18*, *37*). Chlorogenic acid was identified with the comparison of its retention time and UV−vis spectrum

Table 2. Content of Hydroxycinnamic Acids in 11 Cultivars of Fresh Plums[a]

cultivar	neochlorogenic acid	chlorogenic acid
Autumn Sweet	111.6 ± 15.4	11.8 ± 1.7
Beltsville Elite B70197	215.4 ± 7.6	9.5 ± 0.5
Castleton	73.1 ± 8.5	7.1 ± 1.0
Early Magic	18.1 ± 5.4	0.9 ± 0.5
Empress	127.7 ± 13.7	8.0 ± 1.0
Longjohn	128.0 ± 19.9	16.4 ± 1.3
Mirabellier	63.9 ± 7.3	16.4 ± 2.7
NY 6	67.9 ± 11.1	8.8 ± 1.4
NY 9	49.7 ± 4.1	3.7 ± 0.4
NY 101	179.4 ± 20.2	21.0 ± 2.6
Stanley	104.2 ± 16.4	7.8 ± 1.5

[a] Amounts of polyphenolics are expressed in mean ± standard deviation in mg/100 g of fresh weight of six replications. The concentration of neochlorogenic acid is described as chlorogenic acid equivalents.

with the spiked input of its commercial standard. The contents of neochlorogenic acid in the 11 varieties of fresh plums per 100 g broadly ranged from 18.1 to 215.4 mg of CAE, whereas the concentrations of chlorogenic acid were between 0.9 and 21.0 mg/100 g (**Table 2**). Beltsville Elite B70197 showed the highest content (215.4 mg of CAE/100 g) of neochlorogenic acid among various plums tested, whereas the highest amount of chlorogenic acid (21.0 mg/100 g) was found in NY 101. Early Magic had the lowest amounts of neochlorogenic acid and chlorogenic acid at levels of 18.1 mg of CAE and 0.9 mg, respectively. There was a wide difference in the content of hydroxycinnamic acids among cultivars tested this study. Early Magic showed the level of 19.0 mg/100 g of fresh weight hydroxycinnamic acids, whereas Beltsville Elite B70197 was at 224.9 mg/100 g of fresh weight. Neochlorogenic acid was found to be always at a higher level than its isomer, chlorogenic acid, throughout all of the cultivars of plums tested in this study. In previous studies on plums, neochlorogenic acid was reported to be the major hydroxycinnamic acid derivative, whereas chlorogenic acid was the minor (*16*, *18*).

The flavonols found in plums were mainly quercetin derivatives. Rutin was one of the most common and predominant flavonols in the 11 cultivars of plums tested, and its concentration ranged from 2.8 to 7.7 mg/100 g (**Table 3**). Fresh plums previously showed rutin as a principal flavonol, present at the level of 2.5 mg/100 g (*17*). It was also reported that fresh plums exhibited rutin as a predominant polyphenolic among the flavonols (*16*). Quercetin 3-galactoside (hyperoside) and quercetin 3-glucoside (isoquercitrin) were found in Mirabellier at 3.5 and 1.2 mg/100 g of fresh weight, respectively. Quercetin 3-glucoside was also found in Early Magic at the level of 2.2 mg/100 g of fresh weight. Early Magic had two additional quercetin derivatives, which were eluted after quercetin 3-glucoside (data not shown). The individual HPLC peaks of each quercetin derivative were repeatedly collected by multiple injections and hydrolyzed in acidic conditions, and then the resulting hydrolysate was analyzed again by HPLC. On the basis of its UV−vis spectrum, its retention time, and spiked input of quercetin aglycon standard, each peak was identified as a quercetin derivative. The first quercetin derivative was estimated to be at 1.8 mg of QE/100 g, whereas the second was at 0.9 mg of QE/100 g. Various quercetin glycosides, as monosaccharides or disaccharides, were previously found in fresh plums, which included quercetin glucoside, quercetin rhamnoside, quercetin rutinoside, and others (*18*).

The content of anthocyanins in various plums is presented in **Table 4**. Among flavonoids identified in fresh plums, anthocyanins were found to be the principal class, which is in good agreement with the previous study (*14*). As expected, there were no anthocyanins found in the yellow plum varieties, Mirabellier and NY 101. It was previously reported that the yellow plum cultivar Wickson contained no anthocyanins (*18*). The nine cultivars other than Mirabellier and NY 101 commonly contained anthocyanins such as cyanidin 3-glucoside (kuromanin), cyanidin 3-rutinoside (keracyanin), and peonidin derivative. Cyanidin 3-glucoside was eluted before cyanidin 3-rutinoside, suggesting the sugar moiety substituted onto flavonoid aglycon affects the elution order. Cyanidin 3-rutinoside was a predominant anthocyanin. Peonidin derivative, the aglycon of which was identified via acid hydrolysis and then HPLC analysis, was eluted after peonidin 3-glucoside. Peonidin 3-glucoside was additionally found in Castleton and NY 6, at levels of 1.2 and 1.1 mg/100 g, respectively. Besides cyanidin 3-glucoside and cyanidin 3-rutinoside, a cyanidin derivative having the cyanidin aglycon and an unidentified moiety substitution was found only in Early Magic. This cyanidin derivative in Early Magic was eluted prior to cyanidin 3-glucoside, followed by cyanidin 3-rutinoside. Cyanidin 3-rutinoside was found in Early Magic at a concentration level similar to that of neochlorogenic acid. It was previously found that various cyanidin glycosides positively identified in plums were cyanidin 3-glucoside, cyanidin 3-rutinoside, tentative cyanidin 3-galactoside, and tentative cyanidin 3-acetyl-glucoside (*18*). It was reported that anthocyanins in French plums predominantly belonged to rutinoside derivatives such as cyanidin 3-rutinoside and peonidin 3-rutinoside (*16*). Longjohn had the highest amount of cyanidin 3-rutinoside among nine anthocyanin-containing plum cultivars

Table 3. Content of Flavonols in 11 Cultivars of Plums[a]

cultivar	rutin	quercetin 3-galactoside	quercetin 3-glucoside	quercetin derivative 1	quercetin derivative 2
Autumn Sweet	4.5 ± 0.6				
Beltsville Elite B70197	4.3 ± 0.3				
Castleton	3.8 ± 0.5				
Early Magic	6.3 ± 0.6		2.2 ± 0.3	1.8 ± 0.2	0.9 ± 0.1
Empress	3.3 ± 0.4				
Longjohn	7.7 ± 1.3				
Mirabellier	6.7 ± 0.5	3.5 ± 0.4	1.2 ± 0.1		
NY 6	2.8 ± 0.3				
NY 9	4.3 ± 0.3				
NY 101	5.0 ± 0.6				
Stanley	3.8 ± 0.6				

[a] Amounts of polyphenolics are expressed in mean ± standard deviation in mg/100 g of fresh weight of six replications. Contents of the two quercetin derivatives are described as quercetin equivalents.

Table 4. Content of Anthocyanins in 11 Cultivars of Plums[a]

cultivar	cyanidin derivative	cyanidin 3-glucoside	cyanidin 3-rutinoside	peonidin 3-glucoside	peonidin derivative
Autumn Sweet		2.1 ± 0.2	14.1 ± 1.6		2.1 ± 0.3
Beltsville Elite B70197		3.9 ± 0.3	25.7 ± 1.0		11.5 ± 0.5
Castleton		7.0 ± 0.9	16.1 ± 1.9	1.2 ± 0.2	5.0 ± 0.7
Early Magic	3.1 ± 0.3	6.7 ± 0.6	18.9 ± 1.9		1.9 ± 0.3
Empress		1.9 ± 0.8	17.5 ± 2.2		2.0 ± 0.3
Longjohn		5.3 ± 0.9	33.0 ± 2.0		5.4 ± 0.8
Mirabellier					
NY 6		13.5 ± 2.0	23.8 ± 3.5	1.1 ± 0.2	4.4 ± 0.6
NY 9		3.9 ± 0.4	24.2 ± 2.1		8.3 ± 0.7
NY 101					
Stanley		2.1 ± 0.3	21.5 ± 3.9		5.2 ± 0.9

[a] Amounts of polyphenolics are denoted in mean ± standard deviation in mg/100 g of fresh weight of six replications. Concentrations of cyanidin derivatives and peonidin derivative are expressed in cyanidin and peonidin equivalents, respectively.

and showed a 2.3 times higher content than Autumn Sweet. Unlike fresh plums, some reports have shown that prunes (dried plums) possessed no anthocyanins (*17*, *38*). The absence of anthocyanins in dried plums may be mainly due to loss during the dehydration process.

Overall, the polyphenolics identified in the 11 cultivars of plums by HPLC analysis were neochlorogenic acid, chlorogenic acid, rutin, quercetin 3-glucoside, quercetin 3-galactoside, cyanidin 3-glucoside, cyanidin 3-rutinoside, peonidin 3-glucoside, two quercetin derivatives, a cyanidin derivative, and a peonidin derivative. The HPLC analysis of plums showed that the polyphenolic composition was diverse and also that the level of individual polyphenolics was different among test cultivars. On the basis of HPLC analysis, the sum of individual polyphenolics in 100 g of fresh plums widely ranged from 60.2 mg in Early Magic to 270.3 mg in Beltsville Elite B70197. The relative content of neochlorogenic and chlorogenic acids as major phenolics by HPLC analysis varied from 30.8% in Early Magic to 97.6% in NY 101. The flavonols contributed from 1.6% in Beltsville Elite B70197 to 19.0% in Early Magic. In the nine cultivars excluding the two yellow plums, relative contents of anthocyanins ranged from 12.6% in Autumn Sweet to 50.3% in Early Magic. A positive linear relationship with a correlation coefficient, r^2, of 0.686 was displayed between antioxidant capacity (VCEAC) and the sum of individual polyphenolics analyzed by HPLC (data not shown), the correlation coefficient of which was weaker compared to that between antioxidant capacity (VCEAC) and total phenolics by spectrophotometric measurement using Folin−Ciocalteu's phenol reagent. The possible reason for the relatively weaker correlation may be that the HPLC measurement of other polyphenolics such as procyanidins in plums was not achieved here.

In conclusion, various cultivars of plums showed different levels of phenolic acid content, flavonoid content, and antioxidant capacity. There was a strong linear relationship between total phenolics and antioxidant capacity (VCEAC). Polyphenolics in plums possessed the ability to quench the reactive superoxide radical anions. Neochlorogenic acid was identified as a major polyphenolic. Flavonols identified were mainly quercetin derivatives, among which rutin was a common and predominant flavonol. Various anthocyanins, mainly as cyanidin and peonidin derivatives, were found in fresh plums. The results in this study might suggest that plums in our routine diet would be a good source of antioxidants, providing health-promoting effects in human.

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ABBREVIATIONS USED

AAPH, 2,2′-azobis(2-amidinopropane) dihydrochloride; ABTS, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid); CAE, chlorogenic acid equivalent; CE, catechin equivalent; GAE, gallic acid equivalent; NBT, nitro blue tetrazolium; PBE, potassium phosphate buffer containing EDTA; PBS, phosphate-buffered saline; QE, quercetin equivalent; ROS, reactive oxygen species; VCEAC, vitamin C equivalent antioxidant capacity.

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