Identification, Quantitative Determination, and Antioxidative Activities of Chlorogenic Acid Isomers in Prune (*Prunus domestica* L.)

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Neochlorogenic acid (3-CQA) and cryptochlorogenic acid (4-CQA), isolated from prune (*Prunus domestica* L.), were identified by NMR and MS analyses. In addition, the quantity of chlorogenic acid isomers in prune were measured by HPLC. These isomers, 3-CQA, 4-CQA, and chlorogenic acid (5-CQA), were contained in the ratio 78.7:18.4:3.9, respectively. 4-CQA was identified and quantified in prune for the first time, and relatively high amounts of this isomer were characteristic. Antioxidative activities of the chlorogenic acid isomers, such as scavenging activity on superoxide anion radicals and inhibitory effect against oxidation of methyl linoleate, were also evaluated. Each isomer showed antioxidative activities which were almost the same.

Keywords: *Prunus domestica L.; neochlorogenic acid; cryptochlorogenic acid; chlorogenic acid; oxidative stability index (OSI); antioxidative activity; superoxide anion radicals (O_2-); radical scavenging activity*

INTRODUCTION

Prunus domestica L. originated from the Caucasus region in West Asia. It belongs to the Rosaceae family and is one of the species of plum. Generally, plum refers to the fruits of the genus *Prunus*, such as *Prunus domestica*, *P. salicina*, *P. subcordiata*, and *P. insititia* (Pijpers et al., 1986). Among *P. domestica*, the so-called prune is the dried fruit of some cultivars of *P. domestica* which are available for making dried fruits. Therefore, these cultivars are called "prune-making plum". Prune is well-known as a healthy food and has been used medicinally in India in combination with other drugs for the treatment of leukorrhoea, irregular menstruation, and debility following miscarriage (Chopra et al., 1956).

Concerning the chemical constituents of *P. domestica* L., domesticoside (2-*O*-*β*-D-glucopyranosyl-4-*O*-methylphloracetophenone) was isolated from the bark of the tree (Nagarajan and Parmar, 1977). Additional constituents were isolated from the heartwood: isosakuranetin, prudomestin, dihydrokaempferide, naringenin, 3,5,7-trihydroxy-8,4′-dimethoxyflavanone, 5,7,4′-trihydroxy-3-methoxyflavanone, and 3,5,7-trihydroxy-6,4′-dimethoxyflavanone (Parmar et al., 1992). Several glycosides of kaempferol and quercetin were detected from the leaves and fruits, and the total contents of these compounds in the fruits are only 20−52 mg/kg (Henning and Herrmann, 1980). Hexanal, (*E*)-2-hexenal, butyl acetate, butyl butyrate, and other compounds were found as the aroma components of the unprocessed prune-making plum (Horvat et al., 1992). Furfural, benzaldehyde, ethyl cinnamate, and other compounds were also obtained from prune (Moutounet et al., 1975).

It is reported that neochlorogenic acid (3-*O*-caffeoylquinic acid, 3-CQA; Figure 1) was a major hydroxycinnamate (541 mg/kg) in the fruit of plum. Chlorogenic acid (5-*O*-caffeoylquinic acid, 5-CQA; Figure 1) was also contained at a concentration of 73 mg/kg, and cryptochlorogenic acid (4-*O*-caffeoylquinic acid, 4-CQA; Figure 1) was found at 9 mg/kg as a minor component (Herrmann, 1989). Möller and Herrmann (1983) also detected 88−731 mg/kg of 3-CQA, 15−129 mg/kg of 5-CQA, and 56 mg/kg of 4-CQA in fresh plum. However, as described above, plum consists of a number of species and the previous authors did not specify which species of plum was used for their analysis, regardless of whether their samples were fresh or dried.

There are only a few papers on the quantitative analysis of chlorogenic acid isomers in prune. In the exocarp and pulp of fresh prune-making plum, 3-CQA was about half of the amount of total phenolics (Raynal et al., 1989). Recently, Donovan et al. (1998) detected 1300 mg/kg of 3-CQA and 430 mg/kg of 5-CQA in prune. However, 4-CQA was not reported in those papers, thus it seems that it is not contained in prune. In this paper, we describe the identification of 3-CQA and 4-CQA in prune and the quantitative analysis of chlorogenic acid isomers by HPLC.

Chlorogenic acid, 5-CQA, is widely recognized to be an antioxidant for human LDL (Rice-Evans et al., 1996; Nardini et al., 1995). It is also known as a scavenger for reactive species of oxygen and nitrogen (Kono et al., 1997), and an inhibitor against formation of conjugated diene from linoleic acid oxidation (Morishita and Kido, 1995). It is expected that other chlorogenic acid isomers, such as 3-CQA and 4-CQA, may also show antioxidative

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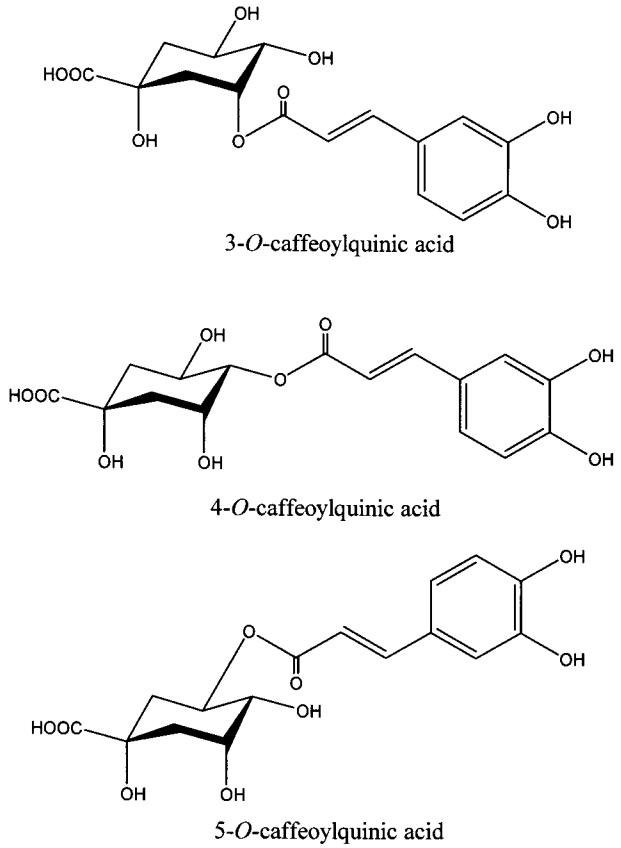

Figure 1. Structure of Chlorogenic Acid Isomers

activities. This paper also describes our findings regarding scavenging activity of these isomers on superoxide anion radicals and their inhibitory effect against oxidation of methyl linoleate.

MATERIALS AND METHODS

General Procedures. ^{1}H-, ^{13}C-, and 2D NMR (H−H COSY, ^{1}H−^{1}H correlation spectroscopy; HMQC, ^{1}H-detected multiple quantum coherence spectrum; HMBC, ^{1}H-detected multiple-bond heteronuclear multiple quantum coherence spectrum) spectra were obtained on a Varian Unity *plus* 500 instrument (^{1}H: 500 MHz, ^{13}C: 125 MHz; Varian Inc., Palo Alto, CA) at 25 °C and referenced to the residual proton solvent resonance (CD_3OD at 3.30 ppm for ^{1}H- and 49.0 ppm for ^{13}C NMR). MS analysis was performed on a HITACHI M-2000 mass spectrometer (HITACHI Ltd., Tokyo, Japan) with atmospheric pressure chemical ionization (APCI) interface using a methanol (MeOH) solvent. Silica gel 60 (70−230 mesh; E. Merck, Darmstadt, Germany), Sephadex LH-20 (Pharmacia Biotech AB, Uppsala, Sweden), and Chromatorex ODS DM1020T (100−200 mesh; Fuji Silysia Chemical, Tokyo, Japan) were used for column chromatography. Silica gel 60 F_{254} plates (E. Merck, Darmstadt, Germany) and ODS plates (E. Merck, Darmstadt, Germany) were used for thin-layer chromatography (TLC). HPLC analysis was carried out on a Waters 600E Multisolvent Delivery System with a 717 plus Autosampler and a 996 Photodiode Array Detector (Waters Co., Milford, MA). The Omnion Oxidative Stability Instrument (Archer Daniels Midland Co., Decatur, IL) was used for the oxidative stability index (OSI) method described by Akoh (1994). ESR spectra were recorded on a JEOL JES-RE1X spectrometer (JEOL Ltd., Tokyo, Japan) using an aqueous quartz flat cell (60 mm × 10 mm × 0.31 mm inner size).

Plant Material. Four lots of prune, whose moisture levels were prepared at 21% and called "natural condition prune" (California Prune Board, 1997), were supplied by MIKI Foods Co., Ltd. (Hyogo, Japan), were imported from the U.S. as the material for prune extract (concentrated prune juice).

Standards. Chlorogenic acid (5-CQA) was purchased from Aldrich Chemical Co. (Milwaukee, WI). 2,6-di-*tert*-butyl-4-methylphenol (butylated hydroxytoluene, BHT), caffeic acid, L-ascorbic acid, rutin, and α-tocopherol were obtained from Wako Pure Chemical Ins. (Osaka, Japan). *p*-Coumaric acid was purchased from Fluka Chemie AG (Buchs, Switzerland). All commercial standards were of the highest analytical grade. Neochlorogenic acid (3-CQA) and cryptochlorogenic acid (4-CQA) were prepared from chlorogenic acid (5-CQA) using the method described by Nagels et al. (1980). The purities of these compounds were higher than 99% for 3-CQA and 96.5% for 4-CQA as determined by HPLC analysis.

Other Chemicals. Other chemicals were obtained from various sources, as appropriate: xanthine oxidase (XOD) from Boehringer Mannheim Co. (Mannheim, Germany); hypoxanthine (HPX) from Sigma Chemical Co. (St. Louis, MO); diethylenetriamine-*N*, *N*′, *N*″, *N*″-pentaacetic acid (DTPA) from Dojindo Laboratories (Kumamoto, Japan); 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO) from LABOTEC Co. (Tokyo, Japan); methyl linoleate from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan); and silicon oil from Toshiba Silicon Co., Ltd. (Tokyo, Japan). These chemicals were all also of the highest grade.

Isolation and Identification of 3-CQA and 4-CQA in Prune. Prune fruit (500 g) was soaked in water overnight at room temperature and then the water layer was eliminated by decantation (2 L × 3 times). After removal of the seeds, the swollen fruit was soaked in acetone (1 L) and allowed to stand overnight at room temperature. After filtration of the acetone extract, the residue was added to another 1-L portion of acetone. This procedure was repeated six times. The combined acetone extract was evaporated in vacuo, then a mixture of water and ethyl acetate (EtOAc) (1:1, v/v, 1 L) was added to the resulting residue to separate the EtOAc-soluble and the H_2O-soluble fractions. The EtOAc-soluble fraction (2.5 g) was subjected to Sephadex LH-20 column chromatography using 2-propanol as the mobile phase to give six fractions under monitoring silica gel TLC. Fraction 5 (325 mg) was rechromatographed on a column of ODS gel (MeOH/H_2O, 2:3) to give seven fractions (fractions 5-1−5-7) by referring ODS TLC analysis. Fraction 5-2 (188 mg) was subjected to silica gel column chromatography and eluted with EtOAc/MeOH/H_2O (21:4:3) to give seven fractions (fractions 5-2-1−5-2-7). Fraction 5-2-4, which was predicted to be a mixture of compounds **1** and **2**, was separated by ODS column chromatography (MeOH/H_2O, 1:9) to give **1** (29 mg) and **2** (11 mg). Compounds **1** and **2** were identified to be 3-CQA and 4-CQA, respectively, by NMR and MS analyses.

3-CQA (**1**): APCI MS, *m*/*z* 355 $[M+1]^+$; ^{1}H NMR (500 MHz, CD_3OD) δ 1.95 (1H, dd, J = 9, 14 Hz, H-6ax), 2.13 (2H, m, H-2eq and 6eq), 2.20 (1H, dd, J = 4, 15 Hz, H-2ax), 3.63 (1H, dd, J = 3, 9 Hz, H-4), 4.14 (1H, ddd, J = 3, 9, 9 Hz, H-5), 5.34 (1H, ddd, J = 3, 3, 4 Hz, H-3), 6.30 (1H, d, J = 16 Hz, H-8′), 6.76 (1H, d, J = 8 Hz, H-5′), 6.93 (1H, dd, J = 2, 8 Hz, H-6′), 7.04 (1H, d, J = 2 Hz, H-2′), 7.58 (1H, d, J = 16 Hz, H-7′); ^{13}C NMR (125 MHz, CD_3OD) δ 36.7 (C-2), 41.5 (C-6), 68.3 (C-5), 73.0 (C-3), 74.8 (C-4), 75.4 (C-1), 115.1 (C-2′), 115.8 (C-8′), 116.4 (C-5′), 122.9 (C-6′), 127.9 (C-1′), 146.79 (C-3′), 146.80 (C-7′), 149.4 (C-4′), 169.0 (C-9′), 178.3 (C-7) [identical to data in the literature (Morishita et al., 1984; Tatefuji et al., 1996)].

4-CQA (**2**): APCI MS, *m*/*z* 355 $[M+1]^+$; ^{1}H NMR (500 MHz, CD_3OD) δ 2.00 (1H, dd, J = 11, 13 Hz, H-6ax), 2.06 (1H, ddd, J = 3, 4, 14 Hz, H-2eq), 2.17 (1H, dd, J = 4, 14 Hz, H-2ax), 2.20 (1H, ddd, J = 3, 5, 13 Hz, H-6eq), 4.27 (1H, ddd, J = 4, 9, 11 Hz, H-5), 4.28 (1H, ddd, J = 3, 3, 4 Hz, H-3), 4.79 (1H, dd, J = 3, 9 Hz, H-4), 6.37 (1H, d, J = 16 Hz, H-8′), 6.77 (1H, d, J = 8 Hz, H-5′), 6.96 (1H, dd, J = 2, 8 Hz, H-6′), 7.06 (1H, d, J = 2 Hz, H-2′), 7.65 (1H, d, J = 16 Hz, H-7′); ^{13}C NMR (125 MHz, CD_3OD) δ 38.4 (C-2), 42.7 (C-6), 65.5 (C-5), 69.6 (C-3), 76.6 (C-1), 79.3 (C-4), 115.1 (C-2′), 115.4 (C-8′), 116.5 (C-5′), 123.0 (C-6′), 127.8 (C-1′), 146.8 (C-3′), 147.1 (C-7′), 149.6 (C-4′), 169.0 (C-9′), 177.3 (C-7) [identical to data in the literature (Morishita et al., 1984; Tatefuji et al., 1996)].

Quantitative Determination of Chlorogenic Acid Isomers in Prune. Prune fruit was pitted and cut into small

pieces. These pieces were crushed in an Oster Blender EX (Sunbeam Co., Delray Beach, FL), and a 10-g portion was homogenized with 100 mL of MeOH in a mixer (Matsushita Electric Co., Osaka, Japan) for 5 min. After homogenization, the mixture was transferred into a flask, 50 mL of MeOH was added, and the mixture was refluxed for 30 min. The mixture was filtered and the residue was re-extracted with an additional 100 mL of MeOH. This procedure was repeated five times. The combined filtrate was evaporated in vacuo to remove the MeOH. The aqueous residue was filled up to 100 mL with water. Standards of 3-CQA, 4-CQA, 5-CQA, caffeic acid, *p*-coumaric acid, and rutin were dissolved in 50% MeOH to make a concentration of 1−6 ppm. The prune extract and standard solutions were filtered through 0.45-μm olefin polymer (OP) syringe-tip filters (Biofield Co., Tokyo, Japan) and analyzed by HPLC: column, Symmetry C18, 4.6 × 250 mm, 5 μm (Waters Co., Milford, MA); column temp, 40 °C; mobile phase, A = 50 mM $NH_4H_2PO_4$ at pH 2.60, B = 80% acetonitrile and 20% A, C = 200 mM *o*-phosphoric acid at pH 1.50; flow rate, 1.0 mL/min; gradient, 0.00 min at %A = 100.0, 4.00 min at %A = 92.0 and %B = 8.0, 10.00 min at %B = 14.0 and %C = 86.0, 22.50 min at %A = 1.5, %B = 16.5, and %C = 82.0, 27.50 min at %B = 21.5 and %C = 78.5, 45.00 min at %B = 50.0 and %C = 50.0, 47.50 min at %A = 100.0, and 55.00 min at %A = 100.0; gradient curve, linear gradient; injection volume, 10 μL; detection, photodiode array (200−600 nm). These conditions refer to the method by Donovan et al. (1998), with some modification to the gradient. Each peak detected in the MeOH extract of prune was identified by comparing retention time and UV−visible spectra given by the PDA detector with the standards. The compounds 3-CQA, 4-CQA, 5-CQA, caffeic acid, *p*-coumaric acid, and rutin in prune were quantified by calibration with the standards.

Evaluation of Scavenging Activity on Superoxide Anion Radicals (O_2^-). The compounds 3-CQA, 4-CQA, 5-CQA, caffeic acid, and L-ascorbic acid (4 μmol of each) were separately dissolved in 20 mL of ultrapure water. XOD and DTPA solutions were prepared with 200 mM phosphate buffer (pH 7.8). The other solution of chemicals was prepared with ultrapure water. XOD (50 μL of 0.4 unit/mL) was added to the mixture of sample (50 μL), 2 mM HPX (50 μL), 5.5 mM DTPA (35 μL), and 9.2 M DMPO (15 μL). Exactly 40 s after the addition, the ESR spectrum of DMPO-O_2^- was recorded: temperature, 20 °C; magnetic field, 335.9 ± 5 mT; power, 8 mW; modulation, 100 kHz; field modulation width, 0.079 mT; sweep time, 2.0 min; receiver gain, 2.5 × 100; time constant, 0.1 s. All analyses were carried out in triplicate. The scavenging ratio of O_2^- at 50 μM was calculated as mean value ± SD.

Evaluation of the Inhibitory Effect against Oxidation of Methyl Linoleate by the OSI Method. The inhibitory effect of chlorogenic acid isomers and caffeic acid against the oxidation of methyl linoleate was evaluated by the OSI method. In this method, the lipid substrate is heated with forced aeration, and the effluent air from the substrate going into 50 mL of distilled water contains the volatile organic acid swept from the oxidizing oil, which increases the conductivity of the water. The OSI value is defined as the point of maximum change of the rate of oxidation and is determined by measuring the conductivity of the distilled water.

One μmol each of 3-CQA, 4-CQA, 5-CQA, caffeic acid, α-tocopherol, and BHT was dissolved in 100 μL MeOH and added to 5 g of silicon oil which contained 10% of methyl linoleate as lipid substrate. For control, the same amount of MeOH alone was added. MeOH was flushed out at 90 °C for 30 min with forced aeration (without linking the conductivity measurement tubes), after which the OSI value of each sample at 90 °C was measured in triplicate. The data are given as mean value ± SD.

RESULTS AND DISCUSSION

Identification of 3-CQA and 4-CQA in Prune. Compound **1** exhibited an $[M+1]^+$ peak at *m*/*z* 355, which was indicative of the molecular weight of 354 corresponding to chlorogenic acid. The ^{13}C NMR data revealed the presence of a quinic acid moiety characterized with two methylenes (δ 36.7 and 41.5), three oxymethines (δ 68.3, 73.0, and 74.8), one quaternary carbon (δ 75.4), and one carboxyl group (δ 178.3), as well as a caffeoyl moiety. In the ^1H NMR spectrum, the downfield shift of the resonance of the equatorial proton signal of H-3 (δ 5.34 ddd, J = 3, 3, 4 Hz) indicated that the hydroxyl group on C-3 was acylated with a caffeic acid. Thus, compound **1** was identified as 3-CQA. Compound **2** gave the same quasi-molecular ion peak as **1** in the APCI mass spectrum and its NMR data resembled those of **1**. In the ^1H NMR spectrum, the axial proton signal of H-4 showed a downfield shift (δ 4.79 dd, J = 3, 9 Hz) due to esterification. Therefore, **2** was identified as 4-CQA. H−H COSY, HMQC, and HMBC measurements allowed the complete assignment of protons and carbons of **1** and **2**.

This is the first report on the isolation of 4-CQA from prune (*Prunus domestica* L.).

Quantitative Determination of Chlorogenic Acid Isomers in Prune. HPLC analysis of prune components was carried out according to the method of Donovan et al. (1998). The compounds 3-CQA, 5-CQA, caffeic acid, *p*-coumaric acid, and rutin were detected. When the authentic standards were subjected to HPLC under the same conditions, 4-CQA and caffeic acid appeared at the same retention time and showed a completely overlapped peak. After several trials to improve the HPLC conditions, the prune components were resolved to separate completely as 4-CQA and caffeic acid as shown in Figure 2.

Each lot of prune was extracted three times and analyzed by HPLC under the modified conditions. The results of the quantitative analyses are shown in Table 1. The total contents of phenolic components in prune were 1595−1922 mg/kg in different lots. The predominant compound was 3-CQA, followed by 4-CQA, 5-CQA, and caffeic acid in all lots tested. *p*-Coumaric acid was not detected in lot no.1, and rutin was detected only in lot no.4. In our results, prune contained 3-CQA, 4-CQA, and 5-CQA in the ratio 77.7:18.4:3.9, respectively (calculated based on the mean value of 4 lots shown in Table 1). This result was apparently different from that of plum (87:1:12, estimated from Herrmann's study, 1989).

In the previous study of other stone fruits, cherries contained 3-CQA and 3-*O*-*p*-coumaloylquinic acid as major hydroxycinnamates, followed by 5-CQA and other subordinate compounds such as 4-CQA. In apricots and peaches, 3-CQA and 5-CQA were predominant and other components were present in slightly lower amounts. In the pome fruits, apples and pears contained principally 5-CQA and other minor compounds (Möller and Herrmann, 1983). Concerning these stone and pome fruits, 4-CQA was contained only in cherries and only as a minor component. Therefore, the content of 4-CQA in prune is fairly high.

It might be supposed that a high amount of 4-CQA was obtained by isomerization among chlorogenic acid isomers, because we previously happened to find that isomerization of some plant components occurred during the extraction with protic solvent (Nakatani et al., 1991). To confirm whether isomerization of prune components occurred, during the extraction with MeOH, we extracted prune with acetone and measured the amount of chlorogenic acid isomers by HPLC analysis.

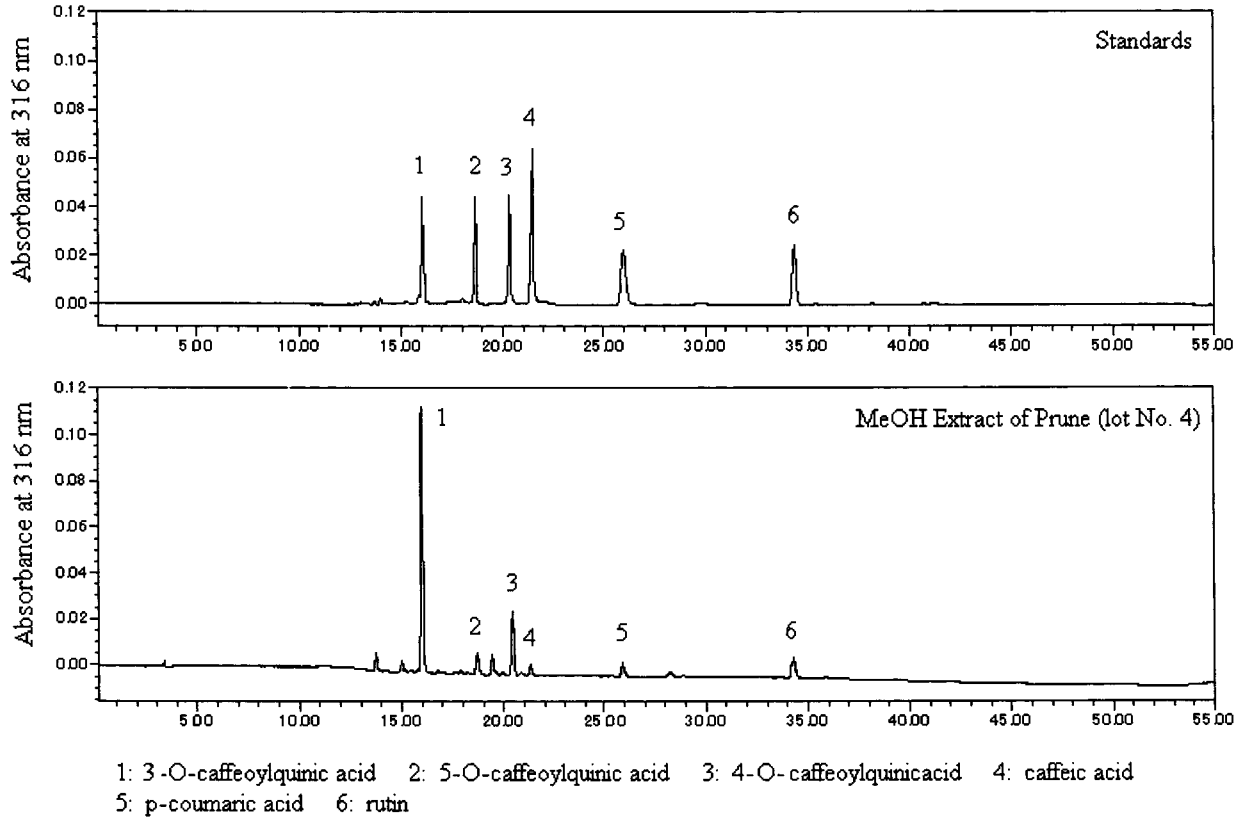

Figure 2. HPLC Chromatogram of Standards and MeOH Extract of Prune

Table 1. Concentrations of the Phenolic Components in Prune[a]

	lot no. 1 ($n = 3$)	lot no. 2 ($n = 3$)	lot no. 3 ($n = 3$)	lot no. 4 ($n = 3$)
3-*O*-caffeoylquinicacid (3-CQA)	1228 ± 8	1233 ± 13	1485 ± 15	1372 ± 41
4-*O*-caffeoylquinicacid (4-CQA)	288 ± 4	322 ± 6	289 ± 6	351 ± 17
5-*O*-caffeoylquinicacid (5-CQA)	53 ± 2	59 ± 1	77 ± 1	77 ± 2
caffeic acid	26 ± 1	25 ± 1	23 ± 1	28 ± 1
p-coumaric acid	nd[b]	28 ± 0	22 ± 1	29 ± 1
rutin	nd	nd	nd	65 ± 1
total	1596 ± 12	1666 ± 20	1894 ± 21	1920 ± 58

[a] Each lot of prune was extracted in triplicate and analyzed by HPLC. The values are expressed as mean concentration ± the standard deviation (mg/kg in edible fruit). [b] nd, not detected.

The content ratio of 3-CQA:4-CQA:5-CQA in acetone extract showed 77.5:18.6:3.9, indicating no difference from that of the MeOH extract. On the basis of these results, it is indicated that the relatively high amount of 4-CQA found in prune is characteristic.

In the chromatogram of the MeOH extract of prune (Figure 2), there are several peaks which were not identified with standard compounds based on their UV−visible spectra given by the PDA detector. We are working on isolation and structural determination of these unknown compounds.

Evaluation of the Scavenging Activity on O_2^-. The scavenging activity on O_2^- of each sample was evaluated by direct ESR measurement as shown in Table 2. The scavenging ratio of 5-CQA at a concentration of 50 μM was 30.1%. Tuchiya et al. (1996) also reported that it's activity was 53% at 100 μM. When comparing our scavenging results among the three chlorogenic acid isomers, they exhibited almost the same activity (30.1−37.0%).

On the other hand, caffeic acid showed stronger scavenging activity (41.1%) than was found for the chlorogenic acid isomers. Chen and Ho (1997) compared the free radical scavenging effect of chlorogenic acid and caffeic acid using the stable 1,1-diphenyl-2-pycrylhydrazyl (DPPH) radicals. The activity of caffeic acid was one and a half times as high as that of 5-CQA. We also obtained similar results to their study when we mea-

Table 2. Scavenging Activity on O_2^- and Inhibitory Effect against Oxidation of Methyl Linoleate of Chlorogenic Acid Isomers and Related Compounds

	scavenging ratio of O_2^- (%)[a]	OSI value (hr)[b]
3-*O*-caffeoylquinic acid (3-CQA)	31.3 ± 3.3	14.03 ± 1.23
4-*O*-caffeoylquinic acid (4-CQA)	37.0 ± 2.2	13.83 ± 1.06
5-*O*-caffeoylquinic acid (5-CQA)	30.1 ± 5.2	15.15 ± 0.39
caffeic acid	41.1 ± 5.5	46.10 ± 1.50
L-ascorbic acid	47.3 ± 1.1	
α-tocopherol		37.83 ± 1.85
BHT		22.85 ± 0.35
control		5.50 ± 0.25

[a] Each sample was measured at 50 μM in triplicate and scavenging ratio of O_2^- was acquired. The data are expressed as mean value ± the standard deviation. [b] Each sample was measured at 1 μmol/5 g of oil in triplicate and the data are expressed as mean value ± the standard deviation. OSI value is defined as the point of maximum change of the rate of oxidation.

sured the DPPH radicals scavenging activity of chlorogenic acid isomers and caffeic acid (data not shown).

Evaluation of the Inhibitory Effect against Oxidation of Methyl Linoleate by the OSI Method. As shown in Table 2, the addition of tested compounds extended the OSI value compared with that of the control (5.50). The values of the chlorogenic acid isomers were close, ranging from 13.83−15.15, which was similar to the result of the scavenging activity on O_2^-. It seems that the position of esterification on the quinic acid molecule with caffeic acid has no influence on the antioxidative activities.

On the other hand, the OSI value of caffeic acid was 46.1, which was much higher than the OSI values of the chlorogenic acid isomers. In the study of Chen and Ho (1997), the antioxidative activity of caffeic acid was higher than that of 5-CQA when lard was used as a lipid substrate. In contrast, the antioxidative activity of caffeic acid and chlorogenic acid were almost the same on the trolox equivalent antioxidant activity (TEAC; Rice-Evans et al., 1997) and against oxidation of linoleic acid (Morishita and Kido, 1995). The disparity among these assays might depend on experimental differences, such as substrate, solubility of sample, and measurement temperature.

In conclusion, three chlorogenic acid isomers, as well as caffeic acid, contribute to the antioxidative activities of prune.

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