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Quantitative Evaluation of Antioxidant Components in Prunes (*Prunus domestica* L.)

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Prunes are known to show high antioxidant activity on the basis of the oxygen radical absorbance capacity (ORAC), and their major antioxidant components are caffeoylquinic acid isomers. The aim of this study is to prove the contribution of caffeoylquinic acid isomers to the ORAC of prunes, and to investigate the existence of other antioxidant components. Caffeoylquinic acid isomers in ethanol (EtOH) extracts of prunes were quantified by HPLC analysis, and the degree of contribution of these isomers to the ORAC was found to be 28.4%; hence, it was speculated that the remaining ORAC is dependent on other antioxidant compounds. EtOH extract was partitioned between hexane and H_2O. The H_2O layer was further separated into H_2O and 2−100% methanol (MeOH) eluates by Diaion HP-20 column chromatography. Both the H_2O and 50% MeOH eluates showed high values of total phenolics and ORAC, although the contribution of caffeoylquinic acid isomers to the ORAC was low. Therefore, it was predicted that unknown antioxidants exist in these fractions, and several compounds were identified by HPLC analysis. Furthermore, hydrolysis of EtOH extract residue led to higher levels of total phenolics and ORAC, and these results suggested the existence of conjugated antioxidant components in prunes.

KEYWORDS: Prunes (*Prunus domestica* L.); oxygen radical absorbance capacity (ORAC); HPLC; caffeoylquinic acid isomers; total phenolics; hydrolysis; proanthocyanidin

INTRODUCTION

Prunes are the dried fruits of certain cultivars of *Prunus domestica* L., which belongs to the Rosaceae family that originated in the Caucasus region in western Asia. Prunes have been used medicinally in India in combination with other drugs for the treatment of leukorrhea, irregular menstruation, and debility following miscarriage (*1*), and in recent years, they have been recognized as a healthy food (*2*). In previous studies concerning prunes, it was reported that low-density lipoprotein (LDL) cholesterol in human plasma (*3*), as well as plasma and liver lipids in rats (*4*), were lowered by high intake of the dietary fibers in prunes. In addition, prune consumption has been seen to induce bone formation in postmenopausal women (*5*) and improved the bone mineral density loss (*6*, *7*) and ovariectomy-induced hypercholesterolemia (*8*) in rats.

Generally, fruits and vegetables are the natural origins of antioxidant components such as flavonoids, flavonolignans, tannins, coumarins, lignans, curcuminoids, phenolic acids, and their derivatives, and they show strong antioxidant activity (*9*−*12*). In particular, the antioxidant activity of prunes is very high in comparison to the antioxidant activities of other fruits and vegetables on the basis of the oxygen radical absorbance capacity (ORAC) (*13*). Antioxidant activities of fruits and vegetables correlate with their total content of phenolic compounds (*14*, *15*), which in prunes is 184 mg/100 g of the edible portion, as determined by HPLC analysis (*16*). The major phenolic compounds in prunes are caffeoylquinic acid isomers such as 3-*O*-caffeoylquinic acid (neochlorogenic acid) and 4-*O*-caffeoylquinic acid (cryptochlorogenic acid), which are isomers of 5-*O*-caffeoylquinic acid (chlorogenic acid) (*16*−*18*) (**Figure 1**), and it is well-known that these isomers are antioxidants for human LDL (*19*, *20*), scavengers for reactive oxygen and nitrogen species (*17*, *21*), and inhibitors against the formation of the conjugated diene from linoleic acid oxidation (*17*, *22*). The antioxidant activity of prunes had been evaluated on the basis of the ORAC (*13*) and against the oxidation of human LDL in vitro (*16*), and in a recent review, it was speculated that the antioxidant activity of prunes is highly dependent on caffeoylquinic acid isomers (*2*). However, the contribution of caffeoylquinic acid isomers to the antioxidant activity of prunes was not proved in those papers, and in our recent study, the

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Table 1. Yields, Total Phenolics, ORAC, Contribution of CQA[a] Isomers, and Proanthocyanidin of Prune Extract and Residue

	yield[b] (g)	total phenolics[c] (mg)	CQA isomers[d] (mg)	total ORAC[e] (units)	ORAC of CQA isomers[f] (units)	contribution of CQA isomers[g] (%)	proanthocyanidin[h] (mg)
EtOH extract	122.0	3380	359	12800	3660	28.4	
hexane layer	1.4	144	nd[i]	312		0	
H_2O layer	119.0	3140	342	11600	3480	30.0	
residue	33.7[j]	3780[k]		21700[l]			108

[a] CQA, caffeoylquinic acid. [b] Recovered weight of each fraction from 205 g of prunes. [c] Total phenolics are expressed as chlorogenic acid equivalents. [d] Sum of the contents of CQA isomers in each fraction determined by HPLC analysis. [e] Total ORAC values are calculated as the ORAC of each fraction (units/mg; determined by ORAC assay) × yield (mg). [f] Calculated as CQA isomers (mg) × ORAC of chlorogenic acid (3.6 units/μmol; determined by ORAC assay). [g] Calculated as ORAC of CQA isomers × total ORAC^{-1} × 100. [h] Proanthocyanidin was expressed as delphinidin equivalents. [i] nd, not detected. [j] Weight of freeze-dried residue. [k] Total phenolics of hydrolyzed residue. [l] Total ORAC of hydrolyzed residue.

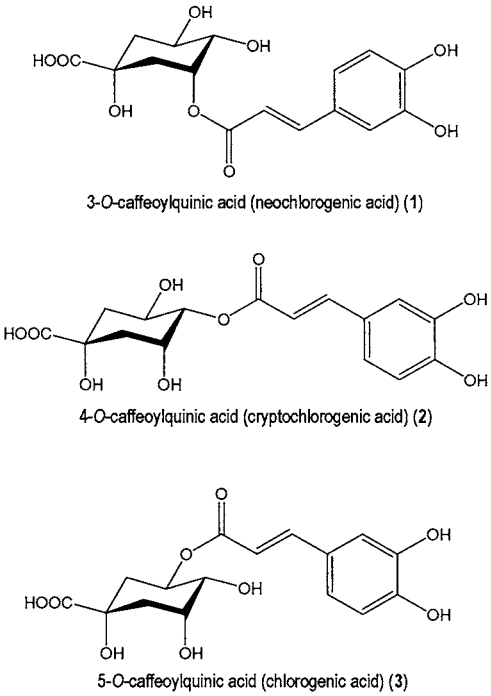

Figure 1. Structures of caffeoylquinic acid isomers.

overall antioxidant activity of prunes seems not to be dependent on caffeoylquinic acid isomers alone (*23*).

Furthermore, in recent studies, total phenolics of fruits and vegetables were measured to determine the sum of free and conjugated phenolics by the hydrolysis method (*24*, *25*). It is known that the ORAC of fruits and vegetables strongly correlates with its total phenolics (*26*, *27*); however, ORAC and total phenolics of prunes have been evaluated only for the soluble fraction. In the present study, we determined the contribution of caffeoylquinic acid isomers to the antioxidant activity of prunes and investigated the existence of unknown antioxidant components in soluble and insoluble fractions by means of ORAC, total phenolics, proanthocyanidin assay, and HPLC analysis.

MATERIALS AND METHODS

General Procedures. HPLC analysis was carried out using a Waters 600E multisolvent delivery system equipped with a 717plus autosampler and a 996 photodiode array detector (Waters Co., Milford, MA). The Arvo 1420sx (Wallac Berthold Japan Co., Tokyo, Japan) microplate reader was used for the measurement of the ORAC. Total phenolics assay and proanthocyanidin assay were carried out on a Beckman DU640 spectrophotometer (Beckman Instruments, Inc., Fullerton, CA). A Diaion HP-20 (Mitsubishi Chemical Co., Tokyo, Japan) was used for column chromatography.

Chemicals. β-Phycoerythrin from *Porphyridium cruentum* was obtained from Molecular Probes, Inc. (Eugene, OR), rutin was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan), and delphinidin chloride was obtained from Funakoshi Co., Ltd (Tokyo, Japan). Chlorogenic acid, (−)-epicatechin, and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were purchased from Aldrich Chemical Co. (Milwaukee, WI). Folin−Ciocalteu reagent, 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH), 5-hydroxymethylfurfural, caffeic acid, *p*-coumaric acid, protcatechuic acid, and 7-methoxycoumarin were obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). All commercial chemicals were of the highest analytical grade available. Neochlorogenic acid and cryptochlorogenic acid were prepared from chlorogenic acid using a method described previously (*28*). The purities of these compounds were >99% for neochlorogenic acid and >96.5% for cryptochlorogenic acid, as determined by HPLC analysis.

Plant Material. Prunes, the d'Agen cultivar, were supplied by Miki Foods Co., Ltd. (Hyogo, Japan), and were imported from the United States as the material for a commercial prune extract (concentrated prune juice). It is called "natural condition prune (NC prune)", with moisture levels adjusted to 21% (*29*).

Extraction and Fractionation of the Prune. Prune fruits (205 g) were pitted (175 g of edible portion), cut into small pieces, and homogenized with 300 mL of 90% aqueous ethanol (EtOH) for 5 min. After filtration of the extract, another portion of aqueous EtOH was added to the residue and re-extracted. This procedure was repeated five times. The combined extract was evaporated in vacuo in order to remove EtOH, followed by dissolution with 300 mL of H_2O, and then 300 mL of hexane was added and the mixture was partitioned between hexane and H_2O. After separation of the hexane layer, another portion of hexane (300 mL) was added to the H_2O layer, and this procedure was repeated five times. The hexane layers were combined and evaporated in vacuo to produce a concentrate. The H_2O layer was separated using Diaion HP-20 column chromatography employing H_2O as an eluting solution, followed by elution with 2%, 5%, 10%, 20%, 50%, and 100% methanol (MeOH) successively, and each solution was evaporated in vacuo to give the H_2O, 2%, 5%, 10%, 20%, 50%, and 100% MeOH eluates (**Figure 2**). Total phenolics and the ORAC value of each fraction were measured, and caffeoylquinic acid isomers in these fractions were quantified by means of HPLC analysis.

Total Phenolics Assay. Total phenolics in each fraction were measured using Folin−Ciocalteu reagent with chlorogenic acid as the standard. Each fraction was dissolved in 50% MeOH at 1−50 mg/mL, and 0.5 mL of the sample solution was diluted with 12 mL of H_2O. After addition of 2 mL of 2 M Na_2CO_3 (in H_2O), sample solutions were stored at 20 °C for 1 h. To the mixture was then added 0.6 mL of Folin−Ciocalteu reagent, which was diluted 2-fold before use. These prepared samples were further diluted with H_2O to 20 mL and stored at 20 °C for a further 1 h, and then the optical density of each sample solution at 675 nm was measured using the spectrophotometer. Total phenolics were quantified by calibration using the standard chlorogenic acid solution (0.05−1.5 mg/mL in H_2O).

ORAC Assay. The ORAC was measured according to a method described previously (*30*). This assay is based on the principle that antioxidant compounds delay the decrease of β-phycoerythrin fluorescence induced by AAPH, a peroxyl radical generator.

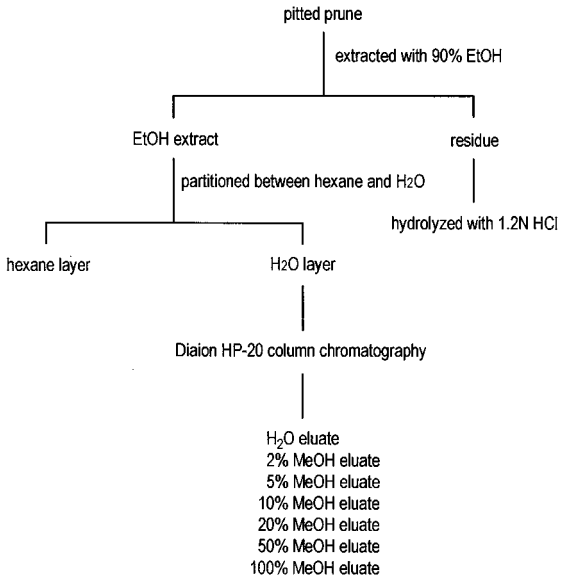

Figure 2. Procedure of extracting and fractionating prune.

The test mixture was prepared with 170 μL of 19.6 nM β-phycoerythrin in 75 mM phosphate buffer, pH 7.0, and 10 μL of sample in 75 mM phosphate buffer or acetone at 20 μM for pure compounds or 20 μg/mL for crude extract, in microwell plates (96-well, black, Corning Coaster Co., Cambridge, MA). Phosphate buffer alone was used as a blank, and 40 μM Trolox was used as a control. After 30 min of incubation at 37 °C, 20 μL of 300 mM AAPH solution was added to the mixture to initiate the assay, and the fluorescence of each well was read every 2 min over a 70-min period at 37 °C. The area under the fluorescence curve was calculated, and the ORAC value of each sample was expressed as 1 unit for 1 μmol equivalent of Trolox. Each sample was measured in triplicate, and the data were determined as mean values. The ORAC values were measured using an Arvo 1420sx instrument at an excitation wavelength of 530 nm and an emission wavelength of 570 nm. In this study, we made some modification to the former measurement conditions, and the ORAC value of chlorogenic acid was changed from 1.6 units/μmol (*23*) to 3.6 units/μmol, which is more similar to the value described in the previous study (*26*).

HPLC Analysis. The quantities of caffeoylquinic acid isomers in prune fractions were measured by HPLC analysis. Ten milligrams of each sample was dissolved in 5 mL of 50% aqueous MeOH. Standards of 3-*O*-caffeoylquinic acid (**1**), 4-*O*-caffeoylquinic acid (**2**), 5-*O*-caffeoylquinic acid (**3**) (**Figure 1**), 5-hydroxymethylfurfural (**4**), caffeic acid (**5**), *p*-coumaric acid (**6**), protocatechuic acid (**7**), rutin (**8**), (−)-epicatechin (**9**), and 7-methocycoumarin (**10**) (**Figure 4**) were also dissolved in 50% MeOH to make a concentration of 1−6 ppm. The sample and standard solutions were filtered through 0.45-μm cellulose syringe-tip filters (Sartorius K.K., Tokyo, Japan) and analyzed by means of HPLC according to a method described previously (*17*). The HPLC conditions were as follows: column, Symmetry C18, 4.6 × 250 mm, 5 μm (Waters Co., Milford, MA); column temperature, 40 °C; mobile phase, A = 50 mM $NH_4H_2PO_4$ at pH 2.60, B = 80% acetonitrile + 20% A, C = 200 mM *o*-phosphoric acid at pH 1.50; flow rate, 1.0 mL/min; gradient, 0.00 min, %A = 100.0, 4.00 min, %A = 92.0, %B = 8.0, 10.00 min, %B = 14.0, %C = 86.0, 22.50 min, %A = 1.5, %B = 16.5, %C = 82.0, 27.50 min, %B = 21.5, %C = 78.5, 45.00 min, %B = 50.0, %C = 50.0, 47.50 min, %A = 100.0, 55.00 min, %A = 100.0; gradient curve, linear gradient; injection volume, 10 μL; detection, photodiode array (200−600 nm). Each peak detected in the sample solutions was identified by comparing the retention time and UV−vis spectra obtained by using a photodiode array detector with the standards and was quantified by calibration with the standards.

Hydrolysis of EtOH Extract Residue. A portion (50 mg) of EtOH extract residue (freeze-dried) was weighed in a screw-capped tube to which was added 5 mL of 1.2 N HCl in 50% aqueous MeOH. The prepared sample was vortexed for 1 min and heated at 90 °C for 3 h. After centrifuging (3000*g*, 15 min), the supernatant was separated, and another portion of 1.2 N HCl was added to the precipitate, rehydrolyzed for 30 min, and centrifuged again. The combined supernatant was diluted to 20 mL with 50% MeOH, and total phenolics and ORAC values were measured.

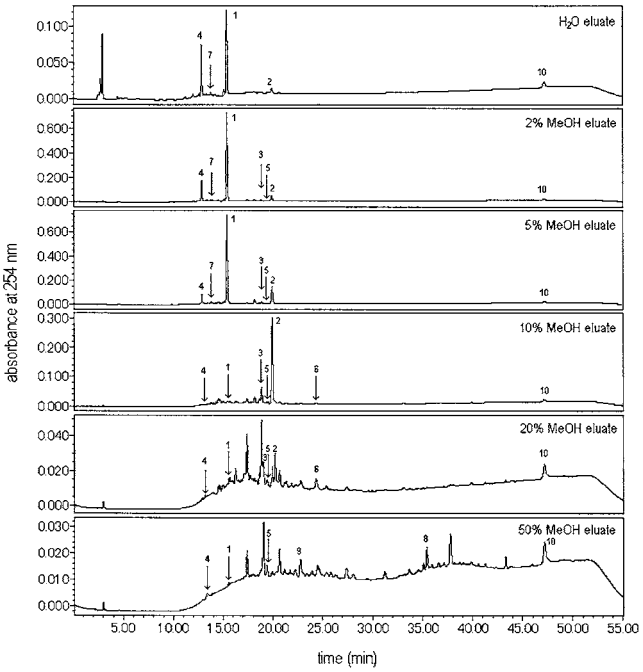

Figure 3. HPLC chromatogram of prune fractions. Peak identification: 1, 3-*O*-caffeoylquinic acid; 2, 4-*O*-caffeoylquinic acid; 3, 5-*O*-caffeoylquinic acid; 4, 5-hydroxymethylfurfural; 5, caffeic acid; 6, *p*-coumaric acid; 7, protocatachuic acid; 8, rutin; 9, (−)-epicatechin; 10, 7-methoxycoumarin.

Proanthocyanidin Assay. Proanthocyanidin in the EtOH extract residue was measured by colorimetry assay according to the method described in an earlier report (*31*). A portion (50 mg) of EtOH extract residue (freeze-dried) was weighed in a screw-capped tube to which was added 7 mL of 0.6 N HCl in *n*-butanol (BuOH) with 0.07% ferrous sulfate. The prepared sample was vortexed for 1 min, heated at 95 °C for 1 h, and filtered. The filtrate was diluted to 20 mL with 0.6 N HCl−BuOH, and then the optical density of the sample solution at 550 nm was measured using the spectrophotometer, and antocyanidin was quantified with delphinidin as the standard (0.5−4 mg/100 mL in 0.6 N HCl−BuOH). The analysis was run in duplicate, and the data were determined as mean values.

RESULTS AND DISCUSSION

Contribution of Caffeoylquinic Acid Isomers to the ORAC of Prune Extract. The procedures for extracting and fractionating prunes are summarized in **Figure 2**, and the yield of each fraction is shown in **Table 1**. The EtOH extract (122.0 g), the recovery of which was about 70% of the pitted prune, was partitioned into a hexane layer and a H_2O layer, and the yields of these fractions were 1.4 and 119.0 g, respectively. Both total phenolics and antioxidant activity on the basis of the ORAC of the H_2O layer were 20−40 times higher than those of the hexane layer (**Table 1**), and it was predicted that almost all antioxidant components in prunes are also entirely in the H_2O layer with sugars and organic acids. The total content of caffeoylquinic acid isomers in the EtOH extract was 359 mg, as measured by HPLC analysis, and the ORAC of these isomers was 3660 units. However, the total ORAC of the EtOH extract was 12 800 units, and the contribution of caffeoylquinic acid isomers to the ORAC of this fraction was as low as 28.4% (**Table 1**). Furthermore, the contribution of these isomers to the ORAC of the H_2O layer was also revealed to be as low as 30.0%; hence, it was suggested

Table 2. Yields, Total Phenolics, ORAC, and Contribution of CQA[a] Isomers of H_2O Layer Fractions

	yield[b] (g)	total phenolics[c] (mg)	total ORAC[d] (units)	ORAC of CQA isomers[e] (units)	contribution of CQA isomers[f] (%)
H_2O layer	119	3140	11600	3480	30.0
H_2O eluate	110	1200	4720	656 (18.9)[g]	13.9
2% MeOH eluate	0.29	209	1430	1010 (29.0)	70.7
5% MeOH eluate	0.27	201	1500	1060 (30.5)	70.2
10% MeOH eluate	0.17	127	928	356 (10.2)	38.3
20% MeOH eluate	0.20	150	880	70 (2.00)	7.9
50% MeOH eluate	0.46	531	1860	1 (0.02)	0.1
100% MeOH eluate	0.19	229	608		0

[a] CQA, caffeoylquinic acid. [b] Recovered weight of each fraction from 205 g of prunes. [c] Total phenolics are expressed as chlorogenic acid equivalents. [d] Total ORAC values are calculated as ORAC of each fraction (units/mg; determined by ORAC assay) × yield (mg). [e] Calculated as CQA isomers (mg, shown in **Tables 1** and **3**) × ORAC of chlorogenic acid (3.6 units/μmol; determined by ORAC assay). [f] Calculated as ORAC of CQA isomers × total ORAC^{-1} × 100. [g] Recovery ratio (%) of ORAC of CQA isomers to that of H_2O layer are given in parentheses.

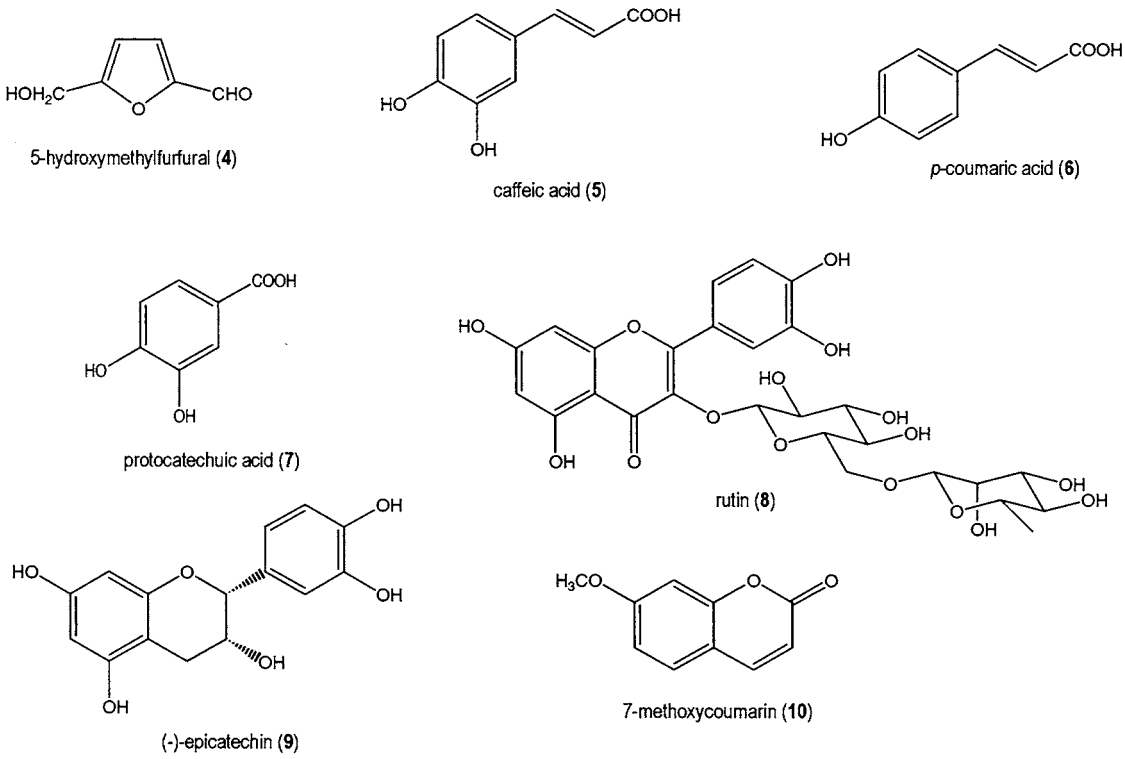

Figure 4. Structures of quantified prune components.

that unknown antioxidants exist in the H_2O layer, and their total ORAC values are higher than those of caffeoylquinic acid isomers.

Properties of Antioxidant Components in H_2O Layer Fractions. The H_2O layer, which showed high total phenolics and total ORAC, was further separated into H_2O and 2%, 5%, 10%, 20%, 50%, and 100% MeOH eluates by means of Diaion HP-20 column chromatography. The yield of the H_2O eluate was as high as 110 g (**Table 2**), and this fraction showed the highest total phenolics (1200 mg) and total ORAC (4720 units) among the separated fractions. Each of these values corresponded to about 40% of those of the H_2O layer (calculated on the basis of the values shown in **Table 2**). In the HPLC analysis, three major peaks were detected in the H_2O eluate (**Figure 3**), two of which were identified as 5-hydroxymethylfurfural (**4**) and 3-*O*-caffeoylquinic acid (**1**). The content of caffeoylquinic acid isomers (**1**−**3**) in the H_2O eluate was 64.6 mg (**Table 3**), and the contribution of these isomers to the ORAC was relatively low, at 13.9% (**Table 2**). In our study, 5-hydroxymethylfurfural, sugars, and organic acids were negative on total phenolics and ORAC assay (data not shown); hence, it was speculated that unknown antioxidant components exist in this fraction. Some other minor peaks were identified as protocatechuic acid (**7**) and 7-methoxycoumarin (**10**), and their contents in this fraction were as low as 1.3−1.5 mg (**Table 3**). It is presumed that the contributions of minor peaks, including **7** and **10**, to the ORAC are small, and other major and unidentified peaks suggest the presence of high polar antioxidant components in this fraction.

The other fractions (2−100% MeOH eluates) had 0.17−0.46 g yields and showed about 60% of the total ORAC of the H_2O layer. Concerning the 2% and 5% MeOH eluates, their total ORACs were as high as 1430−1500 units; however, total phenolics were relatively small, at 201−209 mg (**Table 2**). In the HPLC analysis, about 60% of the caffeoylquinic acid isomers of the H_2O layer existed in these eluates (calculated on the basis of the values shown in **Tables 1** and **3**), and the contributions of these isomers to the ORAC were as high as 70.2−70.7%. The major peaks detected in these fractions by HPLC analysis were identified as 3-*O*-caffeoylquinic acid (**1**),

Table 3. Contents of CQA[a] Isomers and Some Prune Components in H_2O Layer Fractions

	contents[b] (mg)							
	CQA isomers[c] (**1**–**3**)	HMF[d] (**4**)	caffeic acid (**5**)	*p*-coumaric acid (**6**)	protocatechuic acid (**7**)	rutin (**8**)	(–)-epicatechin (**9**)	7-methoxycoumarin (**10**)
H_2O eluate	64.6	38.1	nd[e]	nd	1.5	nd	nd	1.3
2% MeOH eluate	99.3	18.7	0.2	nd	1.4	nd	nd	0.3
5% MeOH eluate	103.8	7.4	0.2	nd	0.3	nd	nd	0.2
10% MeOH eluate	35.0	0.1	0.2	0.3	nd	nd	nd	0.3
20% MeOH eluate	6.9	0.1	0.4	1.5	nd	nd	nd	0.3
50% MeOH eluate	0.1	0.1	0.9	nd	nd	2.6	7.2	0.3
100% MeOH eluate	nd	nd	nd	nd	nd	nd	nd	nd

[a] CQA, caffeoylquinic acid. [b] Contents in each fraction determined by HPLC analysis. [c] Sum of the contents of CQA isomers in each fraction. [d] HMF, 5-hydroxymethylfurfural. [e] nd, not detected.

5-hydroxymethylfurfural (**4**), and 4-*O*-caffeoylquinic acid (**2**), and caffeic acid (**5**), protocatechuic acid (**7**), and 7-methoxycoumarin (**10**) were also quantified as minor components (**Table 3**). Concerning the 10% and 20% MeOH eluates, the contributions of caffeoylquinic acid isomers to the ORAC of these fractions were relatively low, at 7.9−38.3%, and the total phenolics and total ORAC were also low, at 127−150 mg and 880−928 units, respectively. In the HPLC analysis, several unknown peaks were detected, some of which were identified as caffeic acid (**5**), *p*-coumaric acid (**6**), and 7-methoxycoumarin (**10**), and their contents were 0.1−1.5 mg (**Table 3**).

Concerning the 50% MeOH eluate, the contribution of caffeoylquinic acid isomers to the ORAC was small; however, the eluate showed the relatively high total ORAC of 1860 units, corresponding to 16.1% of that of the H_2O layer (calculated on the basis of the values shown in **Table 2**), and this fraction also indicated relatively high total phenolics, at 531 mg. Many peaks were detected by HPLC analysis, and some of them were identified and quantified as 5-hydroxymethylfurfural (**4**, 0.1 mg), caffeic acid (**5**, 0.9 mg), rutin (**8**, 2.6 mg), (−)-epicatechin (**9**, 7.2 mg), and 7-methoxycoumarin (**10**, 0.3 mg). Caffeic acid, rutin, and (−)-epicatechin are known as antioxidant components (*9*, *19*, *26*) and are considered to take part in the ORAC of this fraction, and it is predicted that the detections of other unidentified peaks indicate the presence of unknown antioxidant components in this fraction. The 100% MeOH eluate showed 608 units of total ORAC and no contribution of caffeoylquinic acid isomers, and this fraction also might contain unknown antioxidant components.

Total Phenolics, ORAC, and Proanthocyanidin of Hydrolyzed Residue. In previous studies concerning prunes, antioxidant compounds were characterized only in soluble fractions by means of HPLC analysis, and the evaluation of antioxidants in the residue was insufficient. Therefore, the EtOH extract residue was hydrolyzed, and the total phenolics and ORAC were evaluated to clarify the presence of unknown antioxidants in prunes. Hydrolyzed residue showed high total phenolics, at 3780 mg, which was the same amount as that of the EtOH extract (**Table 1**). This fraction also indicated a high total ORAC (21 700 units), which was 1.4 times higher than that of the EtOH extract. It is presumed that almost all of the soluble antioxidants are entirely in the EtOH extract; hence, large amounts of conjugated compounds such as insoluble tannin or proanthocyanidin exist in the residue and were extracted as lower molecule compounds obtained by hydrolysis. To estimate the contents of insoluble antioxidants in the EtOH extract residue, proanthocyanidin assay was carried out. The content of proanthocyanidin was 108 mg (**Table 1**), and this result is considered to indicate that proanthocyanidin takes part in the antioxidant activity of the residue. However, the contents of proanthocyanidin are apparently smaller than the total phenolics of the hydrolyzed residue, and it is predicted that other conjugated antioxidants still exit in prunes.

In a previous report concerning the prunes' components, proanthocyanidin was detected at 0.79 mg/g in dried pulp by means of colorimetry assay (*19*), and the value is similar to our result in this study (0.78 mg/g, calculated on the basis of the value shown in **Table 1**). On the other hand, no proanthocyanidin was detected by LC/MS/MS analysis in a recent study which analyzed the extract of prunes (*32*). The available studies regarding tannin or proanthocyanidin are insufficient, and thus characterization of conjugated antioxidants in prunes is required.

In this study, we revealed that the contribution of caffeoylquinic acid isomers to the antioxidant activity of the EtOH extract of prunes was 28.4%, as determined by ORAC assay and HPLC analysis, suggesting the existence of unknown antioxidant components in prunes. In addition, the hydrolyzed residue of the EtOH extract also showed high antioxidant activity, and the existence of conjugated compounds such as proanthocyanidin was suggested in this part. It has become apparent that the antioxidant activity of prunes is dependent on not only caffeoylquinic acid isomers but also EtOH-soluble and -insoluble antioxidant components. Concernig insoluble antioxidants, the expression of antioxidant activity might occur during gastroenteric digestion in human. The characterization of unknown antioxidants in prunes, of both soluble and insoluble components, is required and is now in progress.

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