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LC/MS/MS Characterization of Phenolic Constituents in Dried Plums

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Dried plums are known as a healthy food in the West and used as medicine in India. They have been characterized by high concentrations of phenolic compounds, which are believed to play a crucial role in protection against various age-related diseases. Liquid chromatography/mass spectrometry/mass spectrometry (LC/MS/MS) with four different conditions was used to analyze the phytochemicals in commercial dried plums. The major components were neochlorogenic acid and cryptochlorogenic acid. Forty minor components were characterized by their MS/MS spectra and LC retention time. Six of them are novel ester isomers formed by two caffeic acids and one quinic acid. The diagnostic fragmentation patterns of different phenolics are presented on the basis of electrospray ionization (ESI) MS/MS data of components in dried plums and fourteen authentic standards.

KEYWORDS: Dried plums; *Prunus domestica*; hydroxycinnamic acids; chlorogenic acid isomers; LC/MS/MS; MS diagnostic fragmentation pattern

INTRODUCTION

Plums are the fruits of the genus *Prunus* in the Rosaceae family. The fruit of *Prunus domestica* is a common fruit consumed by humans, and the dried fruit of some cultivars of *P. domestica* is called dried plums. Dried plums are known as a healthy food in the West. In combination with other medical plant materials, dried plums are used as a medicine in India to treat leukorrhoea, irregular menstruation, and debility following miscarriage (*1*). California is the world's largest producer of dried plums (ca. 67%) (*2*).

The principal phytochemicals in dried plums are phenolics, which include phenolic acid derivatives, flavonoids, and coumarins (*3*). The total content of phenolics has been reported to be 1840 mg/kg in dried plums (*4*). Phenolics are important components of many fruits and vegetables not only because they contribute to plant color, but also because some contribute to the health functionality of these fruits and vegetables. One of the main potential health benefits of the phenolics in fruits and vegetables is their antioxidant activity, which protects low-density lipoprotein (LDL) from oxidation and, therefore, is thought to prevent various age-related diseases. The major components in dried plums are chlorogenic acid isomers (*4*, *5*) and the mean concentration of these acids in dried plums is as high as 1742 mg/kg, which represents more than 94% of total phenolics (*4*). Chlorogenic acid isomers have high antioxidant activities and inhibit LDL oxidation from 86 to 97% at 5 μM (*6*). For the minor components in plums, nine flavonol glycosides were identified, with rutin as a predominant flavonol (*7*, *8*). Other phenolics, including ethyl cinnamate and coumarins, were also detected in dried plums (*9*, *10*). Recently a number of studies were conducted on the chemistry of anthocyanins in fruits because of their high antioxidant capacity. Although anthocyanins were detected in plums (*11*), dried plums were characterized by the absence of anthocyanins (*4*, *12*, *13*).

The purpose of this study was to analyze systematically the phytochemicals in dried plums with a high-performance liquid chromatograph coupled with on-line mass spectrometry (LC/MS/MS) using an electrospray ionization (ESI) source. Four different ESI MS conditions were employed for the analysis. We report the characterization of major and minor phenolic components in dried plums by LC/MS/MS. The diagnostic fragmentation patterns of hydroxycinnamic acids, hydroxycinnamoylquinic acids, and hydroxybenzoic acids in ESI MS/MS are discussed on the basis of MS/MS data of phenolic acids in dried plums and fourteen authentic standards.

MATERIALS AND METHODS

Standards. Chlorogenic acid, 2,4-hexadienoic acid (sorbic acid), 5-(hydroxymethyl)-2-furaldehyde, salicylic acid, and *trans*-cinnamic acid were obtained from Aldrich Chemical Co. (St. Louis, MO). Gallic acid, protocatechuic acid, gentisic acid, *p*-hydroxybenzoic acid, vanillic acid, caffeic acid, syringic acid, *p*-coumaric acid, ferulic acid, benzoic acid, sinapinic acid, and rutin were purchased from Sigma Chemical Co. (St. Louis, MO).

Dried Plum Sample. The sample of dried plums (pitted prunes) contained 28 g per package and was obtained from the California Prune Board (Pleasanton, CA).

Extraction and Sample Preparation for LC/MS. The dried plums (25 g) were cut into small pieces and homogenized in 100 mL of 80% aqueous methanol at 5 °C for 10 min. The mixture was centrifuged (25 000 rpm for 30 min) and the supernatant was decanted. The extraction process was repeated two more times with the same solvent (100 mL × 2) and twice with 50% aqueous methanol (100 mL × 2). The five supernatants were combined and concentrated on the rotary

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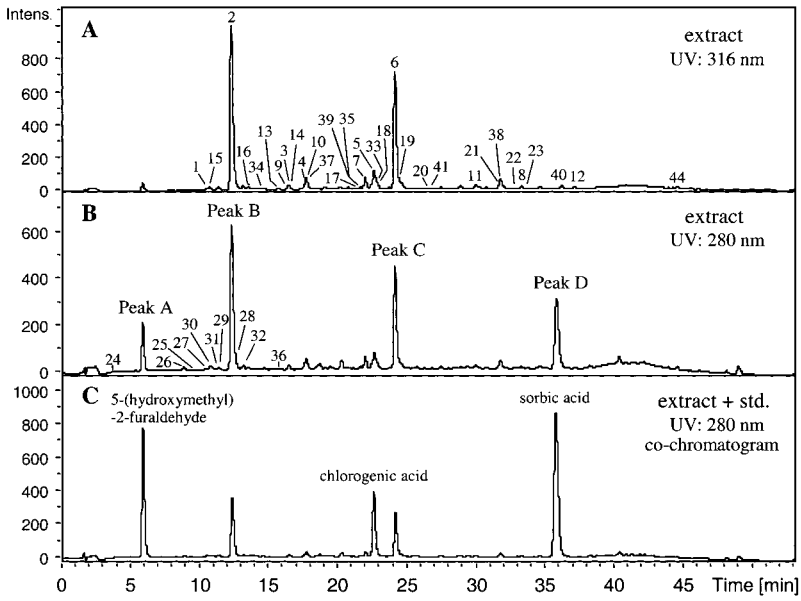

Figure 1. HPLC chromatograms: (A) 316 nm profile of dried plum extract; (B) 280 nm profile of dried plum extract; and (C) co-chromatogram, 280 nm profile of mixture of dried plum extract and standards 5-(hydroxymethyl)-2-furaldehyde, chlorogenic acid, and sorbic acid.

evaporator under reduced pressure at room temperature until methanol was removed from the extract. The concentrated extract was passed through a C18 (60 cm^3/10 gram) Mega Bond Elute cartridge after the cartridge was activated (2 vol methanol, then water). The cartridge was then rinsed with two vol water. The chemicals not retained by the C18 cartridge and eluted with water were sugars and some simple organic acids. The phytochemicals retained by the cartridge were eluted with 50% aqueous methanol, methanol, and acetone. All three effluents were combined to give a fraction of interest, which contained all phenolic and other constituents from dried plums. The combined effluents were rotary evaporated under reduced pressure at room temperature followed by drying in a freeze-dryer. The enriched extract represented 1.68% of the original dried plums (0.42 g/25 g).

Characterization of Phenolic Constituents in Dried Plums. The phytochemicals in the enriched extract were directly analyzed by LC/MS/MS. LC/MS/MS was performed using a Bruker Esquire-LC multiple ion trap mass spectrometer equipped with an Agilent 1100 series liquid chromatograph. A 150 × 4.6 mm i.d. Zorbax C18 column (Agilent Technologies, Wilmington, DE) was used at a flow rate of 1 mL/min. High-performance liquid chromatograpy (HPLC) profiles of extract were measured by a diode-array detector set at four wavelengths of 280, 316, 365, and 520 nm. Conditions for MS analysis of each HPLC peak included a capillary voltage of 4000 V, a nebulizing pressure of 30.0 psi, and the drying gas temperature at 350 °C. The HPLC gradient was methanol in 0.1% formic acid/H_2O as follows: 5 to 15% in 15 min, 15 to 30% from 15 to 35 min, 30 to 40% from 35 to 40 min, 40 to 50% from 40 to 50 min, and finally to reach 95% in 55 min. Phytochemicals were analyzed by both negative and positive ion modes of LC/MS/MS. For detection of anthocyanins, a binary gradient LC solvent composed of methanol and 5% formic acid/H_2O with the same C18 column was used in LC/MS/MS. Proanthocyanidins in dried plums were detected by the method of Lazarus et al. (*15*). In this case, a 250 × 4.6 mm i.d. 5-μ Luna silica column (Phenomenex, Torrance, CA) with mobile phase consisting of dichloromethane, methanol, and acetic acid/water (1:1, v/v) was used. The MS conditions were negative ion mode with 10 mM ammonium acetate used as an ionization reagent at a flow rate of 0.1 mL/min.

RESULTS AND DISSCUSSION

Phenolic constituents in dried plums were monitored by a diode-array detector and MS. A diode-array detector set at four wavelengths of 280, 316, 365, and 520 nm was used to characterize the benzoic acids, hydroxycinnamates, flavonols, and anthocyanins, respectively. Four major peaks, A, B, C and D, were detected at 280 nm and only two of them (peaks B and C) had major absorption at 316 nm (**Figure 1**). There were no significant peaks detected at 360 and 520 nm.

Major Components. HPLC profiles of dried plum extract were essentially identical to those previously reported (*4*). Four major peaks detected at 280 nm have been previously identified as 5-(hydroxymethyl)-2-furaldehyde, neochlorogenic acid, chlorogenic acid, and sorbic acid (**Figure 2**) (*4*). The HPLC profile from co-injection of dried plum extract and standard compounds 5-(hydroxymethyl)-2-furaldehyde, chlorogenic acid, and sorbic acid is shown in **Figure 1C**. Overlap of the peaks of 5-(hydroxymethyl)-2-furaldehyde and sorbic acid with peaks A and D, respectively, supported the assignments of these peaks, and none of the four major components were chlorogenic acid. Mass spectra of peaks B (compound **2**) and C (compound **6**) indicated that these two were chlorogenic acids isomers **(Figure 3)**. The LC retention time (**Figure 1**) and mass spectra of compound **5** are identical to those of standard chlorogenic acid, which suggests the structure chlorogenic acid for **5**. The quantitative ratio 78.7:18.4:3.9 of three isomers, neochlorogenic acid (R_t ~16.0 min), cryptochlorogenic acid (R_t ~20.5 min), and chlorogenic acid (R_t ~18.7 min) has previously been reported in dried plums (*5*). Considering the peak areas and retention times of three isomers **2**, **6**, and **5** on the C18 HPLC column (**Figure 1** and **Table 1**), compounds **2**, **6**, and **5** were identified here as neochlorogenic acid, cryptochlorogenic acid, and chlorogenic acid, respectively (**Figure 2**).

Nonphenolic compounds, 5-(hydroxymethyl)-2-furaldehyde and sorbic acid, in dried plums are not natural products in plums. 5-(Hydroxymethyl)-2-furaldehyde is formed from sugars in the drying process of plums, and sorbic acid is a preservative routinely used in dried plum processing (*4*).

Caffeoylquinic Acid Isomers. Compounds **1**−**6**, in the ESI MS with negative ion mode, gave the same $[M - 1]^-$ ion at *m*/*z* 353 in accord with a molecular formula $C_{16}H_{18}O_9$. Their molecular ions $[M - 1]^-$ yielded four peaks at *m*/*z* 191, 179, 173, and 135 in MS/MS (**Figure 3** and **Table 1**), which suggested that these six compounds were isomers. Structures of components **2**, **6**, and **5** were neochlorogenic acid, cryptochlorogenic acid, and chlorogenic acid, respectively. Chlorogenic acids are the esterified form of caffeic acid and quinic

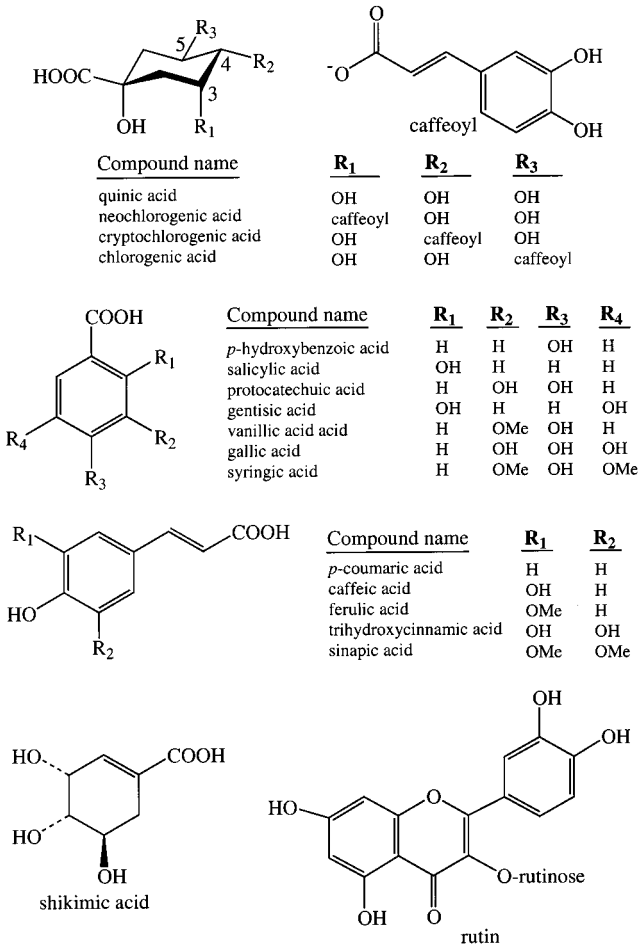

Figure 2. Structures of the phenolic compounds used in this study.

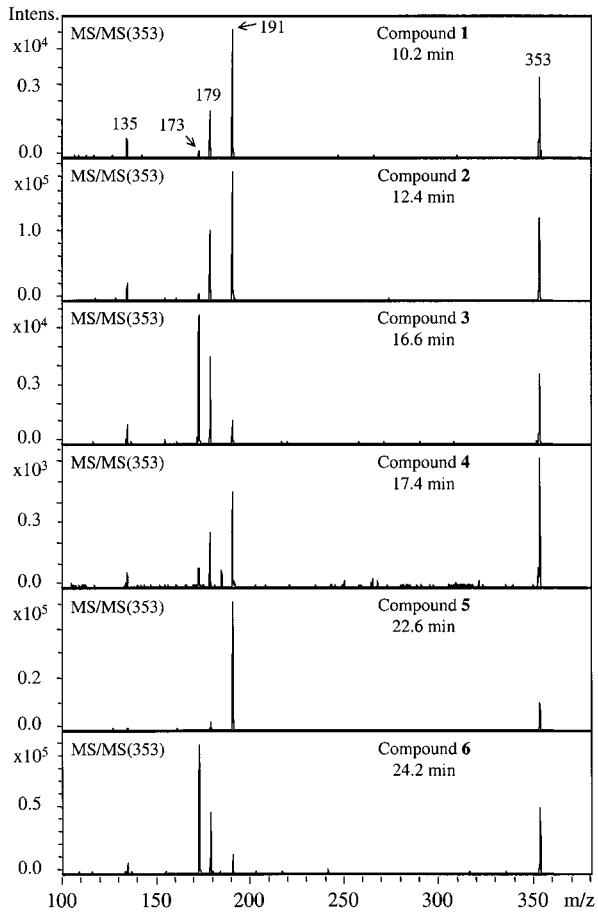

Figure 3. ESI LC/MS/MS of six chlorogenic acid isomers identified in dried plum extract.

acid. The diagnostic fragmentation patterns of chlorogenic acid isomers in ESI MS with negative ion mode involved cleavage of intact caffeoyl and quinic acid fragments. It is likely that two fragmentation pathways I and II are involved in MS/MS, and ions at *m*/*z* 191, 173, 179, and 135 were designated here as $\mathbf{Q_1}$, $\mathbf{Q_2}$, $\mathbf{C_1}$, and $\mathbf{C_2}$, respectively (**Figure 4**). The relative intensities of ions in each spectrum of chlorogenic acid isomers were significantly different using the same MS/MS conditions. MS/MS of cryptochlorogenic acid (compound **6**) was dominated by pathway I to give $\mathbf{Q_2}$ as the base peak. In contrast, pathway II of chlorogenic acid (compound **5**) yielded the base peak $\mathbf{Q_1}$ with $\mathbf{Q_2}$ in very low intensity (0.5%). These two pathways were competitive in the fragmentation process of chlorogenic acid isomers. The MS/MS spectra of compounds **1** and **4** were identical to the spectrum of neochlorogenic acid (compound **2**) (**Figure 3**), and compound **3** had an MS/MS spectra identical to that of cryptochlorogenic acid (compound **6**). Mass spectra indicated that compounds **1**−**6** were chlorogenic acid isomers. Considering neochlorogenic acid (compound **2**), cryptochlorogenic acid (compound **6**), and chlorogenic acid (compound **5**) are the esters of caffeic acid with quinic acid at positions 3, 4, and 5, respectively (**Figure 2**), the minor components **1**, **3**, and **4** might be an ester at position 1 or stereoisomers of neochlorogenic acid, cryptochlorogenic acid, and chlorogenic acid. The stereoisomers could be due to *cis*-isomers of caffeic acid or possibly different conformational forms of quinic acid.

Using ESI MS/MS in the positive ion mode, the protonated molecular ions of chlorogenic acid isomers neochlorogenic acid, cryptochlorogenic acid, and chlorogenic acid gave only one ion at *m*/*z* 163 (designated as $\mathbf{C_3}$) (**Table 1**). The typical fragmentation pathway resulted from the positive ionization of the carbonyl oxygen (**Figure 4**).

Feruloylquinic Acid Isomers. Compound **8**, in its MS spectrum, had a $[M - 1]^-$ ion at *m*/*z* 367 in accord with the $C_{17}H_{20}O_9$ formula of feruloylquinic acid. In the MS/MS spectrum, $[M - 1]^-$ gave an ion at *m*/*z* 173 as base peak with two ions at *m*/*z* 191 (4%) and 193 (8%) corresponding, respectively, to diagnostic fragments $\mathbf{Q_2}$, $\mathbf{Q_1}$, and $\mathbf{C_1}$ (**Table 1**). Diagnostic fragments $\mathbf{Q_2}$ and $\mathbf{Q_1}$ were derived from the quinic acid moiety, and $\mathbf{C_1}$ at *m*/*z* 193 indicated a hydroxy-methoxycinnamoyl moiety (**Figure 4**). Therefore, compound **8** was the ester of quinic acid and hydroxy-methoxycinnamic acid. Compound **7** also exhibited a $[M - 1]^-$ ion at *m*/*z* 367 and two ions at *m*/*z* 193 (100%) and 134 (14%) (**Table 1**). The ion at *m*/*z* 193 was $\mathbf{C_1}$ and ion at *m*/*z* 134 might be formed by loss of CH_3 from $\mathbf{C_2}$, which provided evidence that compound **7** was an isomer of compound **8**. Because feruloylquinic acid was identified previously in plums (*3*), this result might be evidence for the presence of feruloylquinic acids in dried plums. In ESI MS/MS with positive ion mode, base peaks at *m*/*z* 177 ($\mathbf{C_3}$) indicated that the same fragmentation pathway as for chlorogenic acid isomers was involved (**Figure 4**). ESI MS/MS with positive ion mode data is presented in **Table 1**.

Coumaroylquinic Acid Isomers. All MS spectra of compounds **9**−**12** yielded the molecular ions $[M - 1]^-$ at *m*/*z* 337 ($C_{16}H_{18}O_8$). Ions at *m*/*z* 191, 173, 163, and 119 in daughter spectra of $[M - 1]^-$ appeared to be the same significant fragments $\mathbf{Q_1}$, $\mathbf{Q_2}$, $\mathbf{C_1}$, and $\mathbf{C_2}$ observed in the spectra of caffeoylquinic acids (**Table 1**). Fragments $\mathbf{C_1}$ and $\mathbf{C_2}$ at *m*/*z* 163 and 119 established a coumaroyl portion, and thus

Table 1. ESI MS/MS Data for Quinic Acid Esters of Hydroxycinnamic Acids in Dried Plums

		MS/MS, *m/z* values (relative intensity, %) (base peak in MS as parent ion)							
		negative, $[M-1]^-$ as parent ion						positive, $[M+1]^+$ as parent ion	
			pathway I			pathway II			
compd. no.	R_t, min	$[M-1]^-$	C_1	C_2	Q_2	Q_1	other ion	$[M+1]^+$	C_3
caffeoylquinic acid isomers									
1	10.2	353 (62)	179 (35)	135 (15)	173 (2)	191 (100)			
2	12.4	353 (64)	179 (34)	135 (13)	173 (4)	191 (100)		355 (2)	163 (100)
3	16.6	353 (54)	179 (66)	135 (14)	173 (100)	191 (18)			
4	17.4	353 (100)	179 (42)	135 (11)	173 (15)	191 (73)			
5	22.6	353 (21)	179 (6)	135 (1)	173 (0.5)	191 (100)		355 (2)	163 (100)
6	24.2	353 (50)	179 (46)	135 (8)	173 (100)	191 (14)		355 (2)	163 (100)
feruloylquinic acid isomers									
7	22.2	367 (0.1)	193 (100)	149 (2)	173 (3)	191 (3)	$[C_2-CH_3]^-$ (14)	369 (1)	177 (100)
8	33.2	367 (0.0)	193 (8)		173 (100)	191 (4)		369 (0)	177 (100)
coumaroylquinic acid isomers									
9	16.3	337 (6)	163 (100)	119 (6)	173 (8)	191 (13)			
10	17.7	337 (4)	163 (100)	119 (4)	173 (2)	191 (4)			
11	29.8	337 (5)	163 (8)		173 (100)	191 (3)			
12	36.9	337 (6)			173 (100)		$[Q_2-H_2O]^-$ (7)		
trihydroxycinnamoylquinic acid isomers									
13	15.8	369 (0.1)	195 (36)	151 (47)		191 (100)	$[C_1-H_2O]^-$ (19)		
14	16.6	369 (0.4)	195 (100)	151 (12)	173 (18)	191 (55)			

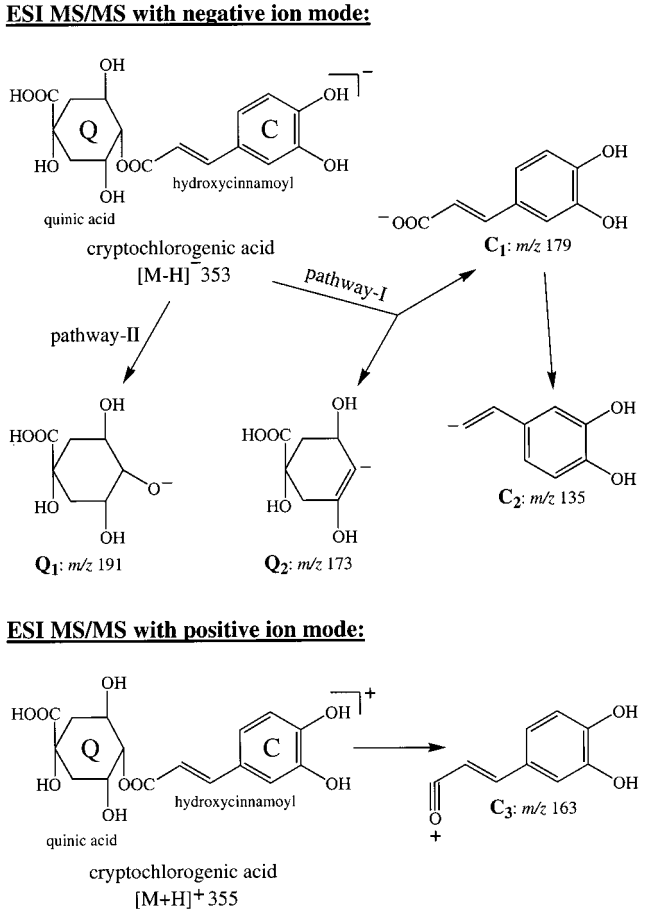

Figure 4. Proposed diagnostic mass spectral fragmentation pathways for chlorogenic acid isomers.

compounds **9**−**12** were coumaroylquinic acids. Since three isomers of *p*-coumaroylquinic acid (esterified at positions 3, 4, and 5 of quinic acid) have been reported in plums (*3*), three of them were likely to be the esters formed by *p*-coumaric acid at the 3, 4, and 5 positions of quinic acids, and one of them might be formed at position 1 of quinic acid or its stereoisomers.

Trihydroxycinnamoylquinic Acid Isomers. Compounds **13** and **14** with the same $[M-1]^-$ at *m/z* 369 commonly produced three ions at *m/z* 195, 191, and 151 in MS/MS (**Table 1**). The ion at *m/z* 191 (**Q_1**) indicated a quinic acid moiety in the structure. Ions at *m/z* 195 and 151 were 16 Da higher than **C_1** and **C_2** of chlorogenic acids, which suggested that there were three hydroxy groups on the hydroxycinnamoyl portion. Compound **14** yielded the ion at *m/z* 173 that was observed in the spectra of the caffeoylquinic acids as **Q_2**. The ion at *m/z* 177 of compound **13** was derived by the loss of H_2O from **C_1**. Thus, the structures of compounds **13** and **14** were trihydroxycinnamoylquinic acid isomers. It should be noted that this is the first characterization of trihydroxycinnamoylquinic acid in nature.

Ester Isomers Formed by Two Caffeic Acids and One Quinic Acid. Compounds **15**, **16**, **17**, **18**, **19** and **20** had the same $[M-1]^-$ ion at *m/z* 515 in accord with a $C_{25}H_{24}O_{12}$ formula of dicaffeoylquinic acid, which has been reported in plums (*3*). MS/MS of this ion from these compounds gave four pairs of ions: $[M-\mathbf{Q_1}]^-$ and **Q_1**; $[M-\mathbf{Q_2}]^-$ and **Q_2**; $[M-\mathbf{C_1}]^-$ and **C_1**; and $[M-\mathbf{C_1}-H_2O]^-$ and $[\mathbf{C_1}-H_2O]^-$ (**Figure 5** and **Table 2**), which indicated that four fragmentation processes were involved. For MS/MS of compound **18**, base peak **Q_1**, which was derived from loss of two caffeoyl fragments in sequence, indicated that compound **18** might be a dicaffeoylquinic acid. However, base peaks $[M-\mathbf{Q_1}]^-$ for compound **17** and $[M-\mathbf{Q_2}]^-$ for compounds **15**, **16**, and **19** indicated a dicaffeoyl fragment and suggested that these compounds should be caffeoylcaffeoyl quinic acids. As neither nuclear magnetic resonance (NMR) data of these minor compounds nor their standards were available, identification of these compounds could not be completed by LC/MS/MS in this study.

Caffeoylshikimic Acids. Compounds **21**, **22**, and **23**, in their mass spectra, had a $[M-1]^-$ ion at *m/z* 335 in accord with a $C_{16}H_{16}O_8$ formula. In the MS/MS spectrum, $[M-1]^-$ of all three compounds gave ions at *m/z* 179, 161, and 135 corresponding, respectively, to diagnostic fragments **C_1**, $[\mathbf{C_1}-H_2O]^-$, and **C_2** derived from a caffeoyl moiety (**Figure 4** and **Table 3**). The deprotonated molecular ions at *m/z* 335 indicated that compounds **21**, **22**, and **23** were one H_2O less than

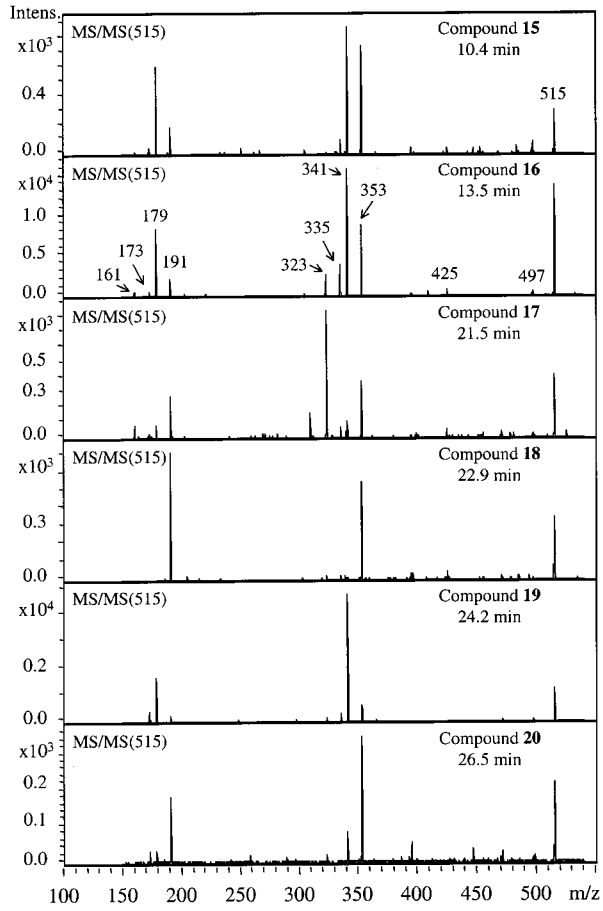

Figure 5. ESI LC/MS/MS of six ester isomers formed from two chlorogenic acids and one quinic acid identified in dried plum extract.

caffeoylquinic acid. Because three chlorogenic acid isomers (neochlorogenic, chlorogenic, and cryptochlorogenic acids) were identified as major components, it was reasonable to assume that compounds **21**, **22**, and **23** were esters of caffeic acid and dehydrated quinic acid (shikimic acid). Therefore, we inferred that compounds **21**, **22**, and **23** were caffeoylshikimic acid isomers. This was the first time that the shikimic acid esters have been detected in dried plums.

Simple Phenolic Acids and their Glycosides. Thirteen phenolic acid standards were used for identification of minor acid components in dried plums by LC/MS/MS. On the basis of the identical MS data and LC retention times of the natural acids and their standards, six phenolic acids (compounds **24**, **26**, **28**, **33**, **38**, and **40**) were identified in dried plums (**Table 4**). These were gallic acid, protocatechuic acid, *p*-hydroxybenzoic acid, caffeic acid, *p*-coumaric acid, and ferulic acid. The common fragment (base peak) for all simple phenolic acids in MS/MS spectra with negative mode was $[M - H - CO_2]^-$, which was formed by elimination of a carboxy group from deprotonated molecular ions. Twelve phenolic acid glycosides (compounds **25**, **27**, **29**, **30**, **31**, **32**, **34**−**37**, **39**, and **41**) were identified in dried plums by the common fragmentation pathway, which involves cleavage of intact sugar and aglycon fragments, and produced an aglycon ion as base peak (**Table 4**). The sugar fragments lost in MS/MS indicated that all glycosides are hexosides, except compound **32**. The sugar fragment lost is 146 Da that suggest a rhamnoside for compound **32**. Two glycosides **34** and **35** were identified as caffeic acid hexoside, and their structures may be different because (1) they are *trans*- or *cis*-caffeic acid isomer glucosides, and (2) they are a glucoside and galactoside, respectively. Because glucosides and galactosides of the same aglycon can be resolved on reverse-phase HPLC, but their retention times (R_t) are close (*14*), it is likely that compounds **34** (R_t 14.6 min) and **35** (R_t 21.6 min) are *trans*- or *cis*-caffeic acid isomer glucosides. Five of these (compounds **27**, **30**, **34, 36**, and **41**) have been previously reported in plums, and might be protocatechuic acid glucoside, vanillic acid glucoside, caffeic acid glucoside, syringic acid glucoside, and ferulic acid glucoside (*3*). It was of note that there were no other fragmentation processes observed before cleavage of aglycon and sugar fragments, as indicated by the absence of any significant ion between the molecular ion and aglycon ion (**Table 4**). The MS/MS data indicated that seven other phenolic acid glycosides identified in this study were gallic acid hexoside (compound **25**), *p*-hydroxybenzoic acid hexoside (compound **29**), methoxybenzoic acid hexoside (compound **31**), methoxybenzoic acid rhamnoside (compound **32**), methoxycinnamic acid hexoside (compound **37**), and *p*-coumaric acid hexoside (compound **39**).

Table 3. ESI MS/MS Data for Caffeoylshikimic Acids in Dried Plums

			relative intensity of ion in MS/MS (*m/z* values)			
compd. no.	R_t, min	MS $[M-H]^-$	$[M-1]^-$ (335)	C_1 (179)	C_2 (135)	$[C_1-18]^-$ (161)
21	31.2	335	100%	18.9%	53.5%	69.7%
22	32.7	335	18.7%	100%	4.5%	15.0%
23	33.6	335	100%	6.0%	75.8%	15.9%

Flavonoids. Compound **42** was identified as quercetin rutinoside by the quercetin ion (*m/z* 301, 100%) resulting from loss of rutinose from $[M - 1]^-$ at *m/z* 609 in the daughter ion spectrum of compound **42**. Furthermore, the structure of compound **42** was confirmed by the identical mass spectra and retention time with standard rutin in the same HPLC conditions (R_t 44.7 min, **Figure 1**), as reported previously (*5*).

Anthocyanins and Proanthocyanidins. For analysis of anthocyanins by LC/MS/MS, a C18 column with a binary gradient composed of methanol and 5% formic acid was used for LC and ESI in the positive ion mode with auto MS/MS. The chromatogram at 520 nm in the UV−Vis and MS and MS/MS spectra did not exhibit any anthocyanins in dried plum

Table 2. ESI MS/MS Data for Esters between Two Caffeic Acids and One Quinic Acid in Dried Plums

			relative intensity of ion in MS/MS spectra (*m/z* values)								
compd. no.	R_t, min	MS $[M-H]^-$	$[M-H]^-$ (515)	$[M-C_1-H_2O]^-$ (353)	$[M-Q_2]^-$ (341)	$[M-C_1]^-$ (335)	$[M-Q_1]^-$ (323)	C_1 (179)	C_1-H_2O (161)	Q_1 (191)	Q_2 (173)
15	10.4	515	33%	84%	100%	10%	1%	68%	1%	21%	5%
16	13.5	515	77%	55%	100%	23%	16%	51%	3%	13%	2%
17	21.5	515	48%	44%	13%	9%	100%	10%	9%	33%	3%
18	22.9	515	49%	76%	1%	2%	4%			100%	
19	24.2	515	26%	13%	100%	6%	3%	34%		4%	8%
20	26.5	515	63%	100%	24%		6%	9%	1%	51%	9%

Table 4. ESI MS/MS Data for Phenolic Acids and Their Glycosides in Dried Plums

compd. no.	R_t, min	MS, *m/z* $[M-H]^-$	MS/MS, *m/z* (rel int.) base ion	MS/MS, *m/z* (rel int.) other ions	sugar, *m/z* M − aglycon	chemical name
24 -acid[a]	3.7	169	125 (100)			gallic acid
25 -glycoside	9.5	331	169 (100)	161 (52)	162	gallic acid hexoside
26 -acid[a]	8.8	153	109 (100)			protocatechuic acid
27 -glycoside	10.6	315	153 (100)		162	protocatechuic acid hexoside
28 -acid[a]	12.7	137	93 (100)			*p*-hydroxybenzoic acid
29 -glycoside	11.8	299	none		162	*p*-hydroxybenzoic acid hexoside
standard	22.1	167	152 (100)	123 (67), 108 (33)		vanillic acid
30 -glycoside	10.9	329	167 (100)	152 (10)	162	vanillic acid hexoside
31 -glycoside	11.2	313	151 (100)		162	methoxybenzoic acid hexoside
32 -glycoside	13.3	297	151 (100)		146	methoxybenzoic acid rhamnoside
33-acid[a]	22.8	179	135 (100)			caffeic acid
34-glycoside	14.6	341	179 (100)	161 (24), 135 (5)	162	caffeic acid hexoside
35-glycoside	21.6	341	179 (100)	135 (15)	162	caffeic acid hexoside
standard	26.9	197	182 (100)	167 (12), 153 (30)		syringic acid
36-glycoside	15.9	359	197 (100)	182 (4), 167 (4), 123 (5)	162	syringic acid hexoside
37 -glycoside	17.7	339	177 (100)		162	methoxycinnamic acid hexoside
38-acid[a]	31.7	163	119 (100)			*p*-coumaric acid
39-glycoside	21.5	325	163 (100)		162	*p*-coumaric acid hexoside
40-acid[a]	36.1	193	149 (100)	178 (73), 134 (60)		ferulic acid
41-glycoside	26.8	355	193 (100)	179 (4), 149 (6), 134 (5)	162	ferulic acid hexoside

[a] The MS data and HPLC R_t of the acids were identical to those of corresponding standard acids in same HPLC condition.

extract, whereas more than twenty anthocyanins were identified in blueberries with this LC/MS/MS condition (*14*). The result supported the previous reports that dried plums do not contain any anthocyanins (*4*, *12*, *13*). Because anthocyanins including glucosides and rutinosides of cyanidin and peonidin have been reported in *Prunus domestica* (*3*), it seems likely that anthocyanins are lost in the process of making dried plums from plums. Analysis of proanthocyanidins in dried plum extract was carried out on LC/MS/MS with a silica column for LC and negative ion mode MS (*15*), but no proanthocyanidins were found in the dried plum extract using these conditions.

Application of LC/MS/MS. In this study, LC/MS/MS with four different conditions was used to analyze the phytochemicals in commercial dried plums. The negative ion mode with methanol in 0.1% formic acid/H_2O as solvent system proved to be a very sensitive method for ionization of phenolics, which characterized 42 components in dried plums. Positive ion mode with the same solvent system ionized the esters of phenolics with much lower sensitivity, and was almost unable to ionize the simple phenolic acids and their glycosides. Molecular ions of all esters were observed by MS in positive ion mode, but only five compounds had daughter spectra because the auto MS/MS used in this study only selected the base peak in MS for MS/MS. Also, the different fragmentation pathways involved cleavage of caffeoyl and quinic acid fragments in negative and positive ion modes (**Figure 4**).

Hydroxycinnamic acids are a class of phenolic compounds ubiquitously present in fruits, beverages, and grains. The common hydroxycinnamic acids, including *p*-coumaric, caffeic, and ferulic acids, predominantly occur in esterified form with quinic acid or glucose in fruits. Chlorogenic acids, esters of caffeic acid and quinic acid, are the most dominant phenolics in plums (*16*). In this study, 31 hydroxycinnamics, including acids, esters, and glycosides, were characterized in dried plums by LC/MS/MS with neochlorogenic acid and cryptochlorogenic acid as major constituents. Ten hydroxybenzoic acids and one flavonoid as minor components were also detected in dried plums. In contrast to some fruits with high antioxidant capacity, there were no detectable anthocyanins or proanthocyanidins in dried plums. The results indicated that hydroxycinnamoylquinic acids and their derivatives were predominant in the chemical constituents of dried plums, and, along with their high antioxidant activity, might be the principal active ingredients for the health functionality of dried plums. Notably, six components, which consist of two caffeic acids and one quinic acid in their structures, were separated by HPLC and characterized by MS/MS. Since caffeic acid and caffeoylquinic acids have been reported to have highest antioxidant activities and inhibited LDL oxidation among the hydroxycinamic acids typically present in fruits (*6*), the esters might be more interesting for nutriceutical use due to two caffeic acids in their structures. For example, cynarin, a dicaffeoylquinic acid, has attracted attention because of its potentiating effect on bile secretion and, therefore, its moderating effect on blood cholesterol levels (*17*). Also, dicaffeoylquinic acids have been studied as a potentially important class of HIV inhibitors that act at a site distinct from that of current HIV therapeutic agents (*18*). Further work is clearly needed to elucidate the final structures, including stereochemistry, for these six esters and to establish their antioxidant potency.

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