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Distribution of Hydroxycinnamic Acid Conjugates in Fruit of Commercial Eggplant (*Solanum melongena* L.) Cultivars

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There is gathering evidence that antioxidant phytonutrients in fruits and vegetables have health-promoting effects. Eggplant fruit have a high content of antioxidant phenolic compounds. We evaluated the main class of eggplant phenolics, hydroxycinnamic acid conjugates, in the fruit of seven commercial cultivars. Fourteen conjugates were quantified and identified by high-performance liquid chromatography, ES$^-$-MS, and ^{1}H NMR data. Significant differences in their content and composition were evident among cultivars and in tissue from stem, middle, and blossom end segments. Chlorogenic acid (5-*O*-caffeoylquinic acid) was the predominant compound, and its 3-*O*-, 4-*O*-, and 5-*O*-cis isomers were also present. The 10 other phenolics fell into four groups, including 3,5- and 4,5-dicaffeoylquinic acid isomers, four amide conjugates, two unknown caffeic acid conjugates, and 3-*O*-acetyl esters of 5-*O*- and 4-*O*-caffeoylquinic acid. Dicaffeoylquinic and 3-*O*-acetyl chlorogenic acids were most variable among the cultivars. Dicaffeoyquinic acids were most abundant in the blossom end, whereas 3-*O*-acetyl esters were highest in the midsection.

KEYWORDS: Eggplant fruit; *Solanum melongena*; chlorogenic acid; caffeoylquinic acid esters; caffeoylpolyamine amides; hydroxycinnamic acid; 3-*O*-acetyl-5-*O*-caffeoylquinic acid

INTRODUCTION

The USDA recommends daily consumption of five servings of fruits or vegetables partly on the basis of accumulated evidence that the phytonutrients in these horticultural products are beneficial to human health (*1*−*3*). The combined antioxidant activity of chemical constituents in fruit and vegetable tissues is thought to be one key factor (*4*−*6*). Eggplant is among the top 10 vegetables in oxygen radical absorbance capacity, and this is attributed to its high content of phenolic antioxidants (*7*). Phenolic compounds extracted from eggplant fruit and administered orally to normal- and cholesterol-fed rats had a significant hypolipidemic action (*8*). Winter and Herrmann (*9*) determined that quinic acid esters of hydroxycinnamic acids are the major class of polyphenols in eggplant fruit, with chlorogenic acid as the predominant compound. However, this study included a sampling of only two cultivars and focused exclusively on monohydroxycinnamic acid quinate esters and glucosides. Accurate quantitation, as well as knowledge of the complete profile of phenolic phytochemicals and their supposed health benefits, is needed to establish future dietary guidelines for recommending phenolic-rich foods such as eggplant as modulators of disease.

The cultivated eggplant, *Solanum melongena* L. (Solanaceae), is a species of considerable economic importance in many tropical and subtropical parts of the world. Consumption of eggplant is growing in the United States as ethnic diversity of urban areas increases and consumer health and diet-related education expands. Eggplant is of tropical origin and produces fruit with many different shapes, sizes, and colors, depending on the cultivar. Eggplant fruit for human consumption are harvested when they are physiologically unripe, similar to cucumbers and winter squash. The purple eggplant is widely used worldwide, but other varieties that differ in color, size, and shape are cultivated and consumed. Scant information is available about the fruit chemical composition and nutritive value among market varieties of eggplant. Studies including data on phenolic phytonutrients in the fruit have focused mainly on changes in the level of total polyphenols during fruit development and postharvest storage or on the role of these compounds as substrates for polyphenol oxidase in the enzymatic browning of cut or injured tissue (*10*−*12*). In this study, we have examined the content and distribution of hydroxycinnamic acid derivatives in fruit flesh of seven commercial eggplant cultivars representing a variety of market types.

Hydroxycinnamic acids are phenolic acids included in the large class of secondary plant metabolites known as phenylpropanoids, which are produced via a pathway initiated primarily by conversion of the amino acid phenylalanine to cinnamic acid by the enzyme phenylalanine ammonia lyase (*13*). Typically,

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hydroxycinnamic acids in fruit tissues are almost entirely esterified to other polyhydroxylated compounds such as quinic acid, tartaric acid, and glucose (*14*, *15*). Esters of caffeic acid predominate in solanaceous species (*16*), and the most abundant of these compounds is almost invariably chlorogenic acid (5-*O*-caffeoylquinic acid). Quinic acid can also be esterified with ferulic acid, *p*-coumaric acid, and (rarely) sinapic acid, and an array of isomers as well as di- and trihydroxycinnamoylquinic acid esters occurs at lower concentrations. Chlorogenic acid and other caffeoyl esters are among the most potent free radical scavengers found in plant tissues (*17*, *18*), and chlorogenic acid acts as an antioxidant agent both in human erythrocytes (*19*) and for human low-density lipoproteins in vitro (*20*). A variety of caffeoyl esters, most notably diesters of quinic and tartaric acid, have been shown to have antiviral activity, including efficacy against human immunodeficiency virus (*21*, *22*). This class of hydroxycinnamic acid derivatives has also been reported to possess antimutagenic activity (*23*) and antimicrobial activity against several species of human pathogenic bacteria (*24*). Our quantitative analysis of hydroxycinnamic acid conjugates in eggplant fruit included four isomers of monocaffeoylquinic acid, two isomers of dicaffeoylquinic acid, four partially characterized amide derivatives, two unidentified caffeic acid conjugates, and two compounds tentatively identified as novel 3-*O*-acetyl esters of caffeoylquinic acid isomers.

MATERIALS AND METHODS

Plant Material and Cultural Methods. Seeds of seven commercial eggplant (*S. melongena* L.) cultivars, Black Magic, Classic, Epic, Ghostbuster, Orient Express, Elondo, and Pirouette, were selected to represent various eggplant market types. Black Magic, Classic, and Epic produce large, black/purple-pigmented, globular to tapered fruit characteristic of the predominant market class in the United States. Elondo is marketed as a large, cylindrical Italian type with dark purple elongated teardrop-shaped fruit. Ghostbuster produces unique white-pigmented, mid-sized, elongated, egg-shaped fruit. Orient Express is an early fruiting cultivar, producing black-pigmented, very elongated, Asian style fruit. Pirouette is a novel, very early producing cultivar bearing small teardrop-shaped, purple-pigmented fruit.

After germination of the seed, plants were grown from seedlings in a greenhouse using standard production practices. Six 7 week old plants of each accession were transplanted to field plots at the Beltsville Agricultural Research Center, Beltsville, MD, into Keyport fine loam soil using standard horticultural practices for eggplant production in Maryland (*25*). Plants were spaced at 0.45 m intervals in single rows on polyethylene-covered raised beds, with beds positioned on 1.5 m centers. Fertilizer and supplemental water was supplied using trickle irrigation.

Three fruit from each of three plants for each cultivar were harvested at market maturity (assessed visually), which ranged from about 30−40 days postanthesis. At harvest, the fruit were washed and peeled, and a 2 cm wide longitudinal section (from stem to blossom end) was excised from the middle. This tissue slice was divided equally into upper (stem end), middle (central mesocarp), and bottom (blossom end) segments, each of which was quickly diced, frozen in liquid N_2, and lyophilized. The freeze-dried tissue samples were pulverized and stored individually in small Ziploc bags at −80 °C.

Tissue Extraction and Sample Preparation. Phenolic acids were extracted from 0.2 g samples of the lyophilized, powdered tissue by sonicating for 15 min in 10 mL of methanol containing 0.5% butylated hydroxytoluene (BHT). The first methanol extract was decanted after centrifugation, and the tissue sample was extracted a second time with 10 mL of methanol plus BHT. The two extracts were combined, filtered through Whatman No. 4 filter paper, and then passed through a Whatman PTFE syringe filter (0.2 μm pore size). Filtered extracts were stored in capped brown glass vials at −80 °C until removed for high-performance liquid chromatography (HPLC) analysis.

A 1.5 mL aliquot of each extract was transferred to a 2 mL amber HPLC vial, followed by addition of 25 μg of sesamol (3,4-methylenedioxyphenol) in 25 μL of methanol as an internal standard (*26*). The solvent was removed by N_2 evaporation at 35 °C, and the residue was resuspended in 1.0 mL of 0.02% (2 mM) phosphoric acid in methanol−water, 1:1 (v/v), with vortexing for 30 s. After centrifugation for 3 min to pellet the insoluble BHT, the supernatant was transferred to a 2 mL microfuge tube and centrifuged at 16 000*g* for 1.5 min to pellet remaining particulates. The supernatant was transferred to a new 2 mL amber HPLC vial, which was flushed with N_2 and sealed with a Teflon-lined septum cap. When not analyzed immediately, samples were stored overnight at −80 °C.

HPLC Analysis. Phenolics in 20 μL injections of the eggplant fruit extracts were separated and quantified by RP-HPLC using an HP 1100 Series instrument with a quaternary pump, autosampler, and photodiode array detector (Agilent Technologies). Data were acquired and analyzed with HP ChemStation software on a pentium PC. A method was developed to achieve separation of the major constituents on a 250 mm × 4.6 mm i.d., 5 μm Luna C18(2) analytical column (Phenomenex, Torrence, CA) within 30 min. A binary mobile phase gradient of methanol in 0.01% aqueous phosphoric acid was used as follows: 0−15 min, linear increase from 5 to 25% methanol, 1.0 mL/min; 15−25 min, linear increase from 25 to 50% methanol, 1.0 mL/min; 25−28 min, 50% methanol, 1.0 mL/min; 28−30 min, linear increase from 50 to 100% methanol and from 1 to 1.2 mL/min; 30−32 min, 100% methanol, 1.2 mL/min; 32−36 min, linear decrease from 100 to 5% methanol, 1.2 mL/min; 36−38 min, 5% methanol, linear decrease from 1.2 to 1.0 mL/min. Quantification was based on absorbance at 325 nm relative to the sesamol internal standard and an external standard of authentic chlorogenic acid purchased from Aldrich (Milwaukee, WI). An isochlorogenic acid standard including 3,4-, 3,5-, and 4,5-dicaffeoylquinic acid isomers was obtained from ICN-K&K Laboratories (Plainview, NY).

LC-MS Analysis. Atmospheric pressure ionization mass spectrometry analysis was performed on a Quattro LC benchtop triple quadrupole mass spectrometer (Micromass Ltd., Manchester, U.K.) operated using the electrospray ionization interface in the negative mode (ES^-). Mass spectrometric data were acquired in the full scan mode over the *m*/*z* 150−600 range. Sensitivity of the mass spectrometer was optimized using the chlorogenic acid standard from Aldrich. A Waters 2690 HPLC system was utilized for separation of phenolics in the eggplant extracts and caffeoylquinic acid standards. The Luna C18(2) column and mobile phase gradient were identical to those used for RP-HPLC-UV analysis with the following exceptions: 0.05% aqueous formic acid was substituted for 0.01% phosphoric acid, and the initial linear increase from 5 to 25% methanol at 1.0 mL/min extended from 0 to 12 min rather than 0−15 min. Eggplant phenolic samples were in methanol−water, 1:1 (v/v), including 0.1% formic acid, and 20 μL was injected per run by a Waters autosampler. Mass spectrometric analyses were performed with MassLynx 3.5 software (Micromass Ltd). UV spectroscopic data were acquired using a Waters model 996 photodiode array detector over the range of 210−400 nm.

NMR Spectroscopy. Caffeoylquinic acid standards and major eggplant fruit phenolics isolated by HPLC were dissolved in 0.8 mL of CD_3OD, and ^1H NMR spectra were acquired deuterium-locked at 25 °C using a Bruker QE 300 MHz NMR spectrometer. Chemical shift values were assigned relative to the frequencies of residual nondeuterated water and methanol externally referenced to tetramethylsilane.

Statistical Analyses. The SAS System (SAS Institute, Cary, NC) was used to perform all statistical analyses. Analysis of variance was obtained with the SAS General Linear Models procedure with cultivars treated as fixed effects.

RESULTS AND DISCUSSION

Fourteen compounds that were present in many but not all fruit and fruit tissue segments were separated by HPLC (**Figure 1**) and identified or tentatively identified as hydroxycinnamic acid derivatives (**Table 1**). Identification was based on HPLC elution times, UV absorbance spectra, ES^--MS mass spectrometric data, and in some cases ^1H NMR data. As indicated in

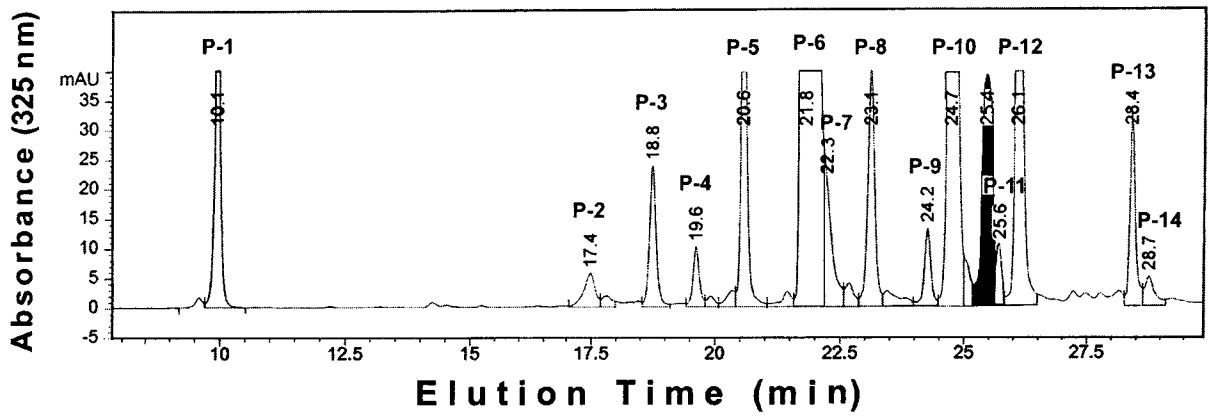

Figure 1. HPLC chromatogram showing separation of the 14 hydroxycinnamic acid conjugates in eggplant fruit extracts that were characterized and quantified (labeled P-1–P-14). Chlorogenic acid (P-6) was invariably the most abundant phenolic acid derivative. The internal standard peak (sesamol) is highlighted in black. Note that in this chromatogram the two compounds tentatively identified as 3-*O*-acetyl esters of 5-*O*- and 4-*O*-caffeoylquinic acid (P-10 and P-12, respectively) are at levels 10–200-fold higher than those in fruit tissue of the seven cultivars examined.

Table 1. Grouping Based on Identification or Tentative Identification of the 14 Hydroxycinnamic Acid Conjugates in Eggplant Fruit Extracts that Were Quantified by HPLC–UV and Subjected to Statistical Analyses

	elution time (min)	UV absorbance maxima (280–330 nm)	ES$^-$-MS $[M-1]^-$ ion	conjugate identification
			group 1	
peak 2	17.4	324, 297 (sh)[a]	353	3-caffeoylquinic acid
peak 6	21.8	326, 296 (sh)	353	5-caffeoylquinic acid
peak 8	23.1	327, 297 (sh)	353	4-caffeoylquinic acid
peak 9	24.2	318	353	5-*cis*-caffeoylquinic acid
			group 2	
peak 13	28.4	328, 298 (sh)	515	3,5-dicaffeoylquinic acid
peak 14	28.7	328, 296 (sh)	515	4,5-dicaffeoylquinic acid
			group 3	
peak 1	10.1	317, 292	249	*N*-caffeoylputrescine
peak 3	18.8	320, 289	470[b]	dihydroxycinnamoyl amide
peak 4	19.6	319, 288	470[b]	dihydroxycinnamoyl amide
peak 5	20.6	319, 293	468[b]	*N*,*N*′-dicaffeoylspermidine
			group 4	
peak 7	22.3	329, 298 (sh)	355	caffeic acid conjugate
peak 11	25.6	326, 296 (sh)	nd[c]	caffeic acid conjugate
			group 5	
peak 10	24.7	327, 297 (sh)	395	3-acetyl-5-caffeoylquinic acid
peak 12	26.1	325, 296 (sh)	395	3-acetyl-4-caffeoylquinic acid

[a] sh, secondary absorbance maximum appearing as a shoulder on the primary maximum. [b] $[M-1]^-$ ion with even mass number suggests that the structure includes an odd number of nitrogen atoms. [c] $[M-1]^-$ ion was not determined.

Table 1, these 14 phenolics were grouped into five classes as follows: chlorogenic acid isomers (group 1), isochlorogenic acid isomers (group 2), hydroxycinnamic acid amide conjugates (group 3), unknown caffeic acid conjugates (group 4), and acetylated chlorogenic acid isomers (group 5).

Group 1 included four compounds with HPLC elution times of ~17.4, 21.8, 23.1, and 24.2 min (**Figure 1**; P-2, P-6, P-8, and P-9). They were identified as the 3-*O*-trans, 5-*O*-trans, 4-*O*-trans, and 5-*O*-cis isomers of caffeoylquinic acid (**Figure 2**; neochlorogenic acid, chlorogenic acid, cryptochlorogenic acid, and *cis*-chlorogenic acid, respectively). Criteria used for identification were as follows: ES$^-$-MS spectra of all four phenolics had a prominent molecular ion minus a proton, $[M-1]^-$, at *m*/*z* 353, required for caffeoylquinic acid ($C_{16}H_{18}O_9 = 354$). For the 17.4 and 23.1 min caffeoylquinic acids, *m*/*z* 353 was the major ion (100% relative intensity), whereas for the 21.8 and 24.2 min isomers, the quinic acid ion, *m*/*z* 191, was predominant. The HPLC peaks at 17.4, 21.8, and 23.1 min had closely similar UV spectra, whereas that of the 24.2 min peak was distinctly different (**Table 1**). The HPLC elution time, ES$^-$-MS spectrum, and ^{1}H NMR spectrum of the highly abundant phenolic at 21.8 min were identical to those of the chlorogenic acid (5-*O*-caffeoylquinic acid) standard. The elution order of the 17.4 and 23.1 min isomers indicated that they were 3-*O*- and 4-*O*-caffeoylquinic acid, respectively (*18*, *27*), and comparison of their ^{1}H NMR spectra with published values confirmed this (*18*, *28*). Compared with chlorogenic acid, the 24.2 min isomer had a similar ES$^-$-MS spectrum but a very different UV spectrum, which suggested that it was 5-*O*-*cis*-caffeoylquinic acid. This was confirmed by the ^{1}H NMR signal for the olefinic proton on C8, a doublet with $J = 13$ Hz at 5.78 ppm, indicative of a cis double bond between C7 and C8 (*29*).

Group 2 consisted of two phenolics with elution times of ~28.4 and 28.7 min (**Figure 1**; P-13 and P-14), both with UV spectra very similar to that of chlorogenic acid (**Table 1**), and $[M-1]^-$ at *m*/*z* 515, required for dicaffeoylquinic acid (isochlorogenic acid; $C_{25}H_{24}O_{12} = 516$). Elution times and mass spectra of the 28.4 and 28.7 min isomers were identical to those of 3,5-di-*O*- and 4,5-di-*O*-caffeoylquinic acid standards (**Figure 2**), which were identified by comparison of their ^{1}H NMR spectra with published values (*30*, *31*).

Group 3 included four phenolics with elution times of ~10.1, 18.8, 19.6, and 20.6 min (**Figure 1**; P-1, P-3, P-4, and P-5). Tentative identification of these constituents as hydroxycinnamic acid amide conjugates was based on their UV spectra, molecular masses, and ^{1}H NMR data. UV spectra of the 10.1 and 20.6 min peaks were very similar, as were those of the 18.8 and 19.6 min peaks (**Table 1**). ES$^-$-MS of all four compounds in group 3 gave the $[M-1]^-$ ion at 100% relative intensity (**Table 1**). This was the sole ion for the 10.1 min phenolic, whereas mass spectra of the 18.8, 19.6, and 20.6 min compounds included a sodium adduct ion at ~10% relative intensity (*m*/*z* 492, 492, and 490, respectively). The almost identical UV and mass spectra of the 18.8 and 19.6 min compounds indicate that they are isomers of the same phenolic, and the 20.6 min compound, with a molecular mass two protons less than these, may be a related phenolic with an additional double bond. Furthermore, the even-numbered mass of the $[M-1]^-$ ion of all three suggests that their structures include an odd number of nitrogen atoms. Amide conjugates of hydroxycinnamic acids with tyramine and polyamines are common in solanaceous

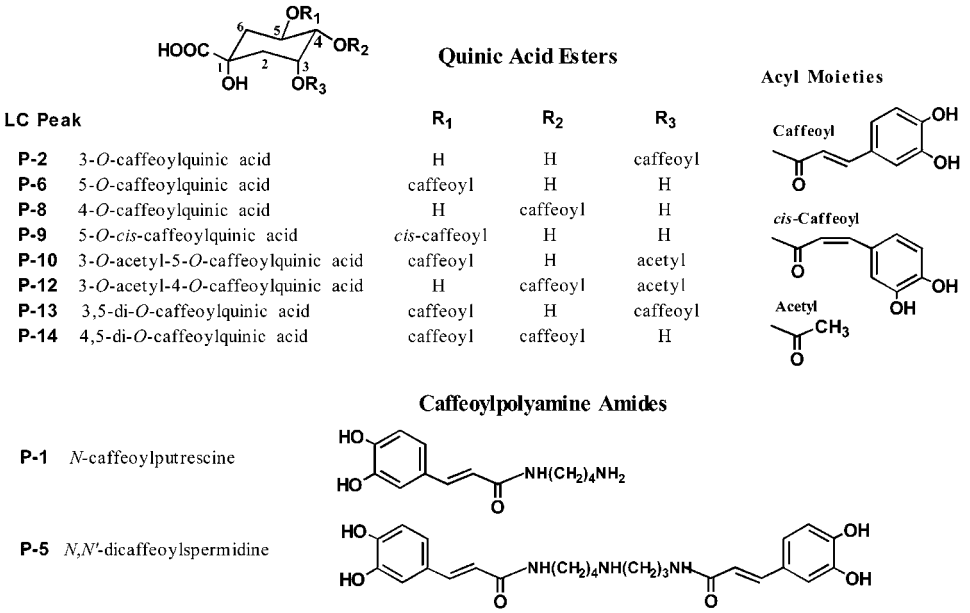

Figure 2. Structures of 10 hydroxycinnamic acid conjugates in eggplant fruit extracts that were identified or tentatively identified and represent four groups classified as chlorogenic acid isomers (group 1), isochlorogenic acid isomers (group 2), hydroxycinnamic acid amide conjugates (group 3), and acetylated chlorogenic acid isomers (group 5). LC peak numbers (P-1–P-14) correspond to those indicated in the HPLC chromatogram in **Figure 1**.

species (*15*, *32*). On the basis of its $[M - 1]^-$ ion (*m*/*z* 468) and ^{1}H NMR spectrum, the 20.6 min compound was identified as *N*,*N*′-dicaffeoylspermidine ($C_{25}H_{31}N_3O_6 = 469$) (**Figure 2**). ^{1}H NMR data were as follows (CD_3OD; δ): 1.91−1.97 (6H, m) [C2−H, C3−H, C6−H], 3.00−3.07 (8H, m) [C1−H, C4−H, C5−H, C7−H], 6.35 (1H, d, $J = 16$ Hz) [C8″-H], 6.36 (1H, d, $J = 16$ Hz) [C8′−H], 6.76 (2H, d, $J = 8$ Hz) [C5′−H, C5″−H], 6.88 (1H, dd, $J = 2$, 8 Hz) [C6″−H],], 6.91 (1H, dd, $J = 2$, 8 Hz) [C6′−H], 7.00 (1H, d, $J = 2$ Hz) [C2″−H], 7.01 (1H, d, $J = 2$ Hz) [C2′−H], 7.40 (1H, d, $J = 8$ Hz) [C7″−H], 7.43 (1H, d, $J = 8$ Hz) [C7′−H]. The 10.1 min phenolic was tentatively identified as *N*-caffeoylputrescine ($C_{13}H_{18}N_2O_3 = 250$) (**Figure 2**) on the basis of its $[M - 1]^-$ ion (*m*/*z* 249), the close similarity of its UV spectrum to that of *N*,*N*′-dicaffeoylspermidine, and partial ^{1}H NMR data in the 6−8 ppm region consistent with a caffeic acid moiety exhibiting chemical shifts similar to those noted in *N*,*N*′-dicaffeoylspermidine.

The two phenolics of group 4 had elution times ∼22.3 and 25.6 min (**Figure 1**; P-7 and P-11). Tentative identification as caffeic acid conjugates (other than esters of quinic acid) was based solely on the similarity of their UV spectra to those of the mono- and di-*O*-caffeoylquinic acid isomers in groups 1 and 2 (**Table 1**). Because both of these compounds were typically very minor constituents and each overlapped with other phenolics, it was not possible to isolate them or obtain reliable mass spectra.

The final group of phenolics, group 5, was composed of two compounds with elution times of ∼24.7 and 26.1 min (**Figure 1**; P-10 and P-12). Their UV spectra differed only slightly from one another and were similar to that of chlorogenic acid (**Table 1**). The ES$^-$-MS spectra of both included *m*/*z* 233 at 100% relative intensity, $[M - 1]^-$ at *m*/*z* 395, and a sodium adduct ion at *m*/*z* 417. Complete ^{1}H NMR data for the 24.7 min phenolic were as follows (CD_3OD; δ): 2.08−2.19 (3H, m) [C2eq−H, C2eq−H, C6ax−H], 2.29 (1H, dd, $J = 4$, 13 Hz) [C6eq−H], 3.57 (3H, s) [O═C−O−Me], 3.92 (1H, dd, $J = 3$, 8 Hz) [C4−H], 5.30 (1H, m) [C3−H], 5.37 (1H, m) [C5−H], 6.24 (1H, d, $J = 16$ Hz) [C8′−H], 6.76 (1H, d, $J = 8$ Hz) [C5′−H], 6.95 (1H, dd, $J = 2$, 8 Hz) [C6′−H], 7.04 (1H, d, $J = 2$ Hz) [C2′−H], 7.54 (1H, d, $J = 8$ Hz) [C7′−H]. Signals from the five protons on C2′, C5′, C6′, C7′, and C8′ in the 6−8 ppm region matched those of the caffeoyl moiety in chlorogenic acid with very slight shifts; signals from the C3, C4, and C5 quinic acid protons indicated that both the 3-OH and the 5-OH were esterified (*28*); and the 3-proton singlet at 3.57 ppm indicated a probable ester-linked methyl group (*33*). These data together with the mass spectrometric data are consistent with the structure 3-*O*-acetyl-5-*O*-caffeoylquinic acid, $C_{18}H_{20}O_{10} = 396$ (**Figure 2**). The principal ion at *m*/*z* 233 in the ES$^-$-MS spectra of both the 24.7 and the 26.1 min phenolics can be accounted for by the 3-*O*-acetylquinic acid moiety, $C_9H_{14}O_7 = 234$. Considering the relative abundance and retention times of the 5-*O*- and 4-*O*-caffeoylquinic acid isomers in group 1, it is likely (but not yet determined) that the 26.1 min compound is 3-*O*-acetyl-4-*O*-caffeoylquinic acid (**Figure 2**).

Analysis of the 14 hydroxycinnamic acid conjugates in extracts from the fruit of *S. melongena* revealed considerable diversity in composition and content among the seven commercial cultivars and among tissue segments within the fruit (**Table 2**). Wide variation in hydroxycinnamic acid content among cultivars has been noted for many other fruits (*15*, *34*). Significant differences between cultivars were evident for composition and content of groups 1, 2, 3, 5, and total hydroxycinnamic acid conjugates (*F* value 3.79*, 12.41***, 96,-89***, 5.77**, and 6.05**, respectively, where *, **, and *** indicate significance at $P \leq 0.05$, 0.01, and 0.001, respectively). Levels of group 4 caffeic acid conjugates were not significantly different between cultivars (*F* value 2.05). Black Magic, Elondo, and Classic contained the highest levels of phenolics. Total phenolic acid content was lowest in the cultivar Orient Express, consistent with the perception by consumers that the Asian type eggplants have a milder, less bitter flavor in comparison with other market types.

As reported by Winter and Herrmann (*9*), chlorogenic acid isomers in group 1 were the major class of hydroxycinnamic acid conjugates, with their content varying 2.3-fold among the cultivars evaluated. Group 1 accounted for 77.6−94.9% of the total conjugates and comprised a greater percentage in cultivars containing low levels of total phenolic acids compared with those having high levels (**Table 2**). With the exception of

Table 2. Composition and Content of Hydroxycinnamic Acid Conjugates in Stem End, Midsection, and Blossom End Segments of Fruit Flesh from *S. melongena* Cultivars

		μmol/100 g dry weight; (% of total)					
cultivar	slice[a]	group 1[b]	group 2	group 3	group 4	group 5	total
Black Magic	1	1875 (77.4)	5.4 (0.2)	512.0 (21.1)	26.5 (1.1)	1.9 (0.1)	2421
	2	3298 (79.2)	9.9 (0.2)	782.8 (18.8)	49.3 (1.2)	24.8 (0.6)	4165
	3	2868 (75.9)	43.8 (1.2)	815.8 (21.6)	43.9 (1.2)	8.1 (0.2)	3779
	$\bar{x}$	2680 (77.6)	19.7 (0.6)	703.6 (20.4)	39.9 (1.2)	11.6 (0.3)	3455
Elondo	1	1692 (76.9)	1.0 (0.04)	484.8 (22.0)	21.8 (1.0)	1.4 (0.1)	2201
	2	3298 (83.7)	3.2 (0.1)	562.7 (14.3)	37.8 (1.0)	39.0 (1.0)	3941
	3	2715 (79.9)	35.2 (1.0)	595.0 (17.5)	33.4 (1.0)	17.7 (0.5)	3397
	$\bar{x}$	2569 (80.8)	13.1 (0.4)	547.5 (17.2)	31.0 (1.0)	19.4 (0.6)	3180
Classic	1	1494 (92.3)	2.3 (0.1)	94.2 (5.8)	27.0 (1.7)	1.4 (0.1)	1618
	2	3249 (91.3)	21.3 (0.6)	214.3 (6.0)	35.9 (1.0)	39.8 (1.1)	3560
	3	3402 (89.4)	68.9 (1.8)	273.1 (7.2)	37.4 (1.0)	25.7 (0.7)	3807
	$\bar{x}$	2715 (90.7)	30.8 (1.0)	193.9 (6.5)	33.4 (1.1)	22.3 (0.7)	2995
Epic	1	1107 (83.9)	0.6 (0.04)	186.6 (14.1)	23.5 (1.8)	1.6 (0.1)	1319
	2	2128 (81.5)	8.0 (0.3)	411.7 (15.8)	39.8 (1.5)	24.0 (0.9)	2612
	3	1562 (77.9)	25.9 (1.3)	369.7 (18.4)	29.2 (1.5)	17.8 (0.9)	2005
	$\bar{x}$	1599 (80.8)	11.5 (0.6)	322.6 (16.3)	30.8 (1.6)	14.5 (0.7)	1979
Ghostbuster	1	1009 (92.7)	1.1 (0.1)	60.2 (5.5)	16.5 (1.5)	1.0 (0.1)	1088
	2	2343 (88.6)	8.3 (0.3)	169.4 (6.4)	24.3 (0.9)	99.4 (3.8)	2644
	3	1783 (85.4)	27.5 (1.3)	194.9 (9.3)	20.5 (1.0)	60.6 (2.9)	2087
	$\bar{x}$	1712 (88.2)	12.3 (0.6)	141.5 (7.3)	20.5 (1.1)	53.7 (2.8)	1940
Pirouette	1	1314 (96.7)	1.5 (0.1)	28.3 (2.1)	13.4 (1.0)	1.3 (0.1)	1359
	2	2030 (94.5)	1.2 (0.1)	95.2 (4.4)	17.6 (0.8)	4.3 (0.2)	2148
	3	2011 (94.3)	6.4 (0.3)	89.0 (4.2)	19.5 (0.9)	7.3 (0.3)	2133
	$\bar{x}$	1785 (94.9)	3.0 (0.2)	70.8 (3.8)	16.9 (0.9)	4.3 (0.2)	1880
Orient Express	1	638 (95.1)	1.5 (0.2)	20.3 (3.0)	10.8 (1.6)	0.8 (0.1)	671
	2	1448 (95.0)	0.2 (0.01)	46.6 (3.1)	28.9 (1.9)	1.1 (0.1)	1524
	3	1506 (93.9)	1.2 (0.1)	60.6 (3.8)	34.6 (2.2)	1.4 (0.1)	1604
	$\bar{x}$	1197 (94.5)	1.0 (0.1)	42.5 (3.4)	24.8 (2.0)	1.1 (0.1)	1267

[a] Slices 1–3 were taken from the stem end, midsection, and blossom end, respectively, of a longitudinal tissue segment cut from the middle of each fruit; $\bar{x}$ is the mean value for all three slices. [b] Group 1, chlorogenic acid isomers; group 2, isochlorogenic acid isomers; group 3, amide conjugates; group 4, unknown caffeic acid conjugates; and group 5, acetylated chlorogenic acid isomers.

Classic, the content of group 2 isochlorogenic acid isomers (3,5-di-*O* > 4,5-di-*O*-caffeoylquinic acid) and group 3 hydroxycinnamic acid amide conjugates decreased as levels of chlorogenic isomers declined. Much less variation in content among cultivars was noted for group 4 caffeic acid derivatives (2.5-fold) than for conjugates in groups 2, 3, or 5 (30.8-, 16.6-, and 48.8-fold, respectively). Second to chlorogenic acid isomers, group 3 amide conjugates accounted for the bulk of the additional phenolic acids (3.4−20.4%) in all cultivars. The levels of these compounds can be highly dynamic, and various types of biotic and abiotic stress can increase their synthesis (*35*, *36*). Although these factors cannot be disregarded, the 16.6-fold range in amide conjugate content appears to be largely a function of cultivar and the level of total phenolic acids. The remaining three groups, isochlorogenic acid isomers, group 4 caffeic acid conjugates, and acetylated chlorogenic acid isomers, comprised only 0.1−2.8% of total hydroxycinnamic acid conjugates in fruit of the seven cultivars.

Significant differences between fruit stem and blossom end tissues and between fruit stem and midsection tissues were observed among all five groups of hydroxycinnamic acid conjugates and in total conjugates across cultivars (**Tables 2** and **3**). Only isochlorogenic acid isomers and acetylated chlorogenic acid isomers differed significantly between the fruit midsection and the blossom end; the former were most abundant in the blossom end, whereas the latter were most concentrated in the midsection. The relatively high total hydroxycinnamic acid content in the fruit midsection and blossom end could be associated with seed development or may simply reflect the advanced physiological maturity of these tissues as compared with the fruit stem end. Isochlorogenic acid isomers and acetylated chlorogenic acid isomers exhibited the greatest variation from stem end to midsection and blossom end (25.9- and 15.9-fold increase, respectively), whereas group 1 chlorogenic acid isomers, group 3 amide conjugates, and group 4

Table 3. Variation in Content of Total Hydroxycinnamic Acid Conjugates in Stem End, Middle, and Blossom End Segments of Eggplant Fruit ($n = 62$ for Slices 1–3 in Each Group)

group[a]	slice [b]	mean content (μmol/100 g dry wt)	*t* value[c] 1 and 2	*t* value[c] 2 and 3	*t* value[c] 1 and 3
1	1	1304	-7.59^{***}		
	2	2550		1.46^{NS}	
	3	2268			-6.22^{***}
2	1	1.9	-3.78^{***}		
	2	7.5		-6.02^{***}	
	3	30.2			-8.10^{***}
3	1	200.8	-2.79^{**}		
	2	329.8		-0.32^{NS}	
	3	346.7			-3.14^{**}
4	1	20.0	-4.93^{***}		
	2	33.6		0.68^{NS}	
	3	31.4			-4.14^{***}
5	1	1.3	-5.93^{***}		
	2	33.7		2.15^{*}	
	3	20.0			-5.69^{***}
total	1	1528	-7.09^{***}		
	2	2954		1.11^{NS}	
	3	2696			-6.17^{***}

[a] Group 1, chlorogenic acid isomers; group 2, isochlorogenic acid isomers; group 3, amide conjugates; group 4, unknown caffeic acid conjugates; and group 5, acetylated chlorogenic acid isomers. [b] Slices 1–3 were from the stem end, middle, and blossom end, respectively. [c] *t* Value for differences between means for slices 1 and 2, 2 and 3, and 1 and 3. $^{NS, *, **, ***}$ signify nonsignificant or significant at $P \leq 0.05$, 0.01, and 0.001, respectively.

caffeic acid conjugates showed much smaller increases of 2.0-, 1.7-, and 1.7-fold, respectively. Possibly, enzymes responsible for the additional esterification of 5-*O*- or 4-*O*-caffeoylquinic acid to yield these diesters are all but absent in stem end tissue or, alternatively, the chlorogenic acids may have to reach a certain threshold level before diester synthesis becomes significant.

In conclusion, this study determined that the content of potentially health beneficial hydroxycinnamic acid conjugates is substantial in eggplant fruit from commercial cultivars, ranging from about 0.5−1.5% on a dry weight basis. This is in accord with the value of about 600−660 mg/kg fresh weight reported by Winter and Herrmann (*9*) and puts eggplant on a par with sweet cherry, kiwi, and several other fruits that are among the highest in phenolic acid content (*15*, *34*). It was also found that the levels and distribution of the individual conjugates vary considerably among fruit from different cultivars and in different tissue zones within each fruit. The content and distribution of hydroxycinnamic acid derivatives in fruit of various eggplant cultivars are likely to be influenced by climate, cultural practices, harvest maturity, and postharvest storage conditions. Total polyphenols declined by about 20−50% in eggplant fruit stored for 20 days at 5, 10, or 20 °C (*11*) and increased by about 30−45% in fruit of three market types between 30 and 42 days after fruit set (42 days being considered physiological maturity) (*12*). Although chlorogenic acid isomers comprised the bulk of the hydroxycinnamic acid derivatives in fruit of all cultivars and in all tissues, several less abundant phenolics showed the highest degree of variability among cultivars and fruit tissues. Two of these were tentatively identified as 3-*O*-acetyl esters of 5-*O*- and 4-*O*-caffeoylquinic acid. Confirmation of these structures will require correlation spectroscopy and ^{13}C NMR analyses, but if correct, this appears to be the first report of these phenolics in any plant tissue. Should they prove to be of particular pharmacological or nutritional interest, it is noteworthy that they were recently found to be abundant in the fruit of a wild eggplant species, *Solanum anguivi* (Stommel and Whitaker, unpublished). Plant polyphenolics are the least well-known but quantitatively the most important phytochemicals (*34*). Because various phytochemicals have different functions and are distributed differently within the body, combinations of compounds are likely to afford greater health protective effects than any individual phytonutrient (*4*, *6*). Our evaluation of eggplant hydroxycinnamic acid conjugates provides data that will be useful in future health-related epidemiological studies and human trials to ascertain the influence of this class of phenolics on human health and in development of new cultivars with optimal hydroxycinnamic acid composition and content.

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