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Identification of Phenolic Compounds in Plum Fruits (*Prunus salicina* L. and *Prunus domestica* L.) by High-Performance Liquid Chromatography/Tandem Mass Spectrometry and Characterization of Varieties by Quantitative Phenolic Fingerprints

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ABSTRACT: Plums (*Prunus domestica* L. and *Prunus salicina* L.) are edible fruits mostly consumed in America, China, and Europe. We have used LC-MS^n to detect and characterize the phenolic compounds in plum varieties. Forty-one phenolics were detected comprising caffeoylquinic acids, feruloylquinic acid, *p*-coumaroylquinic acids, methyl caffeoylquinates, methyl *p*-coumaroylquinate, caffeoylshikimic acids, catechin, epicatechin, rutin, esculin, quercetin, quercetin-3-*O*-hexosides, dimeric proanthocyanidins, trimeric proanthocyanidins, caffeoyl-glucoside, feruloyl-glucoside, *p*-coumaroyl-glucoside, vanillic acid-glucosides, 3,4-dihydroxybenzoyl-glucoside, quercetin-3-*O*-pentosides, quercetin-3-*O*-rhamnoside, quercetin-pentoside-rhamnosides, and 3-*p*-methoxycinnamoylquinic acid. This is the first time when 3-*p*-methoxycinnamoylquinic acid is reported in nature. Chlorogenic acids and proanthocyanidins were the major phenolics present in plums. Furthermore, HPLC with DAD and chemical reaction detection was used to generate quantitative phenolic fingerprints from the fruit flesh of 33 plum varieties. The predominant compound was 3-caffeoylquinic acid in nearly all varieties studied; generally, however, the qualitative and quantitative profiles showed high diversity even among closely related progenies.

KEYWORDS: *chlorogenic acids, plum, proanthocyanidins, phenolics, methyl quinates, caffeoyl shikimates, Prunus domestica L., Prunus salicina L., Prunus cerasifera L., Prunus spinosa L., tandem mass spectrometry, HPLC*

INTRODUCTION

The global annual production of plums is approximately 11,000,000 tons.[1] Plums are considered all over the world to be healthy food. In India, plums are used for medicinal purposes to treat leucorrhea, irregular menstruations, and debility following miscarriage.[2,3] On many occasions, it has been reported that plums are a rich source of phenolics such as chlorogenic acids (CGAs),[3−7] proanthocyanidins (PAs),[6−9] flavonoids, flavonoid glycosides, anthocyanins, cinnamoyl-hexoses, benzoyl-hexoses,[4−8] cinnamic acids, benzoic acids,[3] and coumarins (Figure 1).[10,11] Several pharmacological activities of these phenolics including antioxidant activity,[12] anticancer,[13,14] antimutagenic,[15] antidiabetic,[16] anti-inflammatory,[17] and anti-HIV[18] properties were also reported. These phenolics are also beneficial for wound healing,[19] reduce the risks of cardiovascular diseases,[20] skin diseases,[21] and protect from drug toxicity,[22] UV radiation,[23] and asthma.[24]

The aim of this study was to identify and characterize the phenolics, especially CGAs, PAs, and flavonoid glycosides from plum cultivars by liquid chromatography −multistage mass spectrometry. Recently, LC-MS^n has been used to characterize cinnamoyl-amino acid conjugates,[25] dimeric PAs,[26] CGAs,[27−33] shikimates,[31,33,34] lactones,[34] and methyl quinates of CGAs.[35] For routine analysis, an analytical HPLC method using conventional DAD and chemical reaction detection methods[36] were adopted and applied for a number of cultivars, wild type plums, and hybrids. A former chemometric study of plum skin phenolics revealed the intraspecific heterogeneity of *P. domestica* L., which may be due to its hexaploid nature.[36]

MATERIALS AND METHODS

All chemicals (of analytical grade) were purchased from Sigma-Aldrich (Bremen, Germany). Fresh Japanese plums (*Prunus salicina* L., originating from Chile, Spain, and South Africa) were purchased from the supermarkets in Bremen. The names of the cultivars are unknown. All other varieties (Table 1) were harvested from the experimental orchard of the unit Fruit Science of the Technische Universität München in Freising-Weiehenstephan (Germany). 3-Caffeoylquinic acid (neochlorogenic acid) **1**, 4-caffeoylquinic acid (cryptochlorogenic acid) **3**, 5-caffeoylquinic acid **4**, catechin **15**, epicatechin **16**, rutin **33**, quercetin **39**, quercetin-3-*O*-glucoside **35**, quercetin-3-*O*-galactoside **34**, esculin **24**, proanthocyanidin B1 **17**, and proanthocyanidin B2 **20** were used as authentic standards.

Methanolic Extract of Plums. For the identification of phenolic compounds, whole *P. salicina* L. plum fruits (5 g) of the three origins (Chile, Spain, and South Africa) were crushed to a paste and extracted with aqueous methanol (70%, 100 mL) by a Soxhlet extractor. The methanol and the water were removed in vacuo, and the residue was stored at −20 °C until required, thawed at room temperature, dissolved in methanol (50 mg/10 mL), filtered through a membrane filter, and

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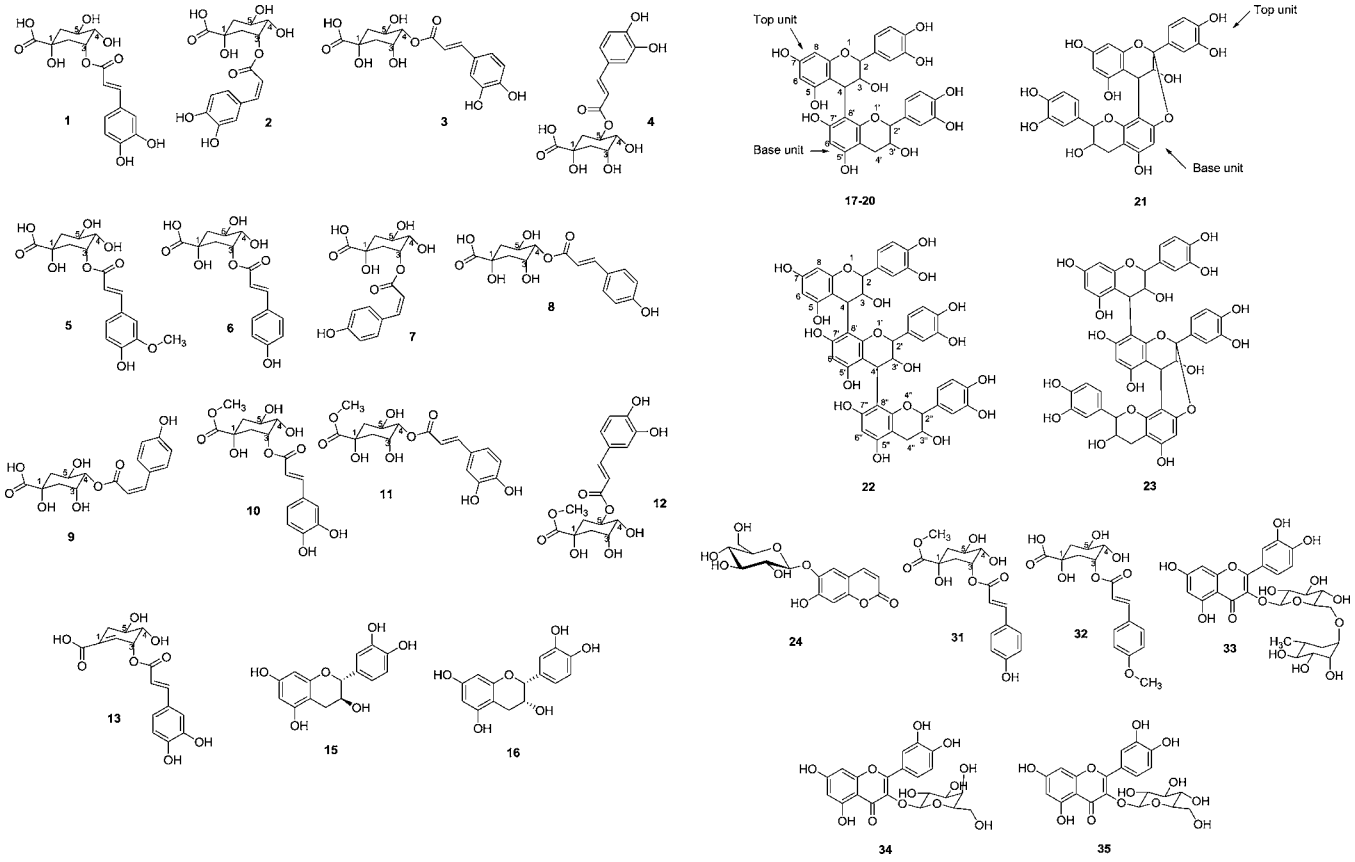

Figure 1. Structures of phenolic compounds in plums.

then used for LC-MS. This extract of the complete fruits was used for the identification of the phenolic compounds.

From the other plum samples, the skin was quickly removed from fresh fruits in order to analyze the part of the fruit which mainly contributes to the uptake of phenolics when consumed. Eight grams of fruit flesh from all varieties was extracted in triplicate with 10 mL of methanol in an ultrasonic water bath for 30 min. After centrifugation, the solvent of the supernatant was evaporated to dryness and redissolved with 2 mL of methanol, which was used for HPLC and LC-MS analysis.

LC-MS^n. The LC equipment (Agilent, Karlsruhe, Germany) comprised a binary pump, an auto sampler with a 100 μL loop, and a DAD detector with a light-pipe flow cell (recording at 320, 280, and 254 nm and scanning from 200 to 600 nm). This was interfaced with an ion-trap mass spectrometer fitted with an ESI source (Bruker Daltonics, Bremen, Germany) operating in full scan, auto-MS^n mode to obtain fragment ion m/z. As necessary, MS^2, MS^3, and MS^4 fragment-targeted experiments were performed to focus only on compounds producing a pseudomolecular ion at m/z 289, 301, 315, 325, 329, 335, 337, 339, 341, 351, 353, 367, 433, 447, 463, 575, 577, 579, 609, 863, and 865. Tandem mass spectra were acquired in the auto-MS^n mode (smart fragmentation) using a ramping of the collision energy. Maximum fragmentation amplitude was set to 1 V, starting at 30% and ending at 200%. The MS operating conditions (negative mode) had been optimized using 5-caffeoylquinic acid **4** and proanthocyanidin B1 **17** with a capillary temperature of 365 °C, a dry gas flow rate of 10 L/min, and a nebulizer pressure of 10 psi. High resolution LC-MS was carried out using the same HPLC equipped with a MicrOTOF Focus mass spectrometer (Bruker Daltonics, Bremen, Germany) fitted with an ESI source,, and internal calibration was achieved with 10 mL of a 0.1 M sodium formate solution injected through a six port valve prior to each chromatographic run. Calibration was carried out using the enhanced quadratic mode, and the mass error was below 5 ppm.

HPLC Coupled to MS. Separation was achieved on a 150 × 3 mm inner-diameter column containing 5 μm diphenyl, with a 5 mm × 3 mm inner-diameter guard column (Varian, Darmstadt, Germany). Solvent A was water/formic acid (1000:0.05 v/v), and solvent B was methanol. Solvents were delivered at a total flow rate of 500 μL/min. The gradient profile was from 10% B to 70% B linearly in 60 min followed by 10 min of isocratic and a return to 10% B at 90 and 10 min isocratic to re-equilibrate.

HPLC-DAD/CRD. The HPLC system used for generating the quantitative phenolic profiles is described by Treutter et al.[36] For chemical reaction detection, the 4-(dimethylamino)cinnamaldehyde reagent was used to specifically detect the flavanols at 640 nm after derivatization.[37,38]

For quantification, phenolic compounds were grouped and monitored at related wavelengths: CGAs at 320 nm and flavanols at 640 nm. The calculation was done on the basis of specific response factors for reference compounds. Hydroxycinnamic acids were calculated as 5-CQA, monomeric flavanols as catechin and epicatechin, respectively, and the remaining flavanols as procyanidin B2 **20**.

RESULTS AND DISCUSSION

Preliminary Assessment of Data. All of the data for CGAs and flavonoids presented in this article use the recommended IUPAC numbering system and nomenclature suggested by Porter,[39,40] respectively; the structures are presented in Figure 1. When necessary, previously published data have been amended to ensure consistency and avoid ambiguity. Not all of the phenolics reported here were present in all of the plum samples (Table 2). The *P. salicina* L. plums from Chile, Spain, and South Africa were extracted as whole fruits including the skin. Flavonols could only be found in these fruits and to some extent in *P. spinosa* 'Haferschlehe' L. and in the *P. domestica* L. × *P. spinosa* L.-hybrid '*P. fruticanus* Slaponice' L. It is assumed that the accumulation of flavonols is restricted to the skin where they occur abundantly.[6,36] With the exceptions mentioned, this study

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Table 1. Plum Varieties and Hybrids Used in This Study

variety	origin	species
unknown cultivar	Chile	*P. salicina* L.
unknown cultivar	Spain	*P. salicina* L.
unknown cultivar	South Africa	*P. salicina* L.
Krichele Dürnau	wild type	*P. domestica* L. (prune)
Wei 1491	Haganta × Jubileum	*P. domestica* L. (prune)
Wei 1492	Haganta × Jubileum	*P. domestica* L. (prune)
Toptaste	Valor × Hauszwetsche	*P. domestica* L. (prune)
Topper	Câcânska najbolja × Auerbacher	*P. domestica* L. (prune)
President	traditional variety	*P. domestica* L. (prune)
Pogauner	traditional variety	*P. domestica* L. (prune)
Miragrande	Herrenhäuser Mirabelle × Gelbe Zwetsche	*P. domestica* L. (mirabelle)
Green Gage	traditional variety	*P. domestica* L. (reineclaude)
Câcânska rodna	Stanley × Pozegaca	*P. domestica* L. (prune)
Ortenauer	traditional variety	*P. domestica* L. (prune)
Katinka	Ortenauer × Ruth Gerstetter	*P. domestica* L. (prune)
Jojo	Ortenauer × Stanley	*P. domestica* L. (prune)
Hoh 6087	Jojo × Hanita	*P. domestica* L. (prune)
Wei 1660	Jojo × *P. Spinosa* L.	*P. domestica* L. × *P. spinosa* L.
Wei 238	Jojo × *P. spinosa* L.	*P. domestica* L. × *P. spinosa* L.
Wei 243	Jojo × *P. spinosa* L.	*P. domestica* L. × *P. spinosa* L.
Wei 244	Jojo × *P. spinosa* L.	*P. domestica* L. × *P. spinosa* L.
Wei 247	Jojo × *P. spinosa* L.	*P. domestica* L. × *P. spinosa* L.
Wei 252	Jojo × *P. cerasifera* L.	*P. domestica* L. × *P. cerasifera*
Wei 261	Jojo × *P. cerasifera* L.	*P. domestica* L. × *P.cerasifera* L.
Wei 256	Jojo × *P. cerasifera* L.	*P. domestica* L. × *P. cerasifera* L.
Wei 266	Jojo × *P. cerasifera* L.	*P. domestica* L. × *P. cerasifera* L.
Wei 267	Jojo × *P. cerasifera* L.	*P. domestica* L. × *P. cerasifera* L.
Prunus fruticanus Slaponice		*P. domestica* L. × *P. spinosa* L.
Haferschlehe	wild type	*P. spinosa* L.
Späte Myrobalane	wild type	*P. cerasifera* L.
Tatjana		*P. cerasifera* L./*P. salicina* L.
Songold		*P. salicina* L. (plum)
Aprimira		*P. domestica* L. × *P. armeniaca* L.

focused on fruit flesh. The distribution of flavanols is highly different among the samples studied. It is striking that this group was not detectable in the *P. salicina* L. samples from overseas, while the variety 'Songold' exhibited a rather complete flavanol profile.

Quantification of CGAs (Table 3) was achieved by inter- and intraday LC-MS experiments.[41]

In general, CGAs and their derivatives can be identified in an all tandem mass spectrum EIC (extracted ion chromatogram) by their unique fragments at m/z 173 and 191. Selected ion monitoring at m/z 335, 337, 351, 353, and 367 immediately located 16 chromatographic peaks eluting between 13 and 29 min, each with a UV spectrum typical of CGAs and cinnamates [λ_{max} 320 nm]. During this study, we did not observe any aliphatic acid-containing monoacyl, diacyl, and triacyl, or tetraacyl quinic acid isomers.[42−44]

Five dimeric and two trimeric PAs were identified in the UV chromatogram (λ_{max} 280 nm) showing the m/z values in the negative ion mode at 575, 577, 863, and 865. Three cinnamoyl-glucoses and three benzoyl-glucoses were also detected in the UV chromatogram. The elemental composition of all of the compounds was determined by high resolution LC-ESI-TOF mass spectrometry. All of the compounds identified displayed mass errors below 5 ppm.

Characterization of Caffeoylquinic Acids (*Mr* 354). Four caffeoylquinic acids eluting from ∼13−18 min were easily located and assigned using the hierarchical keys previously developed[27,30] as the well-known 3-caffeoylquinic acid (**1**), *cis*-3-caffeoylquinic acid (**2**), 4-caffeoylquinic acid (**3**), and 5-caffeoylquinic acid (**4**). Detailed mass spectra have been published previously and are not repeated here.[27,30,31]

Characterization of Feruloylquinic Acid (*Mr* 368) and Methyl Caffeoylquinates (*Mr* 368). The feruloylquinic acid isomer **5** eluting at ∼21 min was assigned as 5-feruloylquinic acid[27] (Table 4). Three peaks located (∼42−52 min) in the extracted ion chromatogram each produced the pseudomolecular ion at m/z 367 (**10**−**12**) and were tentatively assigned as methyl caffeoylquinates. The first (**10**) and the second (**11**) eluting isomers produced the MS^2 base peak at m/z 161 ([caffeic acid-H_2O-H^+]$^-$) and an MS^2 secondary peak at m/z 135 ([caffeic acid-CO_2-H^+]$^-$) (Table 4). The third eluting isomer (**12**) produced the MS^2 base peak at m/z 179 and MS^2 secondary peaks at m/z 135 ([caffeic acid-CO_2-H^+]$^-$), 161 ([caffeic acid-H_2O-H^+]$^-$), and 191 ([quinic acid-H^+]$^-$) (Table 4). It produced the MS^3 base peak at m/z 135 ([caffeic acid-CO_2-H^+]$^-$) by the loss of a CO_2 molecule. The retention times and fragmentation patterns of these isomers (**10**−**12**) were compared to the previously studied methyl caffeoylquinates and were assigned as methyl 3-caffeoylquinate, methyl 4-caffeoylquinate, and methyl 5-caffeoylquinate, respectively.[35]

Characterization of *p*-Coumaroylquinic Acid (*Mr* 338). Four peaks detected in the extracted ion chromatogram

Table 2. Presence of Phenolic Compounds in Different Cultivars/Origin of Plums[a]

		Plum Varieties																
		P. salicina L.[b]			*P. domestica* L.									*P. domestica* L. × *P. spinosa* L.	*P. spinosa* L.	*P. cerasifera* L.	*P. salicina* L.	
phenolic compounds		origin Chile	origin Spain	oriin South-Africa	Krichele Dürnau	Toptaste	President	Miragrande	Green Gage	Cacanska rodna	Ortenauer	Katinka	Jojo	P. frutic. slap.	Haferschlehe	Späte Myrobalane	Tatjana	Songold
Chlorogenic Acids																		
3-caffeoylquinic acid	**1**	P	P	P	P	P	P	P	P	P	P	P	P	P	P	–	P	P
cis-3-caffeoylquinic acid	**2**	P	P	–	P	P	P	P	–	P	–	–	P	P	P	–	P	–
4-caffeoylquinic acid	**3**	P	P	–	P	P	P	P	–	P	P	–	–	P	P	–	–	P
5-caffeoylquinic acid	**4**	P	P	P	P	P	P	P	–	P	P	P	P	P	P	P	–	P
caffeoyl-glucoses	**29**	–	–	–	–	–	P	–	P	–	P	–	P	P	–	–	–	–
methyl 3-caffeoylquinate	**10**	P	P	–	P	P	P	–	P	P	P	–	–	P	P	P	–	P
methyl 4-caffeoylquinate	**11**	P	–	–	–	P	P	–	–	–	–	–	–	–	–	–	–	–
methyl 5-caffeoylquinate	**12**	P	–	–	–	–	–	–	–	–	–	–	P	–	–	–	–	–
3-caffeoylshikimic acid	**13**	P	–	P	P	P	P	P	P	P	P	P	P	P	P	–	P	P
caffeoylshikimic acid	**14**	P	–	–	P	P	P	P	P	P	P		P	P		–	–	P
3-*p*-coumaroylquinic acid	**6**	P	–	–	P	P	P	P	P	–	P	–	P	P	P	–	–	–
cis-3-*p*-coumaroylquinic acid	**7**	P	–	–														
4-*p*-coumaroylquinic acid	**8**	P	P	–														
cis-4-*p*-coumaroylquinic acid	**9**	P	–	–														
p-coumaroyl-glucoses	**25**	–	–	–	–	P	P	–	–	–	P	–	P	P	P	–	–	–
methyl 3-*p*-coumaroylquinate	**31**	–	–	–	P	P	P	P	P	–	P	–	P	P	P	–	–	–
3-feruloylquinic acid	**5**	P	P	–	P	–	P	P	–	P	P	P	P	–	–	–	–	–
feruloyl-glucoses	**26**	–	–	–	–	P	P	–	–	–	P	–	P	P	–	–	–	–
3-*p*-methoxycinnamoylquinic acid	**32**	–	–	–	–	P	P	–	–	–	P	–	–	–	P	–	–	–
Benzoic Acids																		
vanillic acid-glucoses	**27**	–	–	–	P	–	P	P	–	P	–	–	P	–	P	P	–	P
vanillic acid-glucoses	**28**	–	–	–	–	–	–	–	–	–	–	–	–	–	P	–	–	–
3,4-dihydroxybenzoyl-glucoses	**30**	–	–	–	P	–	–	–	–	–	–	–	P	–	–	–	–	P
Coumarin																		
esculin	**24**	–	–	–	P	–	–	–	–	–	–	–	P	P	P	–	–	–
Flavonols																		
rRutin	**33**	P	P	P	–	–	–	–	–	–	–	–	–	–	P	–	–	–
quercetin-3-*O*-galactoside	**34**	P	–	–	–	–	–	–	–	–	–	–	–	–	P	–	–	–
quercetin-3-*O*-glucoside	**35**	P	P	P	–	–	–	–	–	–	–	–	–	–	–	–	–	–
quercetin-pentoside	**36**	P	P	P	–	–	–	–	–	–	–	–	–	P	P	–	–	–
quercetin-pentoside	**37**	P	P	P	–	–	–	–	–	–	–	–	–	–	–	–	–	–
quercetin-rhamnoside	**38**	P	P	P	–	–	–	–	–	–	–	–	–	–	–	–	–	–
quercetin	**39**	P	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
quercetin-pentoside-rhamnoside	**40**	P	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
quercetin-pentoside-rhamnoside	**41**	P	P	P	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Flavan-3-ols																		
catechin	**15**	–	–	–	P	P	P	P	–	–	P	–	P	P	P	–	P	P
epicatechin	**16**	–	–	–	P	P	P	P	–	–	–	–	P	P	P	–	P	–

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Table 2. continued

phenolic compounds		Plum Varieties																					
		P. salicina L.[b]			*P. domestica* L.									*P. domestica* L. × *P. spinosa* L.			*P. spinosa* L.	*P. cerasifera* L.	*P. salicina* L.			
		origin Chile	origin Spain	origin South-Africa	Krichele Dürnau	Toptaste	President	Miragrande	Green Gage	Cacanska rodna	Ortenauer	Katinka	Jojo	P. frutic. slap.	Haferschlehe	Späte Myrobalane	Tatjana	Songold					
Flavan-3-ols																							
procyanidin B1	**17**	–	–	–	P	–	P	–	P	–	P	–	P	P	–	–	–	P					
(epi)catechin-4,8′-(epi)catechin	**18**	–	–	–	P	–	–	–	P	–	–	–	P	–	–	–	–	P					
(epi)catechin-4,8′-(epi)catechin	**19**	–	–	–	P	–	P	–	–	–	–	–	–	–	–	–	–	P					
procyanidin B2	**20**	–	–	–	P	–	P	–	–	–	–	–	–	–	–	–	–	P					
(epi)catechin-4,8′/2,7′(epi)catechin	**21**	–	–	–	–	–	–	–	P	–	–	–	–	–	–	P	–	P					
(epi)catechin-4,8′-(epi)catechin-4′,8″-(epi)catechin	**22**	–	–	–	P	–	–	–	–	–	–	–	P	P	–	–	–	P					
(epi)catechin-4,8′-(epi)catechin-4′,8″/2,7″-(epi)catechin	**23**	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	P					

[a]P, presence; –, absence. [b]Whole fruits were extracted.

Table 3. Amount of CGAs in Plum Samples (μg/g of Flesh Fresh Weight)[a]

plum variety	3-caffeoylquinic acid (μg/g FW)	5-caffeoylquinic acid (μg/g FW)	4-caffeoylquinic acid (μg/g FW)
P. salicina L., Chile	77.4	6.3	7.4
P. salicina L., Spain	2.5	1.1	1.0
P. salicina L., South Africa	1.0	n.d.	n.d.
Krichele Dürnau	521.8	37.4	25.62
Toptaste	142.8	21.1	13.1
President	258.9	18.3	12.8
Miragrande	186.0	19.6	13.0
Green Gage	88.2	24.1	16.4
Cacanska rodna	71.8	16.3	11.2
Ortenauer	63.7	17.5	11.7
Katinka	29.4	17.2	11.7
Jojo	257.2	57.2	11.6
P. fruticanus slaponice	277.4	30.6	21.2
Haferschlehe	140.1	35.9	23.3
Späte Myrobalane	n.d.	22.9	n.d.
Tatjana	19.8	n.d.	n.d.
Songold	48.7	19.2	13.3

[a]n.d. = not detected.[41]

produced a pseudomolecular ion at m/z 337. The first and the second eluting isomers (**6** and **7**) produced the MS^2 base peak at m/z 163 ([*p*-coumaric acid-H^+]$^-$) (Table 4), which is characteristic of 3-*p*-coumaroylquinic acid.[27,30,31] The third and the fourth eluting isomers (**8** and **9**) produced the MS^2 base peak at m/z 173 ([quinic acid-H_2O-H^+]$^-$) (Table 4), which is characteristic of 4-*p*-coumaroylquinic acid.[27,30,31] After the UV treatment, these isomers were assigned as 3-*p*-coumaroylquinic acid **6**, *cis*-3-*p*-coumaroylquinic acid **7**, 4-*p*-coumaroylquinic acid **8**, and *cis*-4-*p*-coumaroylquinic acid **9**.[30,31]

Characterization of Methyl 3-*p*-Coumaroylquinate (*Mr* 352). One peak was detected at m/z 351 in the extracted ion chromatogram. It produced the MS^2 base peak at m/z 145 ([*p*-coumaric acid-H_2O-H^+]$^-$) by the loss of a methyl quinate moiety (206 Da) and secondary peaks at m/z 117 ([*p*-coumaric acid-H_2O-CO-H^+]$^-$) and m/z 119 ([*p*-coumaric acid-CO_2-H^+]$^-$) (Table 4). The presence of an MS^2 secondary peak at m/z 119 is characteristics of 3-*p*-coumaroylquinate **31**, which has been reported by Jaiswal et al.[35]

Characterization of Caffeoyl-Shikimates (*Mr* 336). Two peaks detected in the extracted ion chromatogram produced a pseudomolecular ion at m/z 335. The first eluting isomer **13** produced the MS^2 base peak at m/z 179 ([caffeic acid-H^+]$^-$) and a secondary peak at m/z 135 (Table 4). On the basis of the fragmentation pattern, isomer **13** was assigned as 3-caffeoylshikimic acid. Isomer **14** was tentatively assigned as a caffeoylshikimate despite the lack of authentic standards; it showed, however, different MS^2 and MS^3 spectra (Table 4) if compared to the MS^2 and MS^3 spectra of caffeoylshikimic acids previously reported in maté tea.[31]

Characterization of Putative *p*-Methoxycinnamoylquinic Acid (*Mr* 352). One peak detected at m/z 351 in the extracted ion chromatogram showed a typical UV spectrum of chlorogenic acids at 320 nm (λ_{max}). It produced an MS^2 base peak at m/z 177 ([*p*-methoxycinnamic acid-H^+]$^-$) and secondary peaks at m/z 191 ([quinic acid-H^+]$^-$), m/z 293 ([M-CO_2-CH_3-H^+]$^-$), and m/z 235 (unassigned) (Figure 2 and Table 4). It produced the MS^2 base peak at m/z 133 ([*p*-methoxycinnamic acid-CO_2-H^+]$^-$) (Table 4), which is characteristic of a carboxylic acid, cinnamic

Table 4. Negative Ion MS^4 Fragmentation Data for the Phenolics of Plums

compd. no.	retention time (min)	parent ion (M − H)	characteristic *m/z* of ions in negative ion mode
1	13.0	353	MS^2→ 191 (100), 179 (46), 1359 (11); MS^3 → 127 (100), 173 (80), 111 (65), 85 (55)
2	14.1	353	MS^2→ 191 (100), 179 (45), 135 (9); MS^3 → 127 (100), 173 (85), 111 (65), 85 (50)
3	23.1	353	MS^2→ 191 (100); MS^3 → 127 (100), 173 (58), 111 (38), 93 (48), 85 (61)
4	18.3	353	MS^2→ 191 (100); MS^3 → 127 (100), 173 (50), 111 (62), 93 (50), 85 (61)
5	21.3	367	MS^2→ 193 (100), 134 (14); MS^3 → 134 (100)
6	17.8	337	MS^2→ 163 (100), 191 (8); MS^3 → 119 (100)
7	18.6	337	MS^2→ 163 (100), 191 (9); MS^3 → 119 (100)
8	28.8	337	MS^2→ 173 (100), 163 (8); MS^3 → 71 (100), 111 (85)
9	25.8	337	MS^2→ 173 (100), 163 (10); MS^3 → 111 (100)
10	19.9	367	MS^2→ 161 (100), 135 (10), 193 (15); MS^3 → 133 (100)
11	27.4	367	MS^2→ 161 (100), 135 (28), 193 (12), 335 (10); MS^3 → 133 (100)
12	28.8	367	MS^2→ 179 (100), 191 (20), 161 (10), 135 (40); MS^3 → 135 (100)
13	25.4	335	MS^2→ 179 (100), 135 (10); MS^3 → 135 (100)
14	26.4	335	MS^2→ 161 (100), 179 (14), 135 (72); MS^3 → 133 (100)
15	16.8	289	MS^2→ 245 (100), 205 (38); MS^3 → 203 (100), 227 (26), 161 (23)
16	23.9	289	MS^2→ 245 (100), 205 (42); MS^3 → 203 (100), 227 (23), 187 (20)
17	13.9	577	MS^2→ 407 (100), 451 (20), 425 (78), 289 (32); MS^3 → 285 (100), 389 (27), 297 (29), 283 (90), 255 (24); MS^4 → 257 (100), 283 (24)
18	21.0	577	MS^2→ 407 (100), 451 (20), 425 (96), 289 (27); MS^3 → 285 (100), 389 (26), 297 (33), 283 (95), 257 (10); MS^4 → 257 (100), 283 (25)
19	23.9	577	MS^2→ 407 (100), 451 (20), 425 (96), 289 (27); MS^3 → 285 (100), 389 (26), 297 (33), 283 (95), 257 (10); MS^4 → 257 (100), 283 (25)
20	14.9	577	MS^2→ 407 (100), 451 (25), 425 (98), 289 (26); MS^3 → 285 (100), 389 (26), 297 (24), 283 (69), 255 (18); MS^4 → 257 (100), 283 (30)
21	28.1	575	MS^2→ 423 (100), 449 (52), 425 (96), 327 (10), 285 (31); MS^3 → 285 (100), 405 (10), 257 (8)
22	17.1	865	MS^2→ 695 (100), 577 (65), 543 (31), 425 (28), 407 (67); MS^3 → 543 (100), 525 (45), 451 (25), 407 (25), 289 (17), 243 (46); MS^4 → 525 (100), 391 (43), 243 (17)
23	23.3	863	MS^2→ 575 (100), 711 (39), 449 (13); MS^3 → 449 (100), 539 (33), 423 (43), 285 (42); MS^4 → 287 (100)
24	14.1	339	MS^2→ 177 (100); MS^3 → 133 (100)
25	18.2	325	MS^2→ 145 (100), 187 (60), 163 (99), 119 (13); MS^3 → 117 (100)
26	21.3	355	MS^2→ 193 (100), 217 (69), 175 (45), 295 (16); MS^3 → 117 (100)
27	10.7	329	MS^2→ 261 (100), 167 (64); MS^3 → 119 (100), 145 (15), 203 (30)
28	12.3	329	MS^2→ 209 (100), 261 (63), 167 (9); MS^3 → 167 (100), 125 (14)
29	13.2	341	MS^2→ 179 (100), 271 (11), 161 (27), 135 (15); MS^3 → 135 (100)
30	9.3	315	MS^2→ 153 (100), 245 (85), 109 (8); MS^3 → 109 (100)
31	12.5	351	MS^2→177 (100), 293 (48), 235 (20), 191 (46), 133 (33) ; MS^3 → 133 (100)
32	24.8	351	MS^2→145 (100), 293 (5), 119 (10), 117 (10); MS^3 → 117 (100), 119 (37)
33	36.3	609	MS^2→301 (100); MS^3 → 179 (100), 271 (50), 255 (29), 151 (89)
34	35.8	463	MS^2→300 (100); MS^3 → 151 (100), 271 (71), 255 (26), 179 (95)
35	36.4	463	MS^2→300 (100); MS^3 → 151 (100), 271 (65), 255 (29), 179 (89)
36	38.5	433	MS^2→300 (100); MS^3 → 151 (100), 271 (87), 255 (25), 179 (98)
37	43.1	433	MS^2→300 (100); MS^3 → 151 (100), 271 (70), 255 (28), 179 (73)
38	40.2	447	MS^2→300 (100); MS^3 → 179 (100), 271 (64), 255 (47), 151 (84)
39	30.9	301	MS^2 → 179 (100), 271 (50), 255 (29), 151 (89); MS^3 → 151 (100)
40	46.8	579	MS^2→301 (100); MS^3 → 151 (100), 179 (91)
41	47.5	579	MS^2→301 (100); MS^3 → 151 (100), 257 (16), 179 (70)

acid. The presence of the MS^2 base peak at *m/z* 177 showed that the cinnamoyl residue is attached to C-3 of quinic acid.[27] On the basis of the above points, this compound was assigned as 3-*p*-methoxycinnamoylquinic acid **32**.

Characterization of Dimeric PAs (*Mr* 578 and *Mr* 576). Four peaks were detected at *m/z* 577 in the extracted ion chromatogram (EIC). These four compounds were tentatively assigned as dimeric B-type PAs (**17**−**20**) with (epi)catechin monomeric units. All of these compounds have similar MS^n fragmentation patterns with similar intensities of ions (Table 4). They produced the MS^2 base peak at *m/z* 407 ($[M\text{-}H^+\text{-}170\ Da]^-$) by the loss of a RDA (retero Diels−Alder) fragment (152 Da) followed by the loss of a water molecule (18 Da); the secondary peaks at *m/z* 289 ($[(epi)catechin\text{-}H^+]^-$) originate from a QM (quinone methide) fragment, at *m/z* 425 ($[M\text{-}H^+\text{-}152\ Da]^-$) from the RDA fragment, at *m/z* 451 ($[M\text{-}H^+\text{-}126\ Da]^-$) from a HRF (heterocyclic ring fission) fragment, and at *m/z* 559 ($[M\text{-}H^+\text{-}H_2O]^-$) from the loss of a water molecule (Table 4). On the basis of the above fragmentation, these PAs were assigned as isomers of (epi)catechin-(4,8′)-(epi)catechin dimer. For further evidence, B1- and B2-type PAs were used as authentic standards and showed retention times and fragmentations identical to PAs **17** and **20**, respectively. On the basis of these arguments, isomers **17** and **20** were assigned as B1- and B2-type PAs, respectively. It was not possible to assign the stereochemistry of monomeric units of a PA by tandem mass spectrometry.

One peak was detected at *m/z* 575 and assigned as an A-type dimer of (epi)catechin unit. This compound **21** produced the

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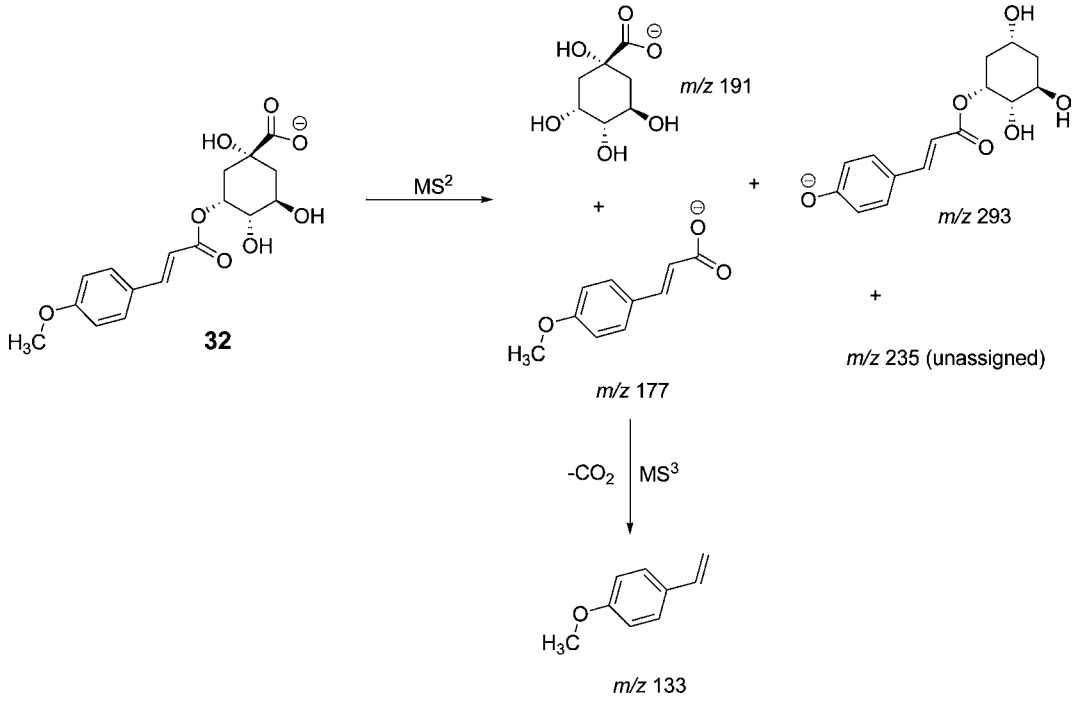

Figure 2. Fragmentation pathway of 3-*p*-methoxycinnamoylquinic acid **32** (*m*/*z* 351) in negative ion mode.

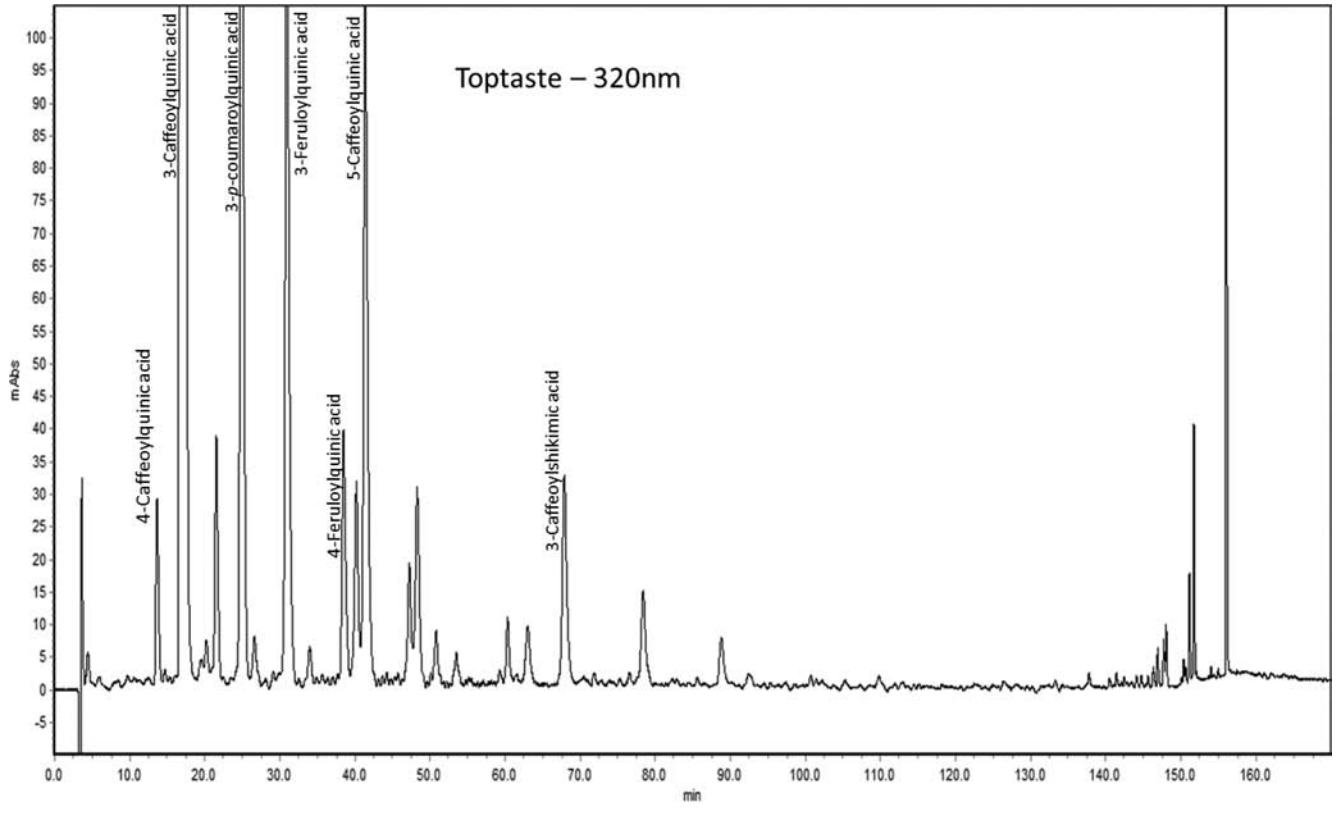

Figure 3. HPLC profile of chlorogenic acids in a fruit flesh extract from *P. domestica* L. 'Toptaste' with detection at 320 nm.

MS^2 base peak at *m*/*z* 449 (HRF) and secondary peaks at *m*/*z* 539 ($[M\text{-}H^+\text{-}2H_2O]^-$), 423 (RDA), 407 (RDA), 327 (a benzofuran formation, BFF), and 289 and 285 (QMs) (Table 4). Detailed mass spectra of dimeric PAs have been published previously and are not repeated here.[26]

Characterization of Trimeric PAs (*Mr* 866 and *Mr* 864). Two peaks were detected at *m*/*z* 865 and 863 in the extracted ion chromatogram (EIC). These compounds were tentatively assigned as trimeric B-type PA (**22**) and A-type PA (**23**), respectively, with (epi)catechin monomeric units. Compound **22** produced the MS^2 base peak at *m*/*z* 695 ($[M\text{-}H^+\text{-}170\ Da]^-$) by the loss of a RDA fragment (152 Da) followed by the loss of a water molecule (18 Da); the secondary peaks at *m*/*z* 577 ($[(epi)catechin\text{-}(epi)catechin\text{-}H^+]^-$) originated from a QM

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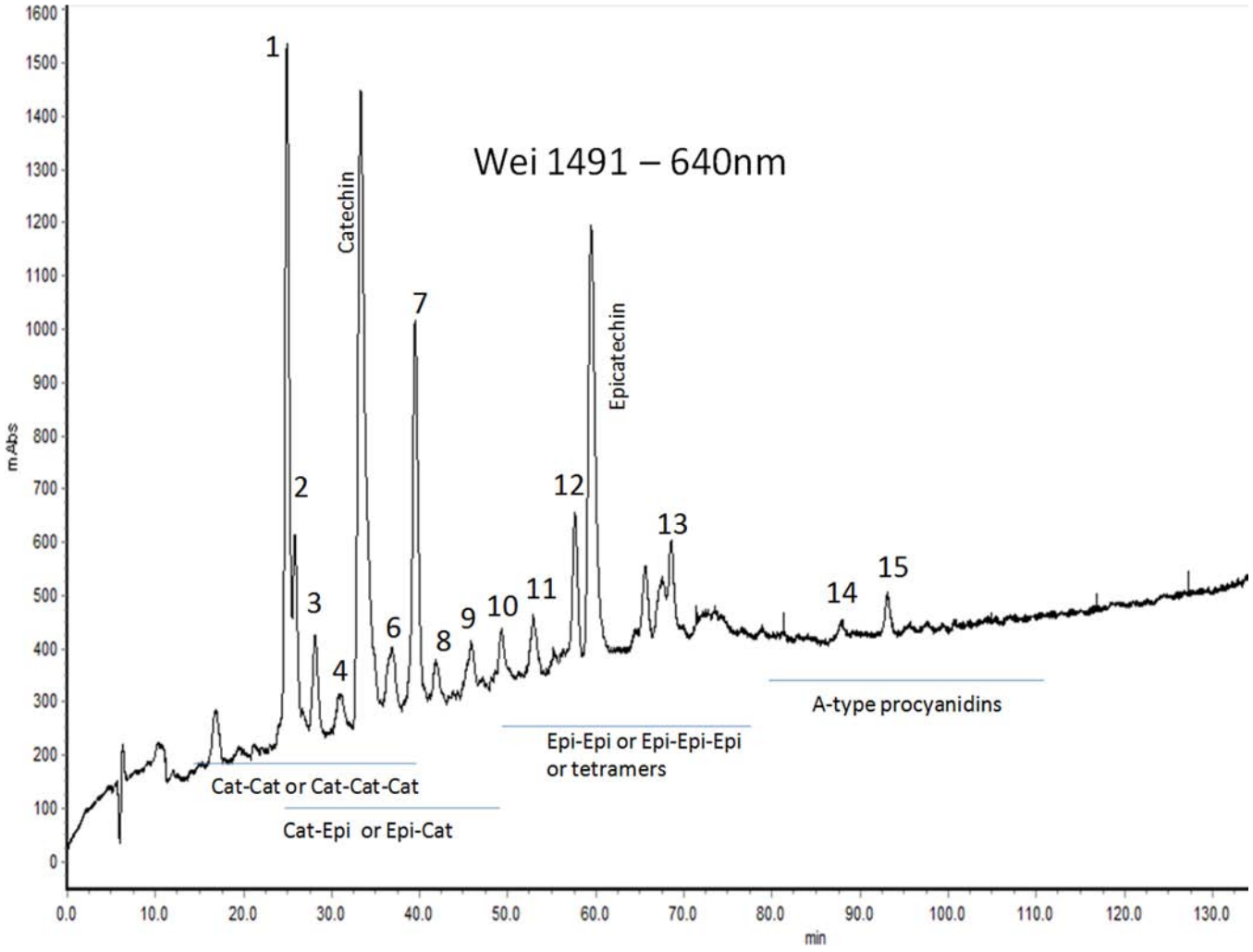

Figure 4. HPLC profile of flavanols in a fruit flesh extract from *P. domestica* L. 'Haganta' × 'Jubileum' Wei 1491 with detection at 640 nm after a chemical reaction with dimethylaminocinnamaldehyde.

fragment, at m/z 739 ($[M\text{-}H^+\text{-}126\ Da]^-$) from a HRF fragment, and at m/z 847 ($[M\text{-}H^+\text{-}H_2O]^-$) from the loss of a water molecule.

Compound **23** produced the MS^2 base peak at m/z 693 ($[M\text{-}H^+\text{-}170\ Da]^-$) by the loss of a RDA fragment (152 Da) followed by the loss of a water molecule (18 Da); the secondary peaks at m/z 711 ($[M\text{-}H^+\text{-}152\ Da]^-$) originated from the loss of a RDA fragment (152 Da), at m/z 449 from a HRF fragment, and at m/z 575 ($[(epi)catechin\text{-}(4',8''/2',7'')\text{-}(epi)catechin\text{-}H^+]^-$) from a QM fragment (Table 4). The presence of a QM fragment at m/z 575 shows that the middle and the bottom units are connected by an A-type linkage, which is also confirmed by the HRF at m/z 449 (Table 4).[9] On the basis of the above points, compound **23** was assigned as (epi)catechin-(4,8′)-(epi)-catechin-(4′,8″/2′,7″)-(epi)catechin.

Identification of Flavonoids and Flavonoid-Glycosides. Two peaks were detected at m/z 289 in the extracted ion chromatogram and were assigned as catechin **15** and epicatechin **16**. Recently, we have reported both isomers in the plants of *Rhododendron* genus.[26] One peak was detected at m/z 609 and was assigned as rutin **33**. It produced the MS^2 base peak at m/z 300 ($[M\text{-}H^+\text{-}309\ Da]^-$) by the loss of the rutinoside moiety attached to quercetin at C-3 (Table 4). The retention time and fragmentation pattern were identical to those of an authentic standard of rutin **33**. One peak was detected at m/z 301 and was assigned as quercetin **39**.

Two peaks were detected at m/z 463 in the extracted ion chromatogram and were tentatively assigned as quercetin-hexosides (**34** and **35**). Both isomers had similar MS^n fragmentation patterns with similar ion intensities (Table 4). They produced the MS^2 base peak at m/z 300 ($[M\text{-}H^+\text{-}163\ Da]^-$) by a neutral loss of a 162 Da (Table 4) hexoside, which is characteristic of quercetin-3-*O*-hexoside. They produced the MS^3 base peak at m/z 151 and secondary peaks at m/z 179, 271, and 255, which is characteristic of a quercetin moiety (Table 4). It was not possible to differentiate between these isomers by tandem mass spectra in negative ion mode. On the basis of their retention times and compared to the authentic standards of quercetin-3-*O*-galactoside and quercetin-3-*O*-glucoside, the first eluting isomer was assigned as quercetin-3-*O*-galactoside **34**, and the second eluting isomer was assigned as quercetin-3-*O*-glucoside **35**.

Two peaks were detected at m/z 433 in the extracted ion chromatogram and were tentatively assigned as quercetin-pentosides (**36** and **37**). Both isomers have similar MS^n fragmentation patterns with similar ion intensities (Table 4). They produced the MS^2 base peak at m/z 300 ($[M\text{-}H^+\text{-}133\ Da]^-$) by a neutral loss of a 132 Da (Table 4) pentoside, which is characteristic of quercetin-3-*O*-pentoside. They produced the MS^3

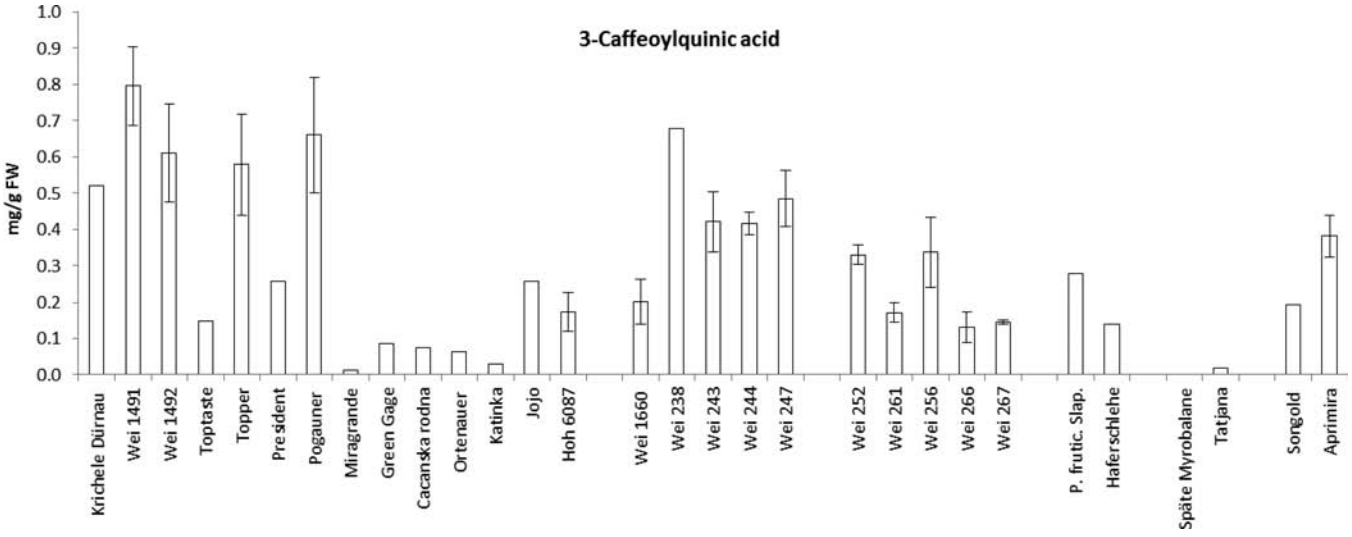

Figure 5. Concentrations of 3-caffeoylquinic acid (neochlorogenic acid) **1** in the fruit flesh of plum varieties estimated by HPLC-DAD (see Table 3 as well for detailed concentrations).

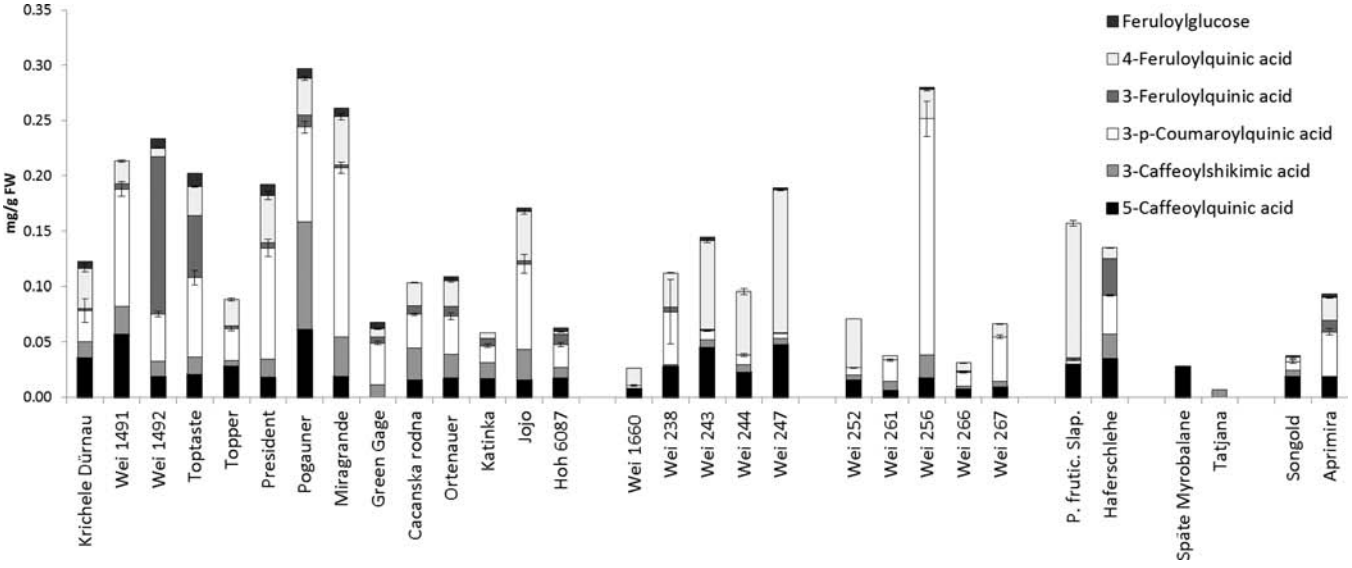

Figure 6. Concentrations of CGAs in the fruit flesh of plum varieties estimated by HPLC-DAD.

base peak at m/z 151 and secondary peaks at m/z 179, 271, and 255, which is characteristic of a quercetin moiety.

One peak was detected at m/z 447 in the extracted ion chromatogram and was tentatively assigned as quercetin-rhamnoside (**38**). It produced the MS^2 base peak at m/z 300 ($[M\text{-}H^+\text{-}147\ Da]^-$) by a neutral loss of a 146 Da rhamnoside (Table 4). They produced the MS^3 base peak at m/z 151 and secondary peaks at m/z 179, 271, and 255, which are characteristic of a quercetin moiety.

Two peaks were detected at m/z 579 in the extracted ion chromatogram and were tentatively assigned as quercetin-pentoside-rhamnosides (**40** and **41**). Both isomers had similar MS^n fragmentation patterns with similar ion intensities (Table 4). They produced the MS^2 base peak at m/z 301 ($[M\text{-}H^+\text{-}132\ Da\text{-}146\ Da]^-$) by a neutral loss of 278 Da (pentoside-rhamnoside).

Identification of Cinnamoyl-Glucosides and Hydroxybenzoyl-Glucoses. One peak was detected at m/z 325 in the extracted ion chromatograms and was tentatively assigned as *p*-coumaroyl-glucoside (**25**). It produced the MS^2 base peak at m/z 163 ($[p\text{-coumaric acid-}H^+]^-$) by the loss of a glucoside moiety and a secondary peak at m/z 119 ($[p\text{-coumaric acid-}CO_2\text{-}H^+]^-$) by the loss of a glucoside moiety and a CO_2 molecule (Table 4). Ions at m/z 163 and 119 in MS^n spectra are characteristic of *p*-coumaric acid derivatives.

One peak was detected at m/z 339 in the extracted ion chromatograms and was assigned as esculin (**24**) after the comparison to standard. It produced the MS^2 base peak at m/z 177 ($[esculetin\text{-}H^+]^-$) by the loss of a glucoside moiety and a secondary peak at m/z 133 ($[esculetin\text{-}CO_2\text{-}H^+]^-$) by the loss of a glucoside moiety and a CO_2 molecule (Table 4).

One peak was detected at m/z 355 and was teantatively assigned as feruloyl-glucoside (**26**). This feruloyl-glucoside produced the MS^2 base peak at m/z 193 ($[ferulic\ acid\text{-}H^+]^-$) by the loss of a glucoside moiety and a secondary peak at m/z 175 by the loss of a glucoside moiety and a H_2O molecule (Table 4).

One peak was detected at m/z 341 in the extracted ion chromatograms and was tentatively assigned as caffeoyl-glucoside (**29**). It produced the MS^2 base peak at m/z 179 ($[caffeic\ acid\text{-}H^+]^-$) by the loss of a glucoside moiety and a secondary peak at m/z 135 ($[caffeic\ acid\text{-}CO_2\text{-}H^+]^-$) by the loss of a glucoside

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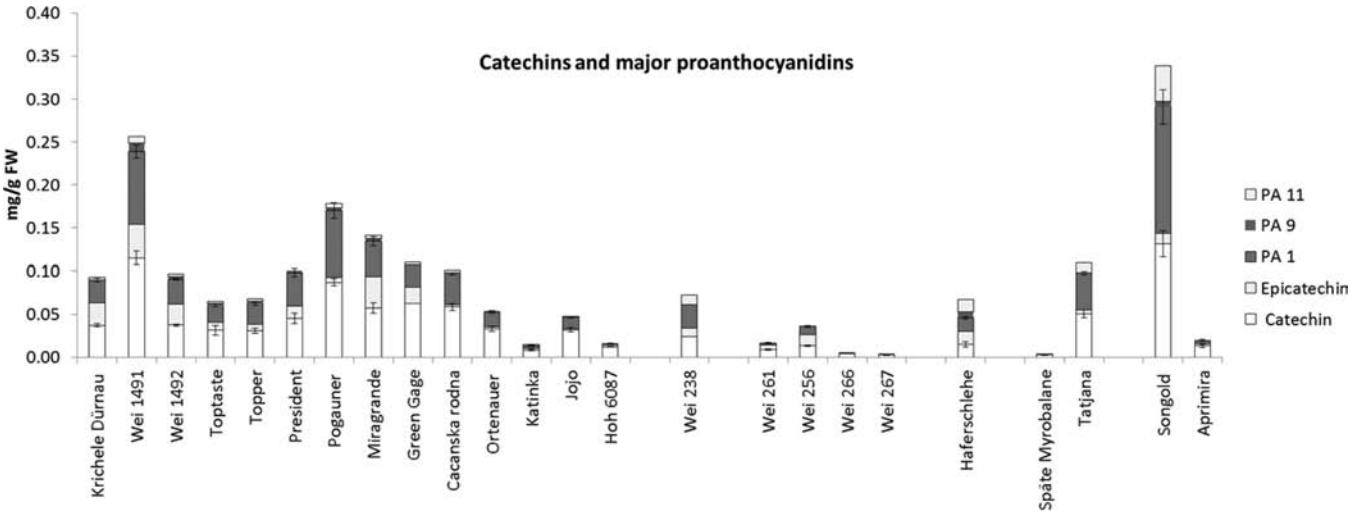

Figure 7. Concentrations of catechins and major PAs in the fruit flesh of plum varieties estimated by HPLC-CRD.

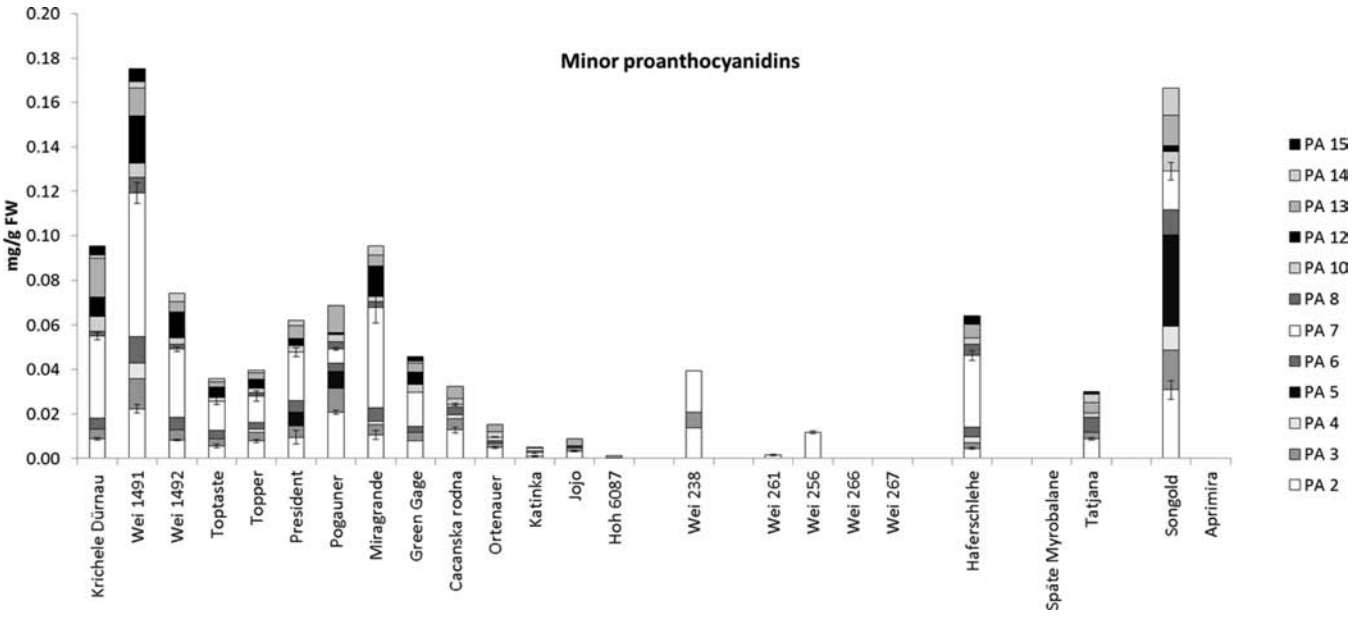

Figure 8. Concentrations of minor PAs in the fruit flesh of plum varieties estimated by HPLC-CRD.

moiety and a CO_2 molecule (Table 4). Ions at m/z 179 and 135 in MS^n spectra are characteristic of a caffeic acid derivative.

Two peaks were detected at m/z 329 in extracted ion chromatograms and were tentatively assigned as vanillic acid-glucosides (**27** and **28**). They produced the MS^2 base peaks at m/z 261 and 209, respectively (Table 4). They both produced an MS^2 secondary peak at m/z 167 ([vanillic acid-H^+]$^-$) by the loss of a glucoside moiety (Table 4).

One peak was detected at m/z 315 in the extracted ion chromatograms and was tentatively assigned as 3,4-dihydroxybenzoyl-glucoside (protocatechuic acid-glucoside) (**30**). It produced an MS^2 base peak at m/z 153 ([protocatechuic acid-H^+]$^-$) by the loss of a glucoside moiety and a secondary peak at m/z 109 ([protocatechuic acid $-CO_2$-H^+]$^-$) by the loss of a glucoside moiety and a CO_2 molecule (Table 4). Ions at m/z 153 and 109 in MS^n spectra are characteristic of protocatechuic acid derivatives.

Quantiative Phenolic Profiles by HPLC-DAD-/CRD Analysis. Figures 3 and 4 show chromatograms obtained by HPLC with detection at 320 nm for CGAs (Figure 3) and after chemical reaction with detection at 640 nm for flavanols (Figure 4). For identification, the individual peaks were collected from several analytical runs and checked by mass spectroscopy as described above. However, not all CGAs could be clearly identified due to low concentrations. Thus, only seven CGAs were quantified (Figures 5 and 6). The HPLC-CRD profiles (Figure 4) show only flavanols due to the selectivity of the reaction with DMACA.[37] Therefore, all peaks of the respective analyses were evaluated (Figures 7 and 8). In Figure 4, the time windows of proanthocyanidins are indicated with respect to their catechin and/or epicatechin components. This information is based on previous studies.[45] When combining this information with the MS results given in Table 2, it is evident that all 4−8 linked dimers of catechin and epicatechin occur in plums. These are procyanidin B1 **17** (epicatechin-(4−8)-catechin), procyanidin B2 **20** (epicatechin-(4−8)-epicatechin), procyanidin B3 (catechin-(4−8)-catechin), and procyanidin B4 (catechin-(4−8)-epicatechin).

Among the two phenolic groups, namely the CGAs and the flavanols, a wide quantitative range was found in the studied fruit flesh samples. Neochlorogenic acid (**1**) is the predominating hydroxycinnamic acid in nearly all varieties. An exception is 'Späte Myrobalane' where this compound could not be detected at all (Figure 5 and Table 2). Together with 'Tatjana' it belongs to the species *P. cerasifera* L. These two varieties show generally very low concentrations of CGAs (Figures 5 and 6). It is noticeable that the interspecific hybrids of *P. domestica* L. 'Jojo' and *P. cerasifera* L., namely, Wei 252, Wei 261, Wei 256, Wei 266, and Wei 267, also show low neochlorogenic acid **1** concentrations as compared to the other samples. In total, the concentrations of neochlorogenic acid **1** range from 0.2 mg/g FW to more than 1 mg/g FW. The sum of the other CGAs is below 0.3 mg/g FW. Their quantitative profiles differ even between closely related hybrids (Figure 6) such as the two groups of 'Jojo'-progenies 'Jojo' × *P. cerasifera* (Wei numbers see above) L. and 'Jojo' × *P. spinosa* L. (Wei 1660, Wei 238, Wei 243, Wei 244, and Wei 247) as well as the 'Haganta' × 'Jubileum'-hybrids Wei 1491 and Wei 1492.

The concentrations of total flavanols range between 0.01 mg/g FW and about 0.5 mg/g FW (Figures 7 and 8). The highest concentrations were found in the flesh of *P. salicina* L. 'Songold' and in the *P. domestica* L. 'Haganta' × 'Jubileum'-hybrid Wei 1491. Lowest levels exhibited the 'Jojo' progenies, 'Katinka', 'Späte Myrobalane', and 'Aprimira'.

We have shown that plums are one of the richest sources of CGAs and PAs reported in nature. We have for the first time quantified the CGAs of a large number of different cultivars of plums (Table 3). This is the first time a *p*-methoxycinnamoyl-containing CGA has been reported in nature.

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Notes

The authors declare no competing financial interest.

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