JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Phenolic Compounds in *Rosaceae* Fruits from Ecuador

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RP-HPLC-DAD was used to study the content of phenolic compounds in four Ecuadorian fruits (strawberry, Andean blackberry, plum, and capulí cherry). Compounds were identified using spectral characteristics of representative standards and reference samples. Further, LC-MS with MS/MS was used to confirm molecular assignments in previously unstudied capulí cherry. Gallic acid was detected in Andean blackberry, and galloyl esters were detected in strawberries. Both these berries contained ellagic acid derivatives as major compounds, followed by anthocyanins, cyanidin, and pelargonidin glycosides. Plums and capulí cherry showed similar profiles of phenolic compounds, with chlorogenic and neochlorogenic acids being the most important hydroxycinnamates. (−)-Epicatechin was found in high amounts in Andean blackberry, plums, and capulí cherry, while (+)-catechin was only found in capulí cherry. Proanthocyanidins were major compounds in all fruits, and all contained considerable amounts of quercetin derivatives and smaller amounts of kaempferol derivatives. LC-MS analysis of capulí cherry revealed dimeric and trimeric procyanidins, quercetin and kaempferol hexosides and pentosides, and a kaempferol-*O*,*C*-dipentoside.

KEYWORDS: Phenolic compounds; strawberry; Andean blackberry; plum; capulí cherry; *Rosaceae*; Ecuador

INTRODUCTION

Fruits are good sources of water, fiber, vitamins, and minerals. In addition, fruits and fruit products, together with tea, red wine, cereals, chocolate, and legumes, are regarded as major dietary sources of polyphenols (*1*). Phenolic compounds in a diet rich in fruits and vegetables have attracted the attention of researchers due to their health-promoting attributes, which include lowering the risk of cardiovascular disease, cancer, or other conditions associated with the aging process. The biological mechanisms behind these effects include protection against free radicals, free radical-mediated cellular signaling, inflammation, allergies, platelet aggregation, microbes, ulcers, viruses, tumors, and hepatotoxicity (*2, 3*).

The *Rosaceae* includes many economically important fruits such as strawberry, raspberry, apple, plum, and pear, among others. Members of this family showed high and intermediate antioxidant activity in a previous study on fruits from Ecuador (*4*). Phenolic classes commonly found in plant-based products are hydroxybenzoic acids, hydroxycinnamic acids, hydrolyzable tannins (i.e., ellagitannins), flavonols, flavan-3-ols, dimeric and trimeric proanthocyanidins, and anthocyanins (**Figure 1**) (*5*). Studies identifying and quantifying the phenolic classes mentioned above and individual compounds have been performed on strawberry (*6*), blackberry (*7, 8*), and plums (*9*−*13*). For example, ellagic acid, which is a major phenolic component in fresh and processed strawberries and blackberries, has been extensively studied for antimutagenic, anticarcinogenic, and antioxidant effects (*14*). In plums, caffeoylquinic acid isomers, i.e. -5-*O*-caffeoylquinic acid (chlorogenic acid), -3-*O*-caffeoylquinic acid (neochlorogenic acid), and -4-*O*-caffeoylquinic acid (cryptochlorogenic acid), predominate (*9, 10*), followed by flavan-3-ols and flavonols (*12*). Macheix et al. (*15*) classified fruits according to their flavan-3-ol content into three groups: low and very low (strawberry and plum), medium (blackberry), and high (some cherry varieties). However, information on the distribution of major compounds and classes of phenolic compounds in *Rosaceae* species consumed in Ecuador is lacking. Very little attention has been paid to capulí cherry in particular, from which only anthocyanins have been purified and identified (*16*).

The aim of this study was to identify and quantify the phenolic compounds in four *Rosaceae* fruits from Ecuador by high performance liquid chromatography (HPLC) using an in-house online HPLC library with retention times and character-

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10.1021/jf802656r CCC: $40.75
Published on Web 01/14/2009

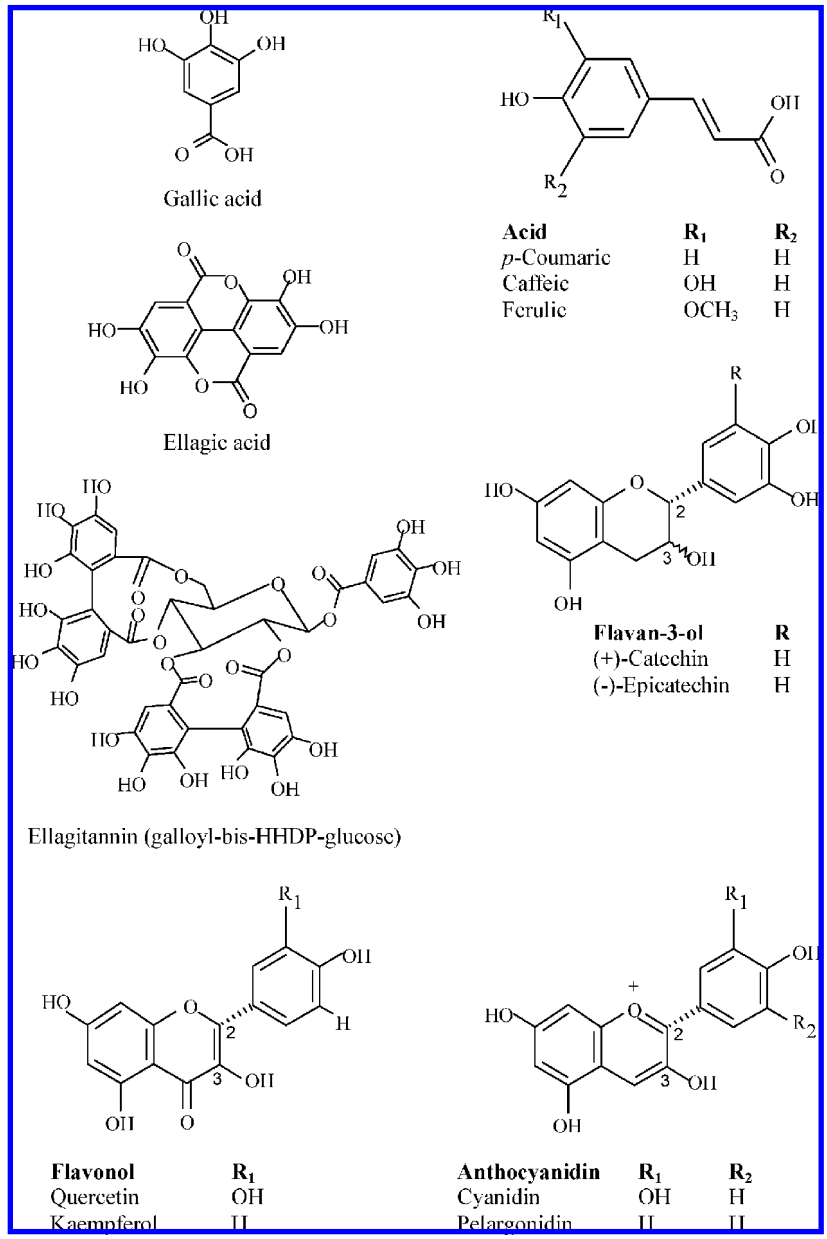

Figure 1. Chemical structures of major phenolic compounds.

istic UV−vis absorption spectra of standards analyzed by LC-DAD and in berry extracts characterized by LC-DAD-MS and supported by literature data (*5*). Phenolic compounds in the less studied capulí cherry were further characterized by LC-MS.

MATERIALS AND METHODS

Fruit Samples. Four types of fruit from the *Rosaceae* were purchased at three markets in Quito, Ecuador, during 2004 and 2005, depending on the season. These fruits were strawberry (*Fragaria ananasa* Duch.), Andean blackberry (*Rubus glaucus* Berth.), two plum varieties "Santa Rosa" and "Beauty" (*Prunus salicina* Lindl.), and capulí cherry (*Prunus serotina* Ehrh. var. Capulí). The fruits and their growing locations are described in detail in a previous study (*4*). The edible parts were chopped and stored at −20 °C until freeze-drying, after which the samples were ground and again stored at −20 °C. A pooled sample was prepared for each market, representing three subsamples from three different purchases. The subsamples were diced, and the same amount of each subsample was added to the pool (*17*).

Chemicals and Reagents. The phenolic compounds *p*-hydroxybenzoic acid, vanillic acid, gallic acid, ellagic acid, chlorogenic acid, *p*-coumaric acid, caffeic acid, ferulic acid, (+)-catechin, (−)-epicatechin, rutin, quercetin, and kaempferol were purchased from Sigma Chemical Co. (St. Louis, MO), myricetin was purchased from Fluka (Buchs, Switzerland), and cyanidin-3-*O*-glucoside chloride and pelargonidin-3-*O*-glucoside chloride were purchased from Extrasynthese (Geney, France). The standards were dissolved in methanol to a concentration of 1 mg/mL, except for rutin and chlorogenic acid (2 mg/mL), and stored at −20 °C as stock solutions. Ellagic acid was first dissolved in dimethyl sulfoxide and then in methanol, as described by Häkkinen et al. (*18*). Ethyl acetate and methanol of analytical grade were used for extraction, and methanol and acetonitrile of HPLC grade were used for analysis.

Extraction of Phenolic Compounds. The extraction of hydroxybenzoic acids, hydroxycinnamic acids, flavan-3-ols, flavonol glycosides, and anthocyanins was performed following a two-step procedure (**Figure 2**) (*19*). Freeze-dried samples (~0.5 g) were remoistened with 4 mL of distilled water containing 1% ascorbic acid under nitrogen atmosphere for 20 min and extracted using ethyl acetate (4 × 10 mL) with intermittent mixing (1 min) and centrifugation (2880*g*) for 2 min. The residue was acidified (2 M HCl) and extracted with methanol (20 mL + 3 × 10 mL). The ethyl acetate extract and 10 mL of the methanolic extract were evaporated separately, and each residue was dissolved into methanol (1−6 mL), passed through a 0.45 μm syringe-filter, and analyzed by HPLC.

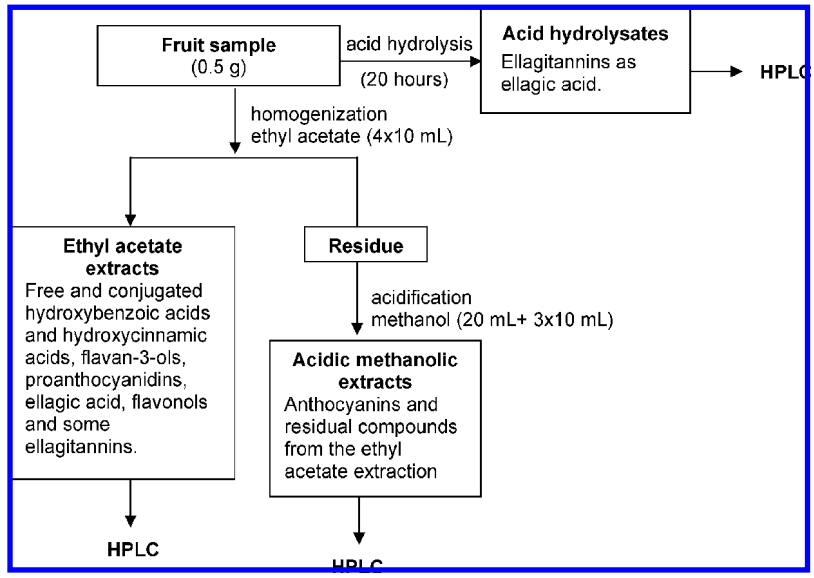

Figure 2. Procedure for extraction of soluble phenolic compounds from fruit samples.

Ellagitannins and ellagic acid derivatives were determined as ellagic acid equivalents after hydrolysis in acidic medium following the extraction method of Koponen et al. (*17*). Freeze-dried samples (~0.5 g) were remoistened (4 mL), mixed with 20 mL of methanol and 5 mL of water, acidified to 0.6 M with concentrated HCl (5 mL), and refluxed for 20 h at 85 °C (**Figure 2**). The mixture was filtered, left to cool to room temperature, made up to 50 mL with methanol, passed through a 0.45 μm syringe-filter, and analyzed by HPLC.

Chromatographic Conditions.

LC-DAD separation was performed on a LiChroCART Purospher RP-18e column (125 mm × 3 mm i.d., 5 μm, Merck, Darmstadt, Germany), on a Hewlett-Packard apparatus with a 1100 series quaternary pump, an autosampler, a diode array detector, and a HP-ChemStation data processing system (Agilent, Waldbronn, Germany).

The ethyl acetate and methanol extracts were analyzed simultaneously by LC-DAD for gallic acid, galloyl esters, flavan-3-ols, and proanthocyanidins (at 280 nm), ellagic acid and ellagitannins (at 250 nm), hydroxycinnamic acids (at 320 nm), and flavonols (at 360 nm). The gradient was a 20 min linear gradient, 5−30% acetonitrile (solvent B) in 1% formic acid in water (solvent A), at a flow rate of 0.5 mL/min. The same gradient was used to analyze ellagitannins as ellagic acid (at 250 nm) after acid hydrolysis. Anthocyanins were analyzed only in the methanolic extracts (at 520 nm) in a separate HPLC run using the same column, a flow rate of 0.5 mL/min, 5% formic acid in water (solvent A) together with acetonitrile (solvent B), and the following gradient: 5−10% solvent B (0−5 min), 10% solvent B (5−10 min), 10−40% solvent B (10−25 min).

Peak identification was based on comparison with the retention times and UV−vis spectra of authentic standards and reference berry samples (Bog whortleberry for flavonol glycoside, blackcurrant and rowanberry for anthocyanins, strawberry and raspberry from the *Rosaceae*), berry phenolic compounds online library (*5*), and MS-MS results for capulí cherry. The identity of flavonol glycosides was confirmed by releasing the aglycones by acid hydrolysis of the ethyl acetate extract. Quantification was based on peak area at the absorption wavelength for each class listed above. The results are reported for the weight of the aglycone and are the sum of the amounts in the ethyl acetate and methanolic extracts.

LC-DAD-MS separation was performed in a Gemini C18 (150 mm × 3 mm i.d., 5 μm, Phenomenex, USA). The mobile phases were acetonitrile−methanol (85:15) (solvent B) and 1% formic acid in water (solvent A) at a flow rate of 1 mL/min and a 25 min linear gradient of 5−30% solvent B. The HPLC was coupled to the DAD detector followed by a Finnigan LTQ linear ion trap mass spectrometer (Thermo, San Jose, CA). Electrospray ionization (ESI)-MS-MS was performed in positive ion mode using a capillary voltage of 3.8 kV, a temperature of 275 °C, and a collision energy of 35%. In the MS analysis (full scan), data were collected over a mass range of *m*/*z* 170−1000.

RESULTS AND DISCUSSION

Prior to chromatographic analysis, phenolic compounds were extracted by the sequential extraction procedure shown in **Figure 2**. The chromatograms of the ethyl acetate extract (**Figure 3**) show all the compounds that were classified according to their spectral characteristics into gallic and ellagic acid derivatives, hydroxycinnamic acid derivatives, flavan-3-ols, proanthocyanidins, and flavonols (*19*). Anthocyanins, extracted with methanol after acidification and analyzed separately by LC-DAD, are shown in **Figure 4**. **Table 1** shows the peaks, numbered according to their retention time, with their characteristic UV−vis absorption maxima and the basis for characterization of the compounds (either a more definite identification with reference to standards, tentative identification with reference to other berry extracts analyzed by LC-MS, or classification on the basis of spectral characteristics). **Table 2** shows the quantification of ellagitannins and ellagic acid derivatives determined as ellagic acid equivalents after acid hydrolysis. For capulí cherry, which was studied for the first time, peaks were further identified by LC-MS (**Table 3**).

Strawberry (*Fragaria ananasa* Duch.). Ecuador produces strawberries throughout the year and exports them as fresh fruit, frozen fruit in syrup, canned fruit, jam, and Individually Quick Frozen (IQF) products. In the strawberry samples analyzed (**Table 1**), gallic acid was not found in the free form, but galloyl esters (peaks 4 and 36 and some minor peaks) were clearly identified by comparison of the characteristic gallic acid spectrum with the corresponding bathochromic shifts (from 282 to 288 nm) due to esterification (*14*) and with reference to samples of Finnish strawberries previously characterized by LC-MS (*14*). Only two hydroxycinnamic acid derivative peaks were detected (peaks 14 and 16; **Figure 3**), and these were classified as *p*-coumaroyl esters according to their retention times and spectra (*14*). Peaks 7 and 8 (**Figure 3**) represented flavan-3-ol spectra and were designated flavan-3-ol derivatives. The exact identity of these compounds is not known, but De Pascual-Teresa et al. (*20*) reported proanthocyanidin B3 in Spanish strawberries. Among the flavonols found in strawberry, peaks 33b and 43 (**Figure 3**) corresponded to quercetin-3-glucuronide

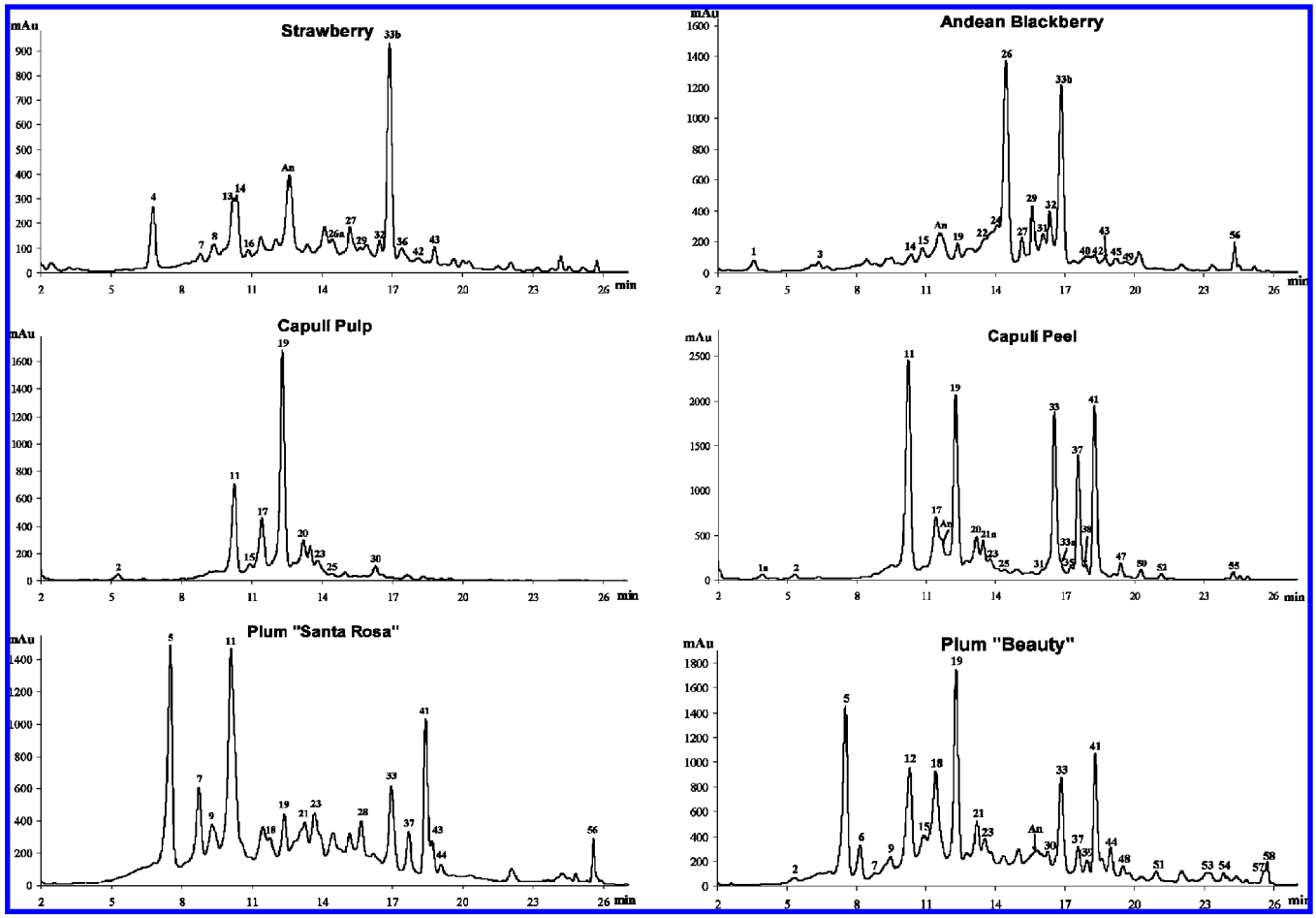

Figure 3. Chromatograms of ethyl acetate extracts at 280 nm of strawberry, blackberry, capulí cherry peel and pulp, and the plum varieties "Beauty" and "Santa Rosa". For peak numbers, see **Table 1**

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and kaempferol-3-glucuronide, respectively, as identified previously in Finnish strawberry (*14*). Anthocyanins An_2, An_4, and An_5, corresponding to cyanidin-3-*O*-glucoside, pelargonidin-3-*O*-glucoside, and pelargonidin-3-*O*-rutinoside, were identified with reference to Finnish strawberry and raspberry and the literature (*14*, *21*). Hybrid strawberry samples cultivated in Turkey (*22*) have been reported to contain 4−58 mg/kg FW of *p*-coumaric acid, 5−24 mg/kg FW of quercetin, 5−22 mg/kg FW of kaempferol, and 58−450 mg/kg FW of anthocyanidins, while our samples contained 18, 34, 5, and ∼72 mg/kg FW of these components, respectively (**Table 4**). In a study of six Finnish strawberries (*23*), the contents were 9−41 mg/kg FW of *p*-coumaric acid, 30−50 mg/kg FW of quercetin, and 20−90 mg/kg FW of kaempferol. Thus, our samples showed comparable values but were in the lower end of the range.

Ellagic acid and its derivatives were also found in the ethyl acetate extract (peaks 26a, 27, 29, 32, and 42), but no clear ellagitannin peaks were detected. More ellagic acid derivatives were found in the methanolic extract, including a distinctive ellagitannin spectrum (*14*). We followed the same method, equipment, and quantification basis as Koponen et al. (*17*). The contents found in our study were lower than those in three Finnish strawberry cultivars, which contained 650−853 mg of ellagic acid/kg FW (*14*, *17*). In our study, the content of ellagic acid and derivatives was the highest for all groups of phenolic compounds (44% of total phenolic compounds studied), in agreement with previous studies reporting ellagic acid to be the main phenolic compound in strawberries (*18*).

Andean Blackberry (*Rubus glaucus* Berth.). Andean blackberry, also called *mora de castilla*, is mainly found in high-altitude areas of South America (1200−3000 m asl), such as Ecuador, Colombia, Panama, Central America, and Mexico. The fruit can be found throughout the year, but the high production season is during winter. The berries are highly perishable, so they have to be collected when they reach commercial ripeness in hardness and texture (*24*). Because of the fragility of the fruit, it is mainly processed into products that include frozen pulp and jam.

Andean blackberry presented peak 1, which was identified as gallic acid by comparison with the pure standard. In this berry, high amounts of ellagitannins were found (peaks 22, 24, and 26; one of the main peaks in the blackberry ethyl acetate chromatogram), giving a total concentration of ellagitannins and ellagic acid derivatives quantified as ellagic acid after acid hydrolysis of 3547 mg/kg FW (**Table 2**). This amount is lower than the value reported in the literature (5379 mg/kg FW) for a sample of Ecuadorian Andean blackberry (*7*). Galloyl esters were not detected in the ethyl acetate extract, but a more polar galloyl ester was detected in the methanolic extract at a concentration of 21 mg gallic acid equivalents (GAE)/kg FW. The total content of free and conjugated forms of gallic acid in our study was 49 mg GAE/kg FW, which is similar to the value of ∼55 mg GAE/kg FW reported by Mertz et al. *(7).* Three major peaks were detected in the ethyl acetate extract at 250 nm, one corresponding to free ellagic acid (peak 32) and two derivatives (peaks 27 and 29), which might be ellagic acid

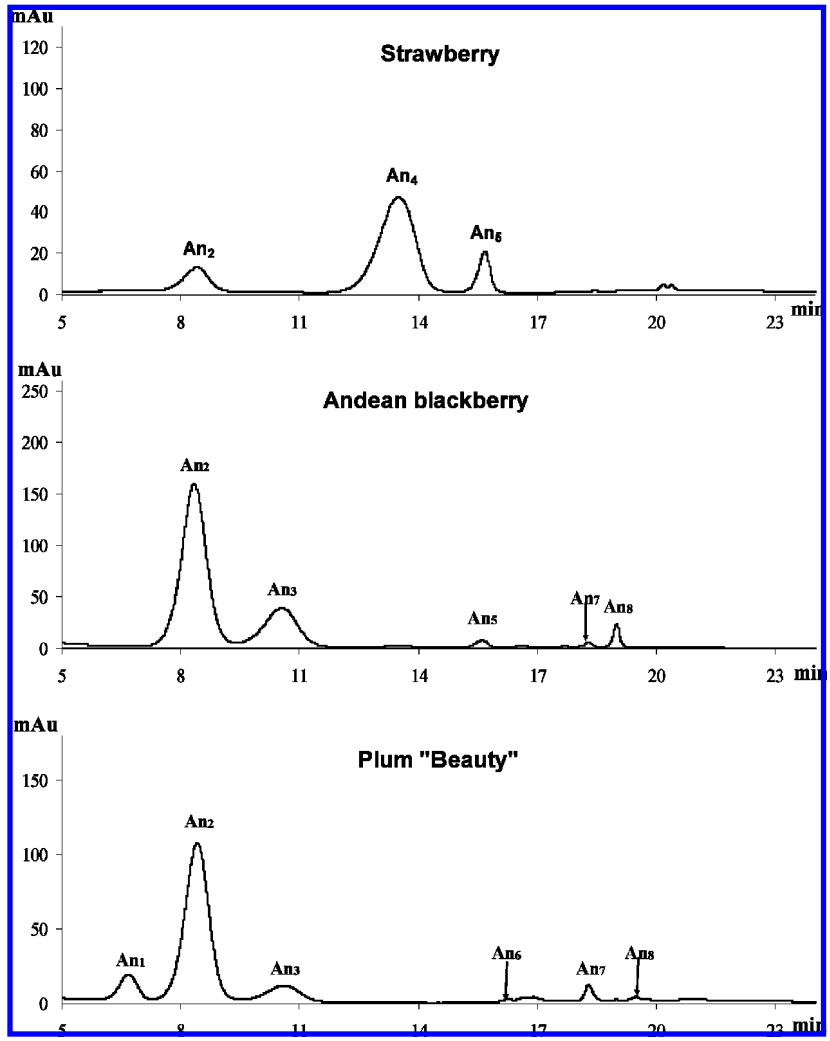

Figure 4. Anthocyanin profiles for the methanolic extracts of strawberry, blackberry, and Beauty plum at 520 nm. Peak identity in **Table 1**.

pentosides according to the literature (*14*). Minor peaks with ellagic acid-like spectra were grouped as "others" (*7, 14*).

Among the hydroxycinnamic acid derivatives, only three peaks were detected (peak 14 and two minor peaks not shown in **Figure 3**). These peaks were tentatively identified as caffeic acid ester, *p*-coumaroyl sugar ester, and *p*-coumaric acid ester (*14, 19*). A flavan-3-ol derivative and (−)-epicatechin were found (peaks 15 and 19, respectively in **Figure 3**), in contrast to results reported by Mertz et al. (*7*) of only (−)-epicatechin being present and at a lower level than in our study (10 compared with 68 mg/kg FW). Peak 33b in **Figure 3** corresponds to quercetin glucuronide, the most abundant flavonol derivative found in blackberry (*7, 14*). Among the minor peaks, a quercetin derivative, quercetin aglycone, and kaempferol derivatives were tentatively identified. Together, they gave a total amount of 76 mg/kg FW, which is in the range reported in the literature (*7*).

The main anthocyanins found in Andean blackberry were cyanidin-3-*O*-glucoside (67% of total anthocyanins) and cyanidin-3-*O*-rutinoside (31% of total anthocyanins). These were identified using the standard and MS data on capulí cherry but were in different proportions than in the literature, which reports 38% glucoside and 62% rutinoside (*7*). The third anthocyanin that eluted at 19.3 min might be the cyanidin-3-*O*-malonyl glucoside found in a previous study (*7*). A compound with the characteristic spectrum of pelargonidin was also detected (An_5), possibly a rutinoside according to the retention time and visible spectrum of this compound found in the strawberry extract, plus reports of the occurrence of pelargonidin glycosides in blackberries (*13, 25*). The total amount of anthocyanins was 510 mg/kg FW, quantified as the weight of the aglycone, which is in agreement with literature values (*7, 26*). Andean blackberry, like other *Rubus* species, e.g. raspberries, has high contents of ellagitannins and ellagic acid derivatives, as well as anthocyanins of the cyanidin type. In our set of samples, this berry and strawberry were the only fruits containing ellagitannins and ellagic acid derivatives, in agreement with the literature (*14*).

Plum (*Prunus salicina* Lindl.). The fruit is known in Ecuador as *reina-claudia*. It is a variety of plum that grows in Tungurahua Province. The fruit is available from December to February, when it is an important fruit in the market and can also be sold on the streets. The plums found in the market are deep purple (peel and flesh) for the variety "Beauty" and have a red peel and yellow flesh for the variety "Santa Rosa".

The total amount of phenolic compounds in Santa Rosa was ∼1170 mg/kg FW, ∼20% lower than that in Beauty (**Table 4**). This difference was due to differences in the contents of anthocyanins and hydroxycinnamic acid derivatives. A galloyl ester was found in the methanolic extract of Santa Rosa but not in Beauty. Neochlorogenic acid (peak 5) was the major hydroxycinnamic acid derivative in both plums, in agreement with the literature (*27*). A second high peak with a caffeoyl

Table 1. Tentative Identification of Phenolic Compounds in the Ethyl Acetate Extract of Andean Blackberry, Strawberry, Plum, and Capulí Cherry

peak no.	t_R (min)	DAD characteristic absorption maxima (nm)	fruit[a]	characterization[c]
		Gallic Acid (280 nm), Ellagitannins, and Ellagic Acid Derivatives (250 nm)		
1	3.6	234, 272	Andean blackberry	gallic acid (std)
4	6.8	232, 288	strawberry	galloyl ester (RS)
36	17.8	234, 282	strawberry	galloyl ester (RS)
22; 24	13.5; 14.1	232, 253	Andean blackberry	ellagitannins (UV−vis)
26	14.5	233, 256	Andean blackberry	ellagitannins (UV−vis)
26a	15.0	254, 370	strawberry	ellagic acid derivative (RS)
27	15.2	254, 362	Andean blackberry, strawberry	ellagic acid glycoside (RS)
29; 42; 45	15.7; 18.3; 19.3	254, 362−364	Andean blackberry, strawberry	ellagic acid glycoside (RS)
32; 49	16.4; 19.8	254, 368	Andean blackberry, strawberry	ellagic acid and ellagic acid derivative (std, RS)
		Hydroxycinnamic Acid Derivatives (320 nm)		
5	7.6	242, 300, 324	plum (B, SR)	neochlorogenic acid (UV−vis)
6	8.2	234, 300, 330	plum (B)	caffeic acid derivative (UV−vis)
11	10.4	238, 300, 326	plum (B), capulí cherry (P, PU)	chlorogenic acid (std, MS)
12	10.4	236, 318	plum(B)	mix of hydroxycinnamic acids (UV−vis)
14; 16	10.4; 10.9	234, 314	Andean blackberry, strawberry	*p*-coumaroyl sugar ester (RS)
39; 48; 53; 54; 57	17.9; 19.5; 23.2; 23.8; 25.7	234, 314	plum (B)	*p*-coumaric acid derivate (UV−vis)
		Flavan-3-ols and Proanthocyanidins (280 nm)		
7	8.8	238, 278	strawberry, plum (SR, B)	flavan-3-ol derivative (UV−vis)
8	9.6	238, 278	strawberry,	flavan-3-ol derivative (UV−vis)
15	10.8	238, 278	Andean blackberry, plum (B)	flavan-3-ol derivative (UV−vis)
17	11.4	238, 278	capulí cherry (P, PU)	(+)-catechin (std, MS)
19	12.4	238, 278	Andean blackberry, capulí cherry (P, PU), plum (B, SR)	(−)-epicatechin (std, MS)
20	13.2	236, 278	capulí cherry (P, PU)	procyanidin trimer B (MS)
23	13.8	234, 278	capulí cherry (P, PU), plum (SR, B)	procyanidin trimer B (MS)
25	14.4	236, 280	capulí cherry (P, PU)	procyanidin dimer B (MS)
28	15.7	238, 278	plum (SR)	proanthocyanidin (UV−vis)
30	16.3	236, 278	capulí cherry (PU), plum (B)	proanthocyanidin (MS)
52	21.2	234, 278	capulí cherry (P)	proanthocyanidin (MS)
		Flavonols (360 nm)		
31	16.1	254, 266, 352	capulí cherry (P), Andean blackberry	quercetin glycoside (rutin) (std, MS)
33	16.9	256, 266, 354	capulí cherry (P), plum (B)	quercetin hexoside (MS)
33a	17.0	256, 355	capulí cherry (P)	quercetin hexoside (MS)
33b	17.2	256, 266, 355	Andean blackberry, strawberry	quercetin-3-*O*-glucuronide (RS, AH)
35	17.3	254, 354	capulí cherry (P)	quercetin-3-*O*-xyloside (MS)
37	17.6	256, 266, 254	capulí cherry (P), plum (SR, B)	quercetin dipentoside (MS)
38	17.9	264, 348	capulí cherry (P)	kaempferol hexoside (MS)
40	18.1	256, 356	Andean blackberry	quercetin glycoside (UV−vis, AH)
41	18.3	256, 352	capulí cherry (P), plum (SR, B)	quercetin-3-*O*-arabinoside (MS)
43	18.6	254, 264, 348	Andean blackberry, strawberry, plum (SR, B)	kaempferol-3-*O*-glucuronide (RS, AH)
44	19.0	256, 266, 354	plum (SR, B)	quercetin glycoside (UV−vis, AH)
47	19.4	264, 348	capulí cherry (P)	kaempferol-*O,C*-dipentoside (MS)
50	20.3	265, 348	capulí cherry (P)	kaempferol pentoside (MS)
51	20.9	254, 266, 348	plum (SR, B)	kaempferol glycoside (UV−vis, AH)
55	24.3	256, 370	capulí cherry (P)	quercetin (std, MS)
		Unknown and Coeluting Compounds		
1a	3.9	234, 294	capulí cherry (P)	unknown
2	5.3	234, 246, 280	capulí cherry (P, PU), plum (B, SR)	unknown
3	6.4	230, 270, 300	Andean blackberry	unknown
9	9.3	(236, 284) (236, 282, 312)[b]	plum (B, SR)	galloyl ester + *p*-coumaric acid (UV−vis)
13	10.4	234, 280, 314	strawberry	(+)-catechin + *p*-coumaric acid (UV−vis)
10	10.4	(236, 278) (234, 298, 325)[b]	plum (SR)	(+)-catechin + chlorogenic acid (UV−vis)
18	11.8	(236, 278) (234, 298, 325)[b]	plum (B, SR)	proanthocyanidin + caffeic/ferulic acid (UV−vis)
21	13.2	238, 280, 328	plum (B, SR)	proanthocyanidin + caffeic/ferulic acid (UV−vis)
21a	13.5	234, 282, 304	capulí cherry (P)	unknown
34	16.9	256, 266, 292, 354	plum (SR)	quercetin glycoside + proanthocyanidin (UV−vis)
56	25.6	232, 256, 268, 316	plum (B)	unknown
		Anthocyanins (520 nm)		
An_1	6.8	278, 516	plum (B)	cyanidin glycoside (UV−vis)
An_2	8.5	280, 516	Andean blackberry, strawberry, capulí cherry (P), plum (SR, B)	cyanidin-3-*O*-glucoside (std, MS)
An_3	10.8	280, 518	Andean blackberry, capulí cherry (P), plum (SR, B)	cyanidin-3-*O*-rutinoside (MS)
An_4	13.8	276, 502	strawberry	pelargonidin-3-*O*-glucoside (std, RS)
An_5	15.8	276, 504	Andean blackberry, strawberry	pelargonidin-3-*O*-rutinoside (RS)
An_6	16.7	280, 518	plum (B)	cyanidin glycoside (UV−vis)
An_7	18.5	278, 526	Andean blackberry, plum (B), capulí cherry (P)	cyanidin (std)
An_8	19.3	282, 520	Andean blackberry, plum (B)	cyanidin glycoside (UV−vis)

[a] Capulí cherry (P = peel; PU = pulp); plum (B = variety Beauty; SR = variety Santa Rosa). [b] Characteristic absorption maxima of coeluting peaks. [c] Characterization based on (std) = standard retention time and standard UV−vis spectra, (RS) = reference sample, (UV−vis) = characteristic absorption maxima for classification in the different phenolic compound groups, AH = aglycone confirmed by release by acid hydrolysis.

Table 2. Contents of Ellagitannins and Ellagic Acid as Free and Derivatives (mg of Ellagic Acid Equivalent/kg of FW)

sample	ellagitannins and ellagic acid derivatives[a]
strawberry	278 ± 45
Andean blackberry	3547 ± 659

[a] Results are presented as mean ± SD. Samples analyzed were collected from different markets ($n = 3$). Result of the content quantified after hydrolysis.

hexose spectrum (peak 6) was found in the deep purple Beauty plums. Although some studies (*9*, *10*, *27*) have reported cryptochlorogenic acid, an isomer of chlorogenic acid, as the second major hydroxycinnamic acid derivative in some plum cultivars, the absorption spectrum (**Table 1**) did not correspond with that of chlorogenic acid. This peak seemed to agree better with the description of Tomas-Barberán et al. (*12*), who found an unidentified caffeic acid derivative in plums, including the varieties Santa Rosa, "Black Beauty", and "Red Beauty", and clearly specified that no cryptochlorogenic acid was detected. In plums, (+)-catechin, chlorogenic acid, and other hydroxycinnamic acids coeluted at 10.4 min (peaks 10 and 12). In Santa Rosa, chlorogenic acid was quantified at 320 nm (40 mg/kg FW), but (+)-catechin could not be quantified at 280 nm due to overlapping spectra. In Beauty, on the other hand, the purity of peak 12 showed a mix of hydroxycinnamic acids which were quantified as a *p*-coumaric derivative according to typical spectral shape. In both plums, several *p*-coumaric acid derivatives were found (peaks 39, 48, 53, 54, and 57) but the amounts were lower in Santa Rosa.

The main proanthocyanidins in plums are B1, B2, B4, and A-type dimers and some trimers (*12*, *28*). In our study, five different proanthocyanidins were found in plums (peaks 7 and 23 in both varieties, peaks 15 and 30 in Beauty, and peak 28 in Santa Rosa), but their exact identities could not be revealed. High amounts of proanthocyanidins, quantified as catechins, were found in both varieties (~880 mg/kg FW), although the individual compound profile differed from one variety to the other. These levels of proanthocyanidins are similar to the 203−680 mg catechin/kg FW reported by Tomás-Barberán et al. (*12*) and to the 755 mg/kg FW reported by De Pascual-Teresa et a.l (*20*) for Spanish plums, but much lower than the 2159−2566 mg/kg FW reported by Gu et al. (*28*) for plums from the United States. Such large variations have been observed for the contents of phenolic compounds and other secondary metabolites in fruits (*12*).

The flavonol profiles in both plum varieties were similar: mainly quercetin derivatives and two kaempferol derivatives. In Santa Rosa, peak 33, identified as quercetin hexoside (-3-glucoside or -3-galactoside), coeluted with one of the proanthocyanidins (peak 34). According to the literature, kaempferol-3-rutinoside, -3-glucoside, -3-arabinoside, -3-arabinoside-rhamnoside, quercetin-3-xyloside, and -3-rhamnoside are characteristic for *P. domestica* and *P. salicina* (*12*). The quercetin derivatives (peaks 37, 41, and 44) corresponded to a quercetin dipentoside, quercetin-3-arabinoside, and a quercetin glycoside (*12*, *19*). Cyanidin glycosides were found at ~250 mg/kg FW in Beauty compared with 75 mg/kg FW in Santa Rosa. This is due to differences between the cultivars since Beauty has anthocyanins in the peel and the pulp, whereas Santa Rosa has a colored peel but yellow flesh. Beauty contained An_1 (tentatively identified as cyanidin-3-*O*-galactoside), which was not found in Santa Rosa. Different authors have reported a wide range (from 140 to 3000 mg/kg FW) of anthocyanin contents for *P. domestica*, *P. salicina*, and other commercial varieties, as reviewed by Cevallos-Casals et al. (*11*).

Capulí (*Prunus serotina* Ehrh. var. Capuli). Capulí cherry is one of the most common trees on the highlands from Venezuela to Peru. Most botanists believe that it was introduced from Mexico or Central America in colonial times. The fruit is available in large amounts in the open market in Ecuador from December to February. Related to the black cherry and Bing cherry, it is similar in shape and taste, but the fruits are found in bunches. The peel is thin with a slightly bitter taste, and the pulp is green. Mostly eaten fresh, it can also be stewed (together with peaches in a traditional Ecuadorian dessert called *jucho*), preserved, or made into jam or wine (*24*).

The main hydroxycinnamic acid in our capulí cherry sample was chlorogenic acid (peak 11 in **Figure 3**), as confirmed by LC-MS and compared with the standard. (+)-Catechin and (−)-epicatechin were identified using LC-DAD, MS, and pure standards. MS confirmed the presence of two trimeric (peaks 20 and 23) and one dimeric proanthocyanidin (peak 25) (*29*), plus another proanthocyanidin that could not be identified (peak 52).

Table 3. Identification of the Phenolic Compounds in Capulí Cherry by LC-DAD, MS, and MS/MS

peak no.[a]	DAD characteristic absorption maxima (nm)	MS (*m*/*z*)	MS/MS (*m*/*z*)	tentative identification
2	234,252,280	482	447, 465, 313	unknown
17	278	291	123, 139, 165, 273	(+)-catechin (std)
11	240,296,325	355	163	chlorogenic acid (std)
19	278	291	123, 139, 165, 273	(−)-epicatechin
20	278	867	577, 715, 697, 409	procyanidin trimer B
23	278	867	577, 715, 409	procyanidin trimer B
25	278	579	427, 409, 291, 301, 247	procyanidin dimer B
31	256, 356	611	303, 465, 355, 593	rutin (std)
52	278	593	441	proanthocyanidin
33	256, 354	465	303	quercetin hexoside
33a	256, 355	465	303	quercetin hexoside
35	256, 354	435	303	quercetin-3-*O*-xyloside
37	256, 352	567	435, 303, 417	quercetin dipentoside
38	266, 348	449	287	kaempferol hexoside
41	256, 354	435	303	quercetin-3-*O*-arabinoside
47	264,294,348	551	419, 401	kaempferol-*O*,*C*-dipentoside
50	264,294,348	419	287	kaempferol pentoside
55	256,300,372	303	257, 229, 285, 165, 247, 165	quercetin aglycone
An2	240,326,516	449	287	cyanidin-3-*O*-glucoside
An3	236,280,518	595	449, 287	cyanidin-3-*O*-rutinoside

[a] Peak numbers correspond to **Figure 1**.

Table 4. Summary of the Content of Phenolic Compounds in the Fruit Samples (mg/kg FW)

			plums		
	strawberry	Andean blackberry	(SR)	(B)	capulí cherry[a]
gallic acid and galloyls	46	49			
neochlorogenic acid			76	92	
chlorogenic acid			40		188
caffeic acid derivatives				29	
p-coumaric acid derivatives	18	4		85	
(−)-epicatechin		68	173	415	972
(+)-catechin					454
proanthocyanidins	78	58	708	469	655
quercetin derivatives	34	59	119	144	72
kaempferol derivatives	5	4	14	5	3
cyanidin glycosides	6	508	76	250	89
pelargonidin glycosides	66	2			
ellagitannins and ellagic acid derivatives	278	3926			
total phenolic compounds	**531**	**4678**	**1206**	**1489**	**2433**

[a] Whole fruit.

The flavonol glycosides (peaks 31, 33, 33a, 35, 37, 38, 41, 47, 50, and 55) correspond to either quercetin or kaempferol derivatives according to their UV−vis spectra. Peak 31 showed the typical fragmentation pattern of the rutin standard, an identification supported by the retention time and UV−vis spectrum. Two peaks (33 and 33a) corresponded to quercetin hexosides due to a loss of 162 mass units, which corresponds to a glucose or galactose sugar moiety. Peaks 35 and 41, both showing molecular ions at *m*/*z* 435 with an MS-MS fragment at *m*/*z* 303 (loss of 132 mass units corresponding to xylose or arabinose), were identified as quercetin pentosides and tentatively designated quercetin-3-xyloside (peak 35) and quercetin-3-arabinoside (peak 41) through comparison with the literature (*19*). Peak 37 was identified as quercetin dipentoside because it had a molecular ion at *m*/*z* 567 in LC-MS which fragmented to ions at *m*/*z* 435 (loss of 132 mass units due to a pentose) and *m*/*z* 303 (again loss of 132 mass units due to loss of a second pentose). Finally, peak 55 corresponds to the aglycone of quercetin, identified using LC-MS and MS-MS and the pure standard.

Among the kaempferol derivatives, peak 38 is a kaempferol hexoside identified by the characteristic fragmentation. Peak 47 presented an interesting MS-MS fragmentation pattern, suggesting mixed *O*,*C*-diglycoside bonds, as evidenced by the fragment at *m*/*z* at 419 (loss of 132 mass units, corresponding to the loss of an O-linked pentose) while retaining another pentose in the molecule (corresponding to the C-linkage) (*30, 31*). Peak 50 is a kaempferol pentoside according to the loss of 132 mass units. Finally, the anthocyanins in capulí cherry An_2 and An_3 were identified as cyanidin-3-*O*-glucoside and -3-*O*-rutinoside, respectively, by the characteristic fragmentation and by comparison with the pure standard for the glucoside and with the literature for the rutinoside in a study on capulin cherry, a variety of *P. serotina* cultivated in Mexico (*14, 16*).

For capulí cherry, the phenolic profile was similar for the pulp and the peel, but the contents were lower in the pulp. Chlorogenic acid (peak 11) was also the main hydroxycinnamic acid in the pulp, and the content was approximately half that in the peel. The proanthocyanidins were the same in the peel and the pulp except for peak 30, which was only found in the pulp. The flavonol glycosides were found in very low concentrations (~14 mg/100 g FW), and no anthocyanins were detected in the pulp. The fruit is consumed whole or peeled, but the intake of the phenolic compounds mainly derives from the pulp, which constitutes ~95% of the edible part of the fruit. The content of the different phenolic compounds, calculated for the whole fruit, is given in **Table 4**. The profiles of phenolic compounds were comparable between capulí cherry and plums.

Comparison of the Different Rosaceae Fruits. The data obtained in this study are on the contents of different phenolic compounds and their classes in four Ecuadorian fruits from the *Rosaceae*; strawberry, Andean blackberry, plum, and capulí cherry are summarized in **Table 4**. Consistent with our previous results on total antioxidant activity in Ecuadorian fruits, Andean blackberry was the richest fruit in phenolic compounds (~4700 mg/kg FW). The phenolic compounds in Andean blackberry were dominated by ellagitannins, ellagic acid derivatives, and cyanidin glycosides. The phenolic compounds in strawberry (mainly ellagitannins, pelargonidin glycosides, and proanthocyanidins) were generally at the lower range of values reported in the literature. The two varieties of plums analyzed differed in their contents of phenolic compounds, with proanthocyanidins, (−)-epicatechin, and neochlorogenic acid dominating. Capulí cherry, a special Andean cherry, contained mainly (+)-catechin and (−)-epicatechin, cyanidin glycosides, dimeric and trimeric proanthocyanidins, and quercetin glycosides. These differences in the phenolic composition of the fruits might explain the differences in their antioxidant capacity (Andean blackberry > capulí cherry > plum > strawberry).

LITERATURE CITED

(1) Scalbert, A.; Johnson, I. T.; Saltmarsh, M. Polyphenols: antioxidants and beyond. *Am. J. Clin. Nutr.* **2005**, *81*, 215S–217S.

(2) Dillard, C. J.; German, J. B. Phytochemicals: nutraceuticals and human health. *J. Agric. Food Chem.* **2000**, *80*, 1744–1756.

(3) Prior, R. L. Fruits and vegetables in the prevention of cellular oxidative damage. *Am. J. Clin. Nutr.* **2003**, *78*, 570S–578S.

(4) Vasco, C.; Ruales, J.; Kamal-Eldin, A. Total phenolic compounds and antioxidant capacities of major fruits from Ecuador. *Food Chem.* **2008**, *111* (4), 816–823.

(5) Määttä-Riihinen, K.; Kamal-Eldin, A.; Mattila, P.; González-Paramás, A. M.; Törrönen, R. Distribution and contents of phenolic compounds in eighteen Scandinavian berry species. *J. Agric. Food Chem.* **2004**, (52), 4477–4486.

(6) Seeram, N. P.; Lee, R.; Scheuller, S. H.; Heber, D. Identification of phenolic compounds in strawberries by liquid chromatography electrospray ionization mass spectroscopy. *Food Chem.* **2006**, *97*, 1–11.

(7) Mertz, C.; Cheynier, V.; Günata, Z.; Brat, P. Analysis of phenolic compounds in two blackberry species (*Rubus glaucus* and *Rubus adenotrichus*) by high-performance liquid chromatography with diode array detection and electrospray ion trap mass spectrometry. *J. Agric. Food Chem*. **2007**, *55*, 8616–8624.
(8) Cho, M. J.; Howard, L. R.; Prior, R. L.; Clark, J. R. Flavonol glycosides and antioxidant capacity of various blackberry and blueberry genotypes determined by high-performance liquid chromatography/mass spectrometry. *J. Sci. Food Agric*. **2005**, *85*, 2149–2158.
(9) Donovan, J. L.; Meyer, A. S.; Waterhouse, A. L. Phenolic composition and antioxidant activity of prunes and prune juice (Prunus domestica). *J. Agric. Food Chem*. **1998**, *46*, 1247–1252.
(10) Kayano, S.-i.; Yamada, N. F.; Suzuki, T.; Ikami, T.; Shioaki, K.; Kizuzaki, H.; Mitani, T.; Nakatani, N. Quantitative evaluation of antioxidant components in prunes (*Prunus domestica* L.). *J. Agric. Food Chem*. **2003**, *51*, 1480–1485.
(11) Cevallos-Casals, B.; Byrne, D.; Okie, W. Cisneros-Zevallos. Selecting new peach and plum genotypes rich in phenolic compounds and enhanced functional properties. *Food Chem*. **2006**, (96), 273–280.
(12) Tomás-Barberán, F. A.; Gil, M. I.; Cremin, P.; Waterhouse, A. L.; Hess-Pierce, B.; Kader, A. A. HPLC-DAD-ESIMS analysis of phenolic compounds in nectarines, peaches and plums. *J. Agric. Food Chem*. **2001**, *49*, 4748–4760.
(13) Wu, X.; Prior, R. L. Systematic identification and characterization of anthocyanins by HPLC-ESI-MS/MS in common food in the United States: Fruits and berries. *J. Agric. Food Chem*. **2005**, *53*, 2589–2599.
(14) Määttä-Riihinen, K.; Kamal-Eldin, A.; Törrönen, R. Identification and quantification of phenolic compounds in berries of *Fragaria* and *Rubus* species (Family *Rosaceae*). *J. Agric. Food Chem*. **2004**, *52*, 6178–6187.
(15) Macheix, J.-J.; Fleuriet, A.; Billot, J. *Fruit Phenolics*; CRC: Boca Raton, FL, 1990; p 378.
(16) Ordaz-Galindo, A.; Wesche-Ebeling, P.; Wrolstad, R. E.; Rodriguez-Saona, L.; Argaiz-Jamet, A. Purification and identification of capulin (*Prunus serotina* Ehrh) anthocyanins. *Food Chem*. **1999**, *65*, 201–206.
(17) Koponen, J. M.; Happonen, A. M.; Mattila, P. H.; Törrönen, A. R. Contents of anthocyanins and ellagitannins in selected foods consumed in Finland. *J. Agric. Food Chem*. **2007**, *55*, 1612–1619.
(18) Häkkinen, S.; Heinonen, M.; Kärenlampi, S.; Mykkänen, H.; Ruuskanen, J.; Törrönen, A. R. Screening of selected flavonoids and phenolic acids in 19 berries. *Food Res. Int*. **1999**, *32*, 345–353.
(19) Riihinen, K. R. Phenolic compounds in berries. Doctoral dissertation, University of Kuopio, Kuopio, 2005.
(20) De Pascual-Teresa, S.; Santos-Buelga, C.; Rivas-Gonzalo, J. Quantitative analysis of flavan-3-ols in Spanish foodstuffs and beverages. *J. Agric. Food Chem*. **2000**, *48*, 5331–5337.
(21) Slimestad, R.; Solheim, H. Anthocyanins from black currants (*Ribes nigrum* L.). *J. Agric. Food Chem*. **2002**, *50*, 3228–3231.
(22) Kosar, M.; Kafkas, E.; Paydas, S.; Can Baser, H. K. Phenolic composition of strawberry genotypes at different maturation stages. *J. Agric. Food Chem*. **2004**, *52*, 1586–1589.
(23) Häkkinen, S. H.; Törrönen, A. R. Content of flavonols and selected phenolic acids in strawberries and Vaccinium species: influence of cultivar, cultivation site and technique. *Food Res. Int*. **2000**, *33*, 517–524.
(24) Popenoe, H.; King, S.; León, J.; Kalinowski, L.; Vietmeyer, N.; Dafforn, M. *Lost Crops of the Incas. Little known plants of the Andes with promise for worldwide cultivation*; National Academy Press: Washington, DC, 1989; p 428.
(25) Dugo, P.; Mondello, L.; Errante, G.; Zappia, G.; Dugo, G. Identification of anthocyanins in berries by narrow-bore high performance liquid chromatography with electrospray ionization detection. *J. Agric. Food Chem*. **2001**, *49*, 3987–3992.
(26) Fan-Chiang, H.-J.; Wrolstad, R. E. Anthocyanin pigments composition of blackberries. *J. Food Sci*. **2005**, *70* (3), C198−C202.
(27) Nakatani, N.; Kayano, S.-i.; Kikuzaki, H.; Sumino, K.; Katagiri, K.; Mitani, T. Identification, quantitative determination, and antioxidative activities of chlorogenic acid isomers in prune (*Prunus domestica* L.). *J. Agric. Food Chem*. **2000**, *48*, 5512–5516.
(28) Gu, L.; Kelm, M. A.; Hammerstone, J. F.; Beecher, G. R.; Holden, J. M.; Haytowitz, D. B.; Prior, R. L. Screening of foods containing proanthocyanidins and their structural characterization using LC-MS/MS and thiolytic degradation. *J. Agric. Food Chem*. **2003**, *51*, 7513–7521.
(29) Määttä-Riihinen, K. R.; Kähkönen, M. P.; Törrönen, A. R.; Heinonen, M. I. Catechins and proanthocyanidins in berries of Vaccinium species and their antioxidant activity. *J. Agric. Food Chem*. **2005**, *53* (22), 8485–8491.
(30) Claeys, M. Mass spectrometry in studies of flavonoid glycosides. In *Fundamentals and applications of gas phase ion chemistry*; Kluwer Academic Publishers: New York, 1999; Vol. 521, pp 512.
(31) Kumazawa, T.; Kimura, T.; Matsuba, S.; Sato, S.; Onodera, J.-i. Cleavage of the C-C linkage between the sugar and the aglycone in C-glycosylphloroacetophenone, and the NMR spectral characteristics of the resulting di-C-glycosyl compound. *Carbohydr. Res*. **2001**, *334*, 207–213.

Received for review August 28, 2008. Revised manuscript received November 26, 2008. Accepted December 12, 2008. This research (Project EC: 01) was supported by grants from the International Programme in the Chemical Sciences (IPICS, Uppsala University, Uppsala, Sweden).

JF802656R