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ARTICLE

Chemical Composition and Phenolic Compound Profile of Mortiño (*Vaccinium floribundum* Kunth)

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The phenolic compounds in mortiño (*Vaccinium floribundum* Kunth, family Ericaceae) from the páramos of Ecuador were studied by LC-DAD−MS/MS for the first time. (−)-Epicatechin, one dimer A and one trimer A were found at a total concentration of 18 mg/100 g FW. Of the flavonol glycosides (38 mg/100 g FW), quercetin and myricetin were found as -3-*O*-hexosides, -3-*O*-pentosides and -3-*O*-deoxyhexosides. Chlorogenic and neochlorogenic acids together with caffeic/ferulic acid derivatives were found as predominant components among the hydroxycinnamic acids in the berry. Anthocyanins, including cyanidin and delphinidin derivatives, were the major phenolic compound class quantified (345 mg cyanidin-3-*O*-glucoside/100 g FW).

KEYWORDS: Mortiño; *Vaccinium floribundum*; phenolic compounds; TEAC; LC−MS; quercetin; anthocyanins

INTRODUCTION

Blueberry and bilberry (*Vaccinium* spp., family Ericaceae) are attracting great interest due to their high content of anthocyanins and other antioxidants and their protective effects against disease, especially cancer (*1*) and age-related neurodegenerative diseases (*2*). Like other berries, blueberries have been characterized as having a complex flavonoid profile with potential use for the food, nutraceutical, and pharmaceutical industries (*3*−*5*). The phenolic compounds that have been characterized in berries include gallic and ellagic acids, ellagitannins, flavan-3-ols, proanthocyanidins, flavonol glycosides, hydroxycinnamic acids and anthocyanins, and they are usually found in very high concentrations (*6*−*8*). Stilbenes, the group of compounds found in grapes and wine, have also been identified in *Vaccinium* berries (*9*). Thorough studies have been performed on the total phenolic contents, antioxidant capacities, and concentrations of different compounds in berries and how they are influenced by variety, maturity, and location (*8*, *10*−*13*). These studies emphasize the importance of genetic background (*11*), environmental factors (*12*), and cultivation practices (*13*) on the biosynthesis of phenolic compounds.

Mortiño (*Vaccinium floribundum* Kunth, syn. *Vaccinium mortinia* Benth), sometimes called Andean blueberry, is a member of the Ericaceae family. This berry is found in Ecuador in the páramos, high altitude grasslands between 3400 and 3800 m above sea level. This berry is black, round, about 7 mm diameter, with many almost undetectable seeds, and sourer than the North American cultivated highbush blueberry (*Vaccinium corymbosum* L.) (*14*). It is very glaucous, with green flesh (**Figure 1**), and the anthocyanins are located in the peel. The average mass is 0.4 g. Mortiño berries are usually collected from wild bushes and sold in the markets during a short season from October to December. In Ecuador, mortiño is used as an ingredient in a special dish called "mazamorra morada" prepared every year for 2 November, All Souls' Day or the "Day of the Dead". In this study, we characterized for the first time the chemical composition, total phenolic compounds, antioxidant capacity, and phenolic compound profile of mortiño. The aim was to find the true potential of this Andean berry compared with previously studied dark purple-colored *Vaccinium* berries (*V. angustifolium*, *V. corymbosum, V. ashei* Reade, and *V. myrtillus* L.).

MATERIALS AND METHODS

Standards and Chemicals. Gallic acid, *p*-hydroxybenzoic acid, vanillic acid, (+)-catechin, (−)-epicatechin, chlorogenic acid, caffeic acid, ferulic acid, *p*-coumaric acid, quercetin, Folin−Ciocalteu reagent (2.0 N), ABTS (2,2′-azinobis (3-ethylbenzothiaoline-6-sulfonic acid)), Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and potassium persulfate were from Sigma Aldrich (St. Louis, MO), myricetin was from Fluka (Buchs, Switzerland), sodium carbonate anhydrous was from JT Baker (Phillipsburg, NJ) and cyanidin-3-*O*-glucoside chloride was from Extrasynthese (Geney, France). Methanol, ethanol and acetonitrile were HPLC grade, and ethyl acetate and acetone were analytical grade. Standard stock solutions (1 mg/mL) were prepared in methanol, and stored at −20 °C for quantification of the different phenolic compounds. Calibration curves were constructed for each standard. Galloyl esters were quantified as gallic acid, hydroxybenzoic acids as *p*-hydroxybenzoic acid or vanillic acid, flavan-3-ols as (+)-catechin or (−)-epicatechin, proanthocyanidins as (+)-catechin, flavonols as quercetin or myricetin, hydroxycinnamic acids as chlorogenic, caffeic, *p*-coumaric acids and anthocyanins as cyanidin aglycon.

Sample Preparation. Ripe mortiño berries (1.0 kg, 12 °Brix) were purchased on three different occasions from three different open markets

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Figure 1. Mortiño berries.

in Quito during the berry season (October−December). The berries were cleaned by removing leaves, stems, and unripe and damaged berries. Every purchase was divided into four portions (~250 g/tray), weighed and freeze-dried, and then the trays were immediately packed in plastic and weighed to determine the moisture content of fresh mortiño berries. A domestic mill was used to grind the samples to a fine powder, which was packed and stored at −20 °C until analysis. For chemical characterization, total soluble phenolic compounds and antioxidant activity, one sample from each market was prepared by pooling the three subsamples from every purchase.

Chemical Characterization. Proximate analysis was performed on the freeze-dried pooled samples using the following official AOAC methods (*15*): moisture (AOAC method 934.06), ash (AOAC method 920.46), protein (AOAC method 2001.11), and fat (AOAC method 922.06). Total carbohydrate content was calculated as 100 − % (moisture + fat + protein + ash), and caloric value was estimated using conversion factors according to FAO (*16*). Reference methods were used for soluble and insoluble dietary fiber (*17*). Sugars were analyzed by high performance liquid chromatography (HPLC) according to the instruction manual for the column ASTEC NH_2 series (250 mm × 4.6 mm i.d., 5 μm, Advance Separation Technologies Inc., USA) on the water extract of the samples with a flow of 1 mL/min of acetonitrile:water (80:20, v/v) for 30 min and connected to a refractive index detector IR HP 1047A. Organic acids were analyzed by HPLC with an Aminex HPX-87H (300 mm × 7.8 mm i.d., 9 μm) column in a 30 min isocratic separation at a flow rate of 0.5 mL/min with 0.02 N H_2SO_4 mobile phase and UV detection at 210 nm on the extracts prepared with 0.05% metaphosphoric acid and 0.02% homocysteine in 0.02 N H_2SO_4 (*18*), while minerals were analyzed using an atomic absorption spectrometer Perkin-Elmer 3300 (PerkinElmer Inc., USA) after microwave digestion with 65% HNO_3 and 98% H_2SO_4 (*19*).

The chromatography systems consisted of a Hewlett-Packard 1050 multisolvent delivery HPLC system connected to a 1050 PDA and equipped with Peak 96 and HP-ChemStation Chromatography Software (Waldbronn Analytical Division, Germany) and was also the equipment used for vitamin C and β-carotene determinations.

Antioxidant Components and Antioxidant Activity. *Vitamin C* was determined as ascorbic acid by HPLC following a modification of the method described by Van Niekerk (*20*) and validated in the laboratory. In brief, an extraction with 30 mL of metaphosphoric acid (3%) and 5 mL of homocysteine (0.2%) under nitrogen using an ultrasonic bath for 15 min was carried out using 3 g of freeze-dried sample. The volume was made up to 50 mL and centrifuged (3417*g*, 4 °C). The supernatant was filtered and injected into the HPLC. Separation was achieved in a LiChrospher 100 NH2 (250 mm, 4 mm i.d., 5 μm) column with a flow of 1 mL/min using a mixture of KH_2PO_4 (0.25%, pH 3.5) and methanol 50:50 (v/v) for 7 min and UV−vis detection at λ = 244 nm.

β-Carotene was measured by HPLC using the method described by Pettersson and Jonsson (*21*). Freeze-dried samples (0.250 g) were remoistened and extracted using acetone:ethanol (50:50 v/v) until colorless. The fractions were pooled and extracted with petroleum ether. The ether extract was then evaporated to dryness and dissolved in 10 mL of the mobile phase (methanol:*tert*-butyl ether:water, 56:40:4). An isocratic separation was run in a YMC carotenoid column (250 mm × 4.5 mm i.d. × 5 μm) at 1 mL/min flow for 25 min using UV−vis detection at λ = 450 nm.

Total Soluble Phenolic Compounds. Freeze-dried sample (0.5 g) was extracted at room temperature with 20 mL of methanol:water (50:50 v/v) for 1 h, centrifuged, extracted again for 1 h with 20 mL of acetone:water (70:30 v/v), and centrifuged, and the supernatants from both extractions were combined in a volumetric flask and made up to 50 mL with distilled water (*22*). The total soluble phenolic content was measured using a modified Folin−Ciocalteu method (*23*), and quantification was done as gallic acid equivalents (GAE).

Antioxidant activity was measured on the same extract as for total phenolic compounds with the modified TEAC assay using the ABTS radical cation decolorization reported by Re et al. (*24*) and expressed as Tolox equivalents.

All determinations were performed in triplicate, and the mean value was determined for each of the three pooled samples analyzed.

Extraction and HPLC Analysis of Phenolic Compounds. For LC-DAD−MS/MS, the two-step extraction described by Määttä et al. (*25*) was used. In brief, about 0.6 g of the freeze-dried sample was remoistened and extracted four times with ethyl acetate (10 mL), carefully mixed (1 min) and centrifuged (2880*g* × 2 min). The extracts were pooled, evaporated, dissolved into 2 mL of methanol, filtered and injected into the HPLC to analyze gallic acid and galloyl esters, hydroxybenzoic and hydroxycinnamic acids and derivatives, flavonol glycosides, flavan-3-ols and oligomeric proanthocyanidins. The solid residue of the previous extraction was acidified with 2 mL (2 M, HCl) and extracted first with 20 mL of methanol and then with 10 mL on three occasions. The methanol extracts were pooled, and an aliquot of 10 mL was evaporated, reconstituted in methanol, filtered and injected into the HPLC to analyze residues from the previous extraction. Anthocyanins were analyzed directly in the methanol extract. All determinations were performed in triplicate, and the mean value was determined for the nine samples analyzed.

Table 1. Proximate Analysis, Sugars, Organic Acids, Minerals, Total Phenolic Content and Antioxidant Capacity in Mortiño from Ecuador[a]

component	level
proximate components	
moisture (g/100 g FW)[b]	81.0 ± 2.0
fat (g/100 g FW)	1.0 ± 0.04
protein (g/100 g FW)	0.7 ± 0.02
ash (g/100 g FW)	0.4 ± 0.03
total carbohydrates (g/100 g FW)	16.9 ± 0.1
total dietary fiber (g/100 g FW)	7.6 ± 2.2
soluble dietary fiber (g/100 g FW)	1.2 ± 1.0
insoluble dietary fiber (g/100 g FW)	6.5 ± 2.5
soluble sugars	
fructose (g/100 g FW)	4.4 ± 0.4
glucose(g/100 g FW)	2.6 ± 0.3
caloric value (kcal/100 g FW)	84.0 ± 0.4
organic acids	
citric acid (mg/100 g FW)	3142 ± 614
malic acid (mg/100 g FW)	1823 ± 274
metal ions	
Fe (mg/100 g FW)	0.64 ± 0.2
K (mg/100 g FW)	607 ± 73
Ca (mg/100 g FW)	17.0 ± 2.3
Mg (mg/100 g FW)	10.2 ± 1.1
Cu (mg/100 g FW)	0.12 ± 0.02
Zn (mg/100 g FW)	0.13 ± 0.02
antioxidant components	
ascorbic acid (mg/100 g FW)	9.0 ± 2.0
β-carotene (μg/100 g FW)	36.0 ± 6.0
total soluble phenolic content (mg GA/100 g FW)	882 ± 38
TEAC (mg Trolox/100 g FW)	1203 ± 94

[a] Results presented as mean ± SD (n = 3). [b] Moisture was measured on fresh mortiño, n = 9. Moisture content in freeze-dried mortiño was 4 g/100 g. For conversion from dw to FW, the moisture contents in fresh and freeze-dried mortiño were used.

A Hewlett-Packard liquid chromatography system equipped with a 1100 series quaternary pump, autosampler, DAD and HP-Chemstation data processing system (Waldbronn Analytical Division, Germany) was used with a Gemini C18 column (150 × 3 mm i.d., 5 μm, Phenomenex, USA) protected with a guard column of the same material.

For identification of the compounds, the HPLC system was simultaneously connected to a DAD and a Finnigan LTQ linear ion trap mass spectrometer (Thermo, San Jose, CA). Electrospray ionization (ESI)-MS/MS was run in the positive mode, capillary voltage 3.8 kV, 275 °C and collision energy of 35% in the MS/MS. Analysis was full scan mode, and data were collected in the range m/z 170−1000.

The ethyl acetate and methanol extracts were analyzed for gallic acid, galloyl esters, flavan-3-ols and proanthocyanidins, hydroxycinnamic acid and derivatives and flavonols using a 25 min linear gradient: 5−30% acetonitrile:methanol (85:15, solvent B) and 1% formic acid in water (solvent A) at a flow rate of 1 mL/min. Anthocyanins were quantified in a separate run with a different gradient: 8.5% formic acid in water (solvent A), acetonitrile:methanol (85:15, solvent B), flow rate 1 mL/min and the following program: 0−2 min 4−10% B, 2−20 min 10% B, 20−35 min 10−15% B, 35−40 min 15−35% B, 40−50 min 35−80% B, 50−52 min 80% B, and 52−60 min 80−4% B. Diode array detection scans from 240 to 520 nm were used for identification and quantification, and LC-DAD data were simultaneously recorded at four different wavelengths: 280 nm for gallic acid, galloyl esters, flavan-3-ols and proanthocyanidins, 320 nm for hydroxycinnamic acid and derivatives, 360 nm for flavonols and 520 nm for anthocyanins. MS/MS data were only collected using the 25 min linear gradient described above with 1% formic acid in water as solvent A and acetonitrile:methanol (85:15) as solvent B. Calibration curves were constructed using the standard stock solutions, and quantification was based on peak area at the wavelength of maximum absorption for the different classes and calculated as the weight of the aglycon. Anthocyanins were all quantified as cyanidin aglycon and oligomeric proanthocyanidins as (+)-catechin.

RESULTS AND DISCUSSION

Mortiño berries, which are available in Ecuadorian markets during October−December, were characterized as shown in **Table 1**. The tentative identification of the phenolic compounds using the LC-DAD−ESI-MS/MS results is shown in **Table 2**, where compounds were classified according to their spectral characteristics into galloyl esters, hydroxybenzoic acid derivatives, flavan-3-ols, proanthocyanidins, flavonols, hydroxycinnamic acid derivatives and anthocyanins. Finally, quantification (**Table 3**)

Table 2. Tentative Identification of Phenolic Compounds in Mortiño, UV−Vis Spectra and LC−ESI$^+$ MS/MS

peak number[a]	t_R (min)	DAD characteristic absorption maxima (nm)	MS (m/z)	MS/MS (m/z)	tentative identification
		Galloyl Esters and Hydroxybenzoic Acid Derivatives			
2	8.2	260, 296			vanillic acid derivative
4	10.8	262			*p*-hydroxybenzoic acid derivative
12	17.0	232, 288			gallic acid derivative
		Flavan-3-ols and Proanthocyanidins			
9	15.4	236, 278	291	**123**, **139**, 165, 151, 273	(−)-epicatechin
10	16.0	236, 280	865	**713**	trimer A
		Flavonols			
16	19.9		451	**319**, 288, 393, 433	myricetin pentoside
19	21.3	242, 262, 350	451	**319**, 393, 415, 329	myricetin pentoside
21	21.8	254, 354	465	**303**, 447	quercetin hexoside
22	22.1	254, 354	465	**303**, 447	quercetin hexoside
24	23.1	258, 356	435	**303**	quercetin pentoside
25	23.6	256, 355	435	**303**	quercetin pentoside
27	24.3	256, 352	435	**303**	quercetin pentoside
28	24.5	256, 350	449	**303**, 413, 431, 287	quercetin deoxyhexoside
35	28.4	256, 348	593	**413**, 345, 575, 547, 303	quercetin derivative
36	30.0	254, 372	303	**257**, 229, 165, 285, 247	quercetin aglycon
		Hydroxycinnamic Acid Derivatives			
3	9.2	240, 306, 325	355	**163**	neochlorogenic acid
5	11.3	236, 312			*p*-coumaric acid derivative
6	12.7	240, 300, 325	355	**163**	chlorogenic acid
7	13.7	240, 306, 328			caffeic/ferulic acid derivative
8	14.4	242, 306, 324			caffeic/ferulic acid derivative
11	16.6	240, 306, 326	337	**163**	caffeoylshikimic acid
13	18.3	240, 310,326	369	**163**	caffeic acid ester
17	20.4	242, 306,326	325	**163**, 307	caffeoyl hexoside
31	26.0	242, 306,328	367	**163**, 349	caffeic acid ester
33	26.6	242, 306,328	367	**163**, 349	caffeic acid ester
		Anthocyanins			
An_1	6.9	276, 526	465	**303**	delphinidin hexoside
An_2	8.7	280, 518	449	**287**	cyanidin hexoside
An_3	9.4	276, 526	435	**303**	delphinidin pentoside
An_4	10.5	280, 518	449	**287**	cyanidin hexoside
An_5	12.4	280, 518	419	**287**	cyanidin pentoside
An_6	18.8	272, 530	303	**257**, 303, 229	delphinidin aglycon
An_7	30.7	274, 524	287	**287**, 213, 259	cyanidin aglycon

[a] For peaks 2−36, see **Figure 3**, and for peaks An_1−An_7, see **Figure 4**. Boldface peaks are the most abundant MS/MS ions.

Table 3. Concentration of Phenolic Aglycons in Mortiño from Ecuador[a]

phenolic aglycon	concentration (mg/100 g FW)
gallic acid and galloyl esters (280 nm)	
gallic acid	3.1 ± 1.9
hydroxybenzoic acid derivatives (280 nm)	
vanillic acid	5.0 ± 1.9
p-hydroxybenzoic acid	2.1 ± 0.9
flavan-3-ols and proanthocyanidins (280 nm)	
(+)-catechin	10 ± 3
(−)-epicatechin	8.0 ± 4.1
flavonol derivatives (360 nm)	
quercetin	35 ± 11
myricetin	2.6 ± 1.0
hydroxycinnamic acid derivatives (320 nm)	
chlorogenic acid	17 ± 10
caffeic acid/ferulic acid	15 ± 8
p-coumaric acid	1.7 ± 1.2
anthocyanins (520 nm)	
cyanidin[b]	203 ± 47
total quantified aglycons	303

[a] Results are expressed as mean ± SD (n = 9). The wavelength used for quantification is given in brackets. [b] Using cyanidin as standard for delphinidin, the quantification is underestimated by ~27% (*34*). Anthocyanins as cyanidin-3-*O*-glucoside (345 mg/100 g FW).

was based on peak area at the absorption wavelength for each class and reported as the weight of the aglycon. Anthocyanins are reported as cyanidin.

Chemical Composition and Antioxidant Capacity. The mortiño berries contained ~17% of total carbohydrates, defined as 100% less the moisture content (81%), fat (1%), protein (0.7%) and ash (0.4%) (**Table 1**). Mortiño berries showed a caloric value of 84 kcal/100 g FW, which is intermediate between banana (101 kcal/100 g) and other fruits such as grape (75 kcal/100 g) and mango (69 kcal/100 g) (*26*), but the berries are not usually consumed in high amounts. The main sugars, representing ~59% of the total soluble solids, were glucose (2.6 g/100 g) and fructose (4.4 g/100 g). The berries are mildly sweet, in comparison with the 9.7 g/100 g of fructose and 4.6 g/100 g of glucose reported for *V. corymbosum* L. grown from organic culture and the 7.9 g/100 g fructose and 3 g/100 g glucose reported for the conventionally grown equivalent (*13*).The dietary fiber content (7.6%) was high compared with blueberry (3.1%), blackberry (5.9%) and lingonberry (2.5%) (*26*). The amount of potassium found in mortiño berries was high, and a serving of 100 g could provide 13% of the recommended adequate intake (AI) of 4.7 g/day for all adults (*27*).

The total soluble phenolic content (882 mg GAE/100 g FW) placed this berry in the group of Ecuadorian fruits with high phenolic content, such as banana passion fruit (1010 mg GAE/100 g FW). The amount of phenolic compounds was almost twice that in guava fruit (462 mg GAE/100 g FW) and plum (440 mg GAE/100 g FW), four times that in strawberry (238 mg GAE/100 g FW) and less than half that in Andean blackberry (2167 mg GAE/100 g FW) (*28*). The content was comparable to values reported in the literature for lowbush blueberry (295−495 mg GAE/100 g FW, *V. angustifolium*), highbush blueberry (181−585 mg GAE/100 g FW, *V. corymbosum*), and Rabbiteye blueberry (231−930 mg GAE/100 g FW, *V. ashei* Reade) (*10*, *29*) as well as bilberry (525 mg GAE/100 g FW, *V. myrtillus* L.) (*10*). The antioxidant capacity of mortiño berries (1200 mg Trolox/100 g FW) was also high according to the classification reported in our previous study (*28*) and comparable to that of Andean blackberry (1302 mg Trolox/100 g FW) and guava (1051 mg Trolox/100 g FW). In comparison with reported values for *V. corymbosum* L. hybrids (200−675 mg Trolox/100 g FW) and *V. ashei* Reade (500−950 mg Trolox/100 g FW) (*29*), our TEAC value for mortiño was high.

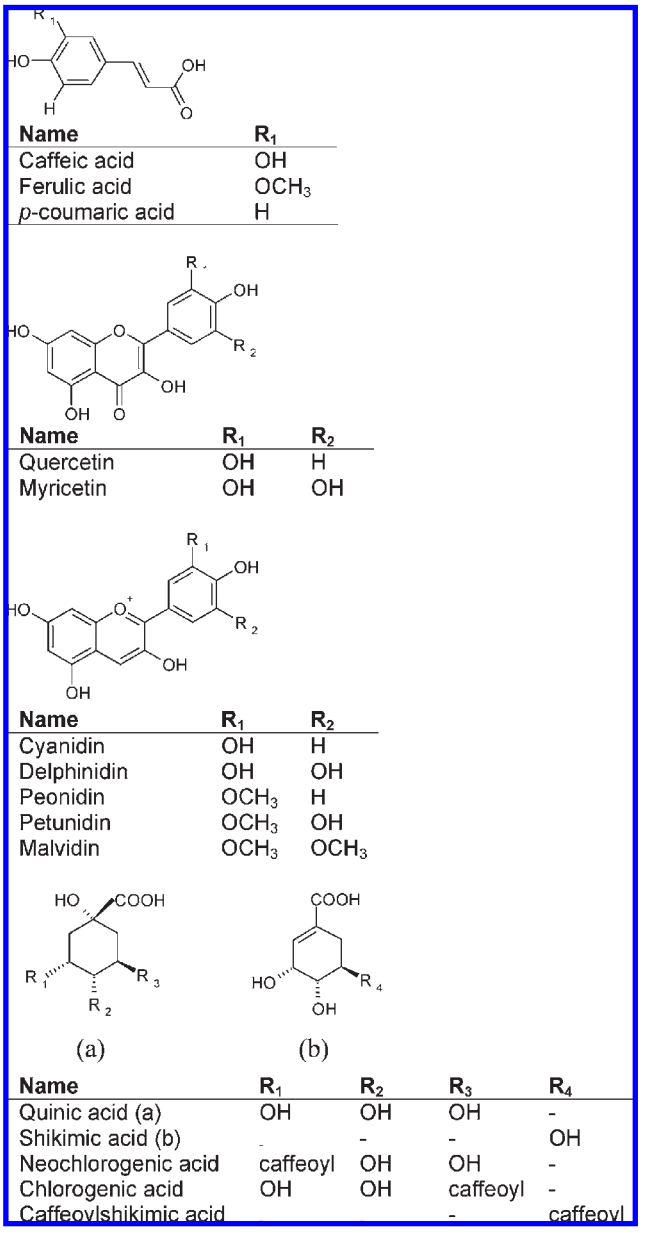

Figure 2. Structures of the phenolic compounds discussed in this study.

Identification of Phenolic Compounds. Identification of the chromatographic peaks in the ethyl acetate extract (Figure **3**) and methanol extract (**Figure 4**) is given in **Table 2**.

Galloyl Esters and Hydroxybenzoic Acid Derivatives. This group of compounds, which was minor in mortiño berry, did not ionize or form sodium adducts under our LC−MS conditions. The UV−vis spectra were used in tentative identification of the peaks, and only a general classification could be made. Peak 2 showed the typical spectrum of vanillic acid derivatives, with a bathochromic shift from 292 nm in the vanillic acid aglycon to 296 nm in the derivative, presumably sugar ester (*30*). A similar shift was observed for peak 12 (a gallic acid derivative) and peak 4 (a *p*-hydroxybenzoic acid derivative) with bathochromic shifts 272 → 288 nm and 255 → 262 nm, respectively. Thus, peak 2 can be tentatively identified as vanilloyl hexoside, peak 12 as galloyl hexoside and peak 4 as *p*-hydroxybenzoyl hexoside.

Flavan-3-ols and Proanthocyanidins. (−)-Epicatechin (peak 9) and one trimer A (peaks 10) were detected. Peak 9 was

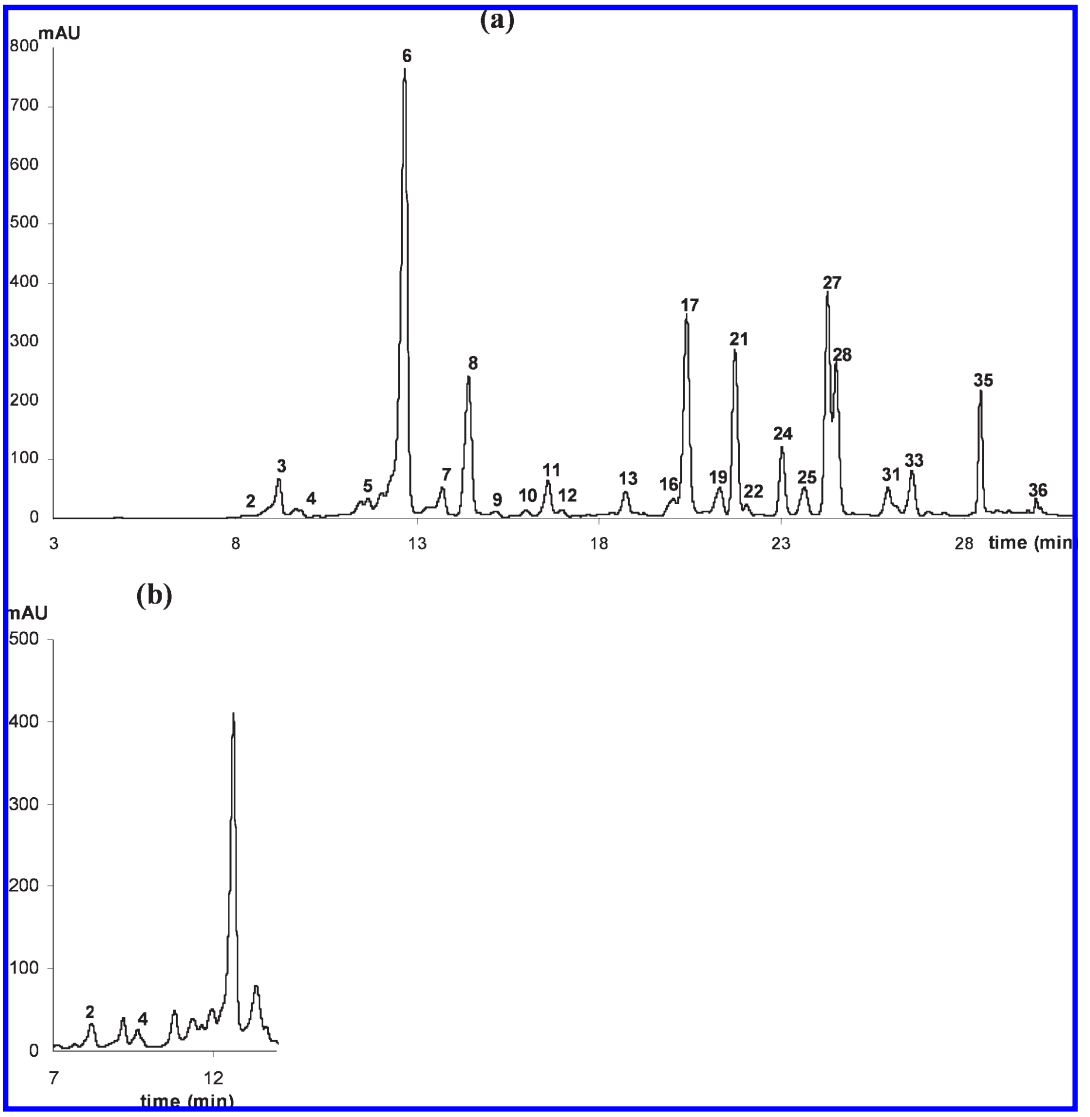

Figure 3. LC-DAD chromatogram of the ethyl acetate extract (320 nm) (**a**). Peaks 2 and 4 in the LC-DAD chromatogram of ethyl acetate at 280 nm (**b**). Peak numbers are referred to in **Table 2**.

identified as (−)-epicatechin by LC-DAD, comparing the retention time and spectrum with the pure standard, and confirmed by the molecular ion at *m*/*z* 291 in the MS. Peak 10 was identified as a trimer A (*m*/*z* 865) according to the molecular ion and characteristic fragmentation previously established for *V. vitis-idea* L., *V. oxycoccos* L., *V. myrtillus* L., and *V. uliginosum* L. species (*30*).

Flavonols. Ten flavonols were identified by LC-DAD, LC−MS and LC−MS/MS as shown in **Table 2**. The compounds were mainly quercetin derivatives (peaks 21, 22, 24, 25, 27, 28, 35 and 36) or myricetin derivatives (peaks 16 and 19). The major peaks corresponded to a quercetin-hexoside (peak 21), two quercetin-pentosides (peaks 24 and 27) and a quercetin-deoxy-hexoside (peak 28). These respective peaks can be tentatively assigned as quercetin-3-*O*-glucoside or quercetin-3-*O*-galacto-side, quercetin-3-*O*-xyloside, quercetin-3-*O*-arabinoside and quercetin-3-*O*-rhamnoside, all reported to be present in berries from the Ericaceae family such as *V. vitis-idea* L., *V. oxycoccos* L., *V. myrtillus* L., and *V. uliginosum* L. (*6*). Peak 35 was also identified as a quercetin derivative as the LC−MS/MS showed the fragment at *m*/*z* 303 typical of quercetin. The bathochromic shift to lower wavelengths for a quercetin derivative (348 nm) can result from additional methylation, glycosylation or acylation of the hydroxyl groups in the molecule (*6*). Among the minor peaks, myricetin derivatives were identified as myricetin-pentosides (peaks 16 and 19) with a loss of 132 mass units due to a pentose (xylose or arabinose). The rest of the minor peaks were identified as quercetin hexoside (peak 22), quercetin pentoside (peak 25) and quercetin aglycon (peak 36).

Hydroxycinnamic Acid Derivatives. Ten hydroxycinnamic acid derivatives (peaks 3, 5, 6, 7, 8, 11, 13, 17, 31 and 33) were identified according to their characteristic UV−vis spectra from the LC-DAD, but identity was only confirmed for seven compounds (peaks 3, 6, 11, 13, 17, 31, and 33) with LC−MS/MS. All the identified peaks had a major fragment at *m*/*z* 163, the caffeoyl residue [caffeic acid − OH]$^+$, by cleavage of the ester bond (*31*). Peaks 3 and 6 corresponded to neochlorogenic acid and chlorogenic acid *m*/*z* 355 (fragment due to a loss of 192 mass units corresponding to quinic acid, **Figure 2**), peak 11 to a caffeoyl-shikimic acid (loss of 174 mass units from the shikimic acid, **Figure 2**) (*32*), and peaks 13, 31, and 33 were identified as caffeic acid esters. Peak 17 was identified as caffeoyl hexoside (loss of 162 mass units due to the sugar moiety). Among the peaks that could not be identified by LC−MS, peaks 7 and 8 were classified according to the characteristic UV−vis spectra as caffeic/ferulic acid derivatives and quantified as caffeic acid equivalents. Peak 5,

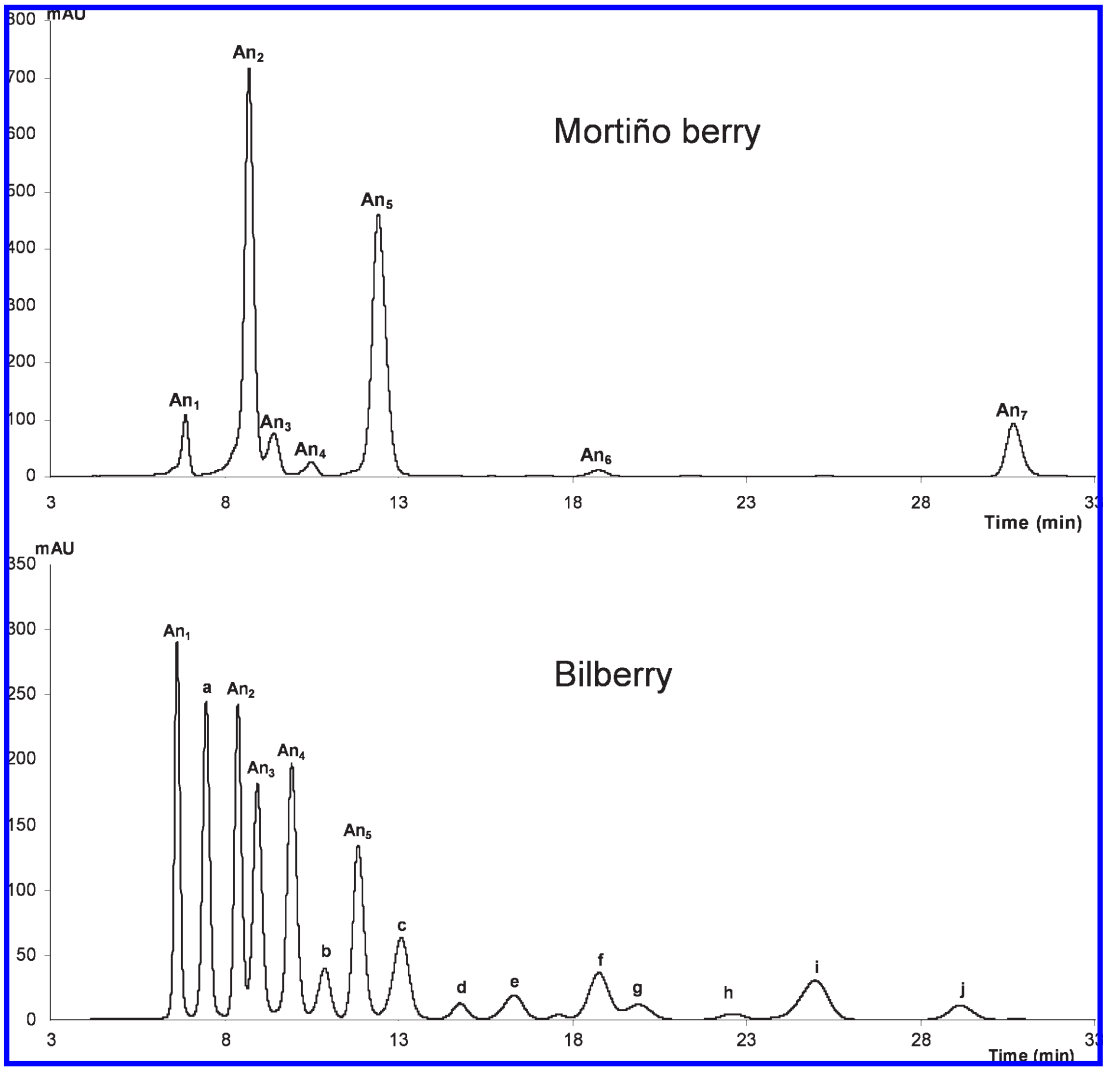

Figure 4. Anthocyanin profile of mortiño berry (*Vaccinium floribundum*) and Finnish bilberry (*Vaccinium myrtillus*) at 520 nm. Peak identity in **Table 2** and the rest of the peaks are (a) delphinidin glucoside, (b) petunidin galactoside, (c) petunidin glucoside, (d) peonidin galactoside, (e) petunidin arabinoside, (f) peonidin glucoside, (g) malvidin galactoside, (h) peonidin arabinoside, (i) malvidin glucoside, and (j) malvidin arabinoside.

a minor component, was classified according to the UV−vis spectra as a *p*-coumaric acid derivative.

Anthocyanins. The anthocyanin profile of mortiño is shown in **Figure 4**. Glycosides of cyanidin (peaks An_2, An_4, and An_5) and delphinidin (peaks An_1 and An_3) were found in addition to peaks An_6 and An_7, which are the delphinidin and cyanidin aglycons, respectively. Identification was made using the data reported from the LC−MS/MS and by comparison with the anthocyanin profile of the Finnish bilberry (*V. myrtillus*) used as a reference sample (**Figure 4**). In the cyanidin group, peaks were identified as cyanidin galactoside (An_2), cyanidin glucoside (An_4) and cyanidin arabinoside (An_5). In the delphinidin group, delphinidin galactoside (An_1) and delphinidin arabinoside (An_3) were identified.

Quantification of the Phenolic Classes. The concentrations of the different phenolic compounds estimated by reference to their aglycons using representative standards are shown in **Table 3**. Gallic acid and galloyl esters (3.1 mg/100 g FW) were found in comparable amounts to those in *V. ashei* Reade (1.5−4.2 mg/100 g FW) and *V. corymbosum* L. hybrids (2−4.8 mg/100 g FW) described in the literature (*29*), and in mortiño they corresponded to 1% of the total phenolic compounds in the berry.

The concentrations of flavan-3-ols and proanthocyanidins in the ethyl acetate extract were very low (6−14 mg/100 g FW), in the same order as flavan-3-ol values reported for *V. angustifolium* Aiton *x corymbosum* L. and *V. corymbosum* L. berries in the United States (4−25 mg catechin/100 g FW) (*7*). The amount of quercetin derivatives was dominant in mortiño berries, making up 93% of the total flavonols (**Table 3**). This value is within those reported in previous studies, where the concentration of quercetin derivatives was found to be between 77 and 97% of the total flavonols for different blueberry genotypes (*V. corymbosum x V. angustifolium* and *V. corymbosum* L.) and 95% for *V. myrtillus* L. (*8*, *33*). The total flavonol content (26−50 mg/100 g FW) was higher than the amounts reported by Cho et al. (19−32 mg/100 g) for *V. corymbonsum* and hybrids with the contribution of *V. darrowi*, *V. ashei* Reade, and *V. atrococcum* Heller (*8*) and than values (9−37 mg/100 g FW) for different *Vaccinium* species including *V. corymbosum*, *V. deliciosum*, *V. membranaceum*, *V. ovalifolium*, *V. ovatum*, *V. oxycoccus*, *V. parvifolium*, and *V. uliginosum* (*7*).

Taruscio et al. (*9*) reported that the concentration of hydroxycinnamic acids quantified as caffeic, ferulic and *p*-coumaric acids as representative standards for nine *Vaccinium* species (*V. angustifolium* Aiton x *corymbosum* L., *V. corymbosum*, *V. deliciosum*, *V. membranaceum*, *V. ovalifolium*, *V. ovatum*, *V. oxycoccus*, *V. parvifolium* and *V. uliginosum*) ranged from 12 to 163 mg/100 g FW, while Määttä-Riihinen et al. (*6*, *33*) reported

33−53 mg/100 g FW for the Finnish blueberries *V. corymbosum* and *V. corymbosum x V. angustifolium* and ∼16 mg/100 g FW for *V. myrtillus* L. The hydroxycinnamic acid content of mortiño berries (∼34 mg/100 g FW), mainly from chlorogenic acid (17 mg/100 g FW) and caffeic acid derivatives (15 mg/100 g FW), was in the range reported in those previous studies (*6*, *7*, *33*).

Anthocyanins accounted for ∼67% of the total phenolic compounds quantified in this study. The major peaks were cyanidin derivatives and represented ∼89% of the total anthocyanins present in the berry. The profile and distribution of anthocyanins in mortiño differed greatly from those reported for blueberry (*V. corymbonsum*) or bilberry (*V. myrtillus*), which include peonidin, petunidin and malvidin, and which have cyanidin contents less than 50% (*6*, *34*). The total amount of anthocyanins in mortiño (345 mg cyanidin/100 g FW), including a small amount of the cyanidin glycosides that were extracted with ethyl acetate (∼1%), is five times the concentration in Andean blackberry (71.4 mg cyanidin/100 g FW) (*35*) and comparable to the amounts reported for 20 wild populations of the Finnish bilberry *V. myrtillus* (350−525 mg/ 100 g FW) (*34*). The only common features that the profile of anthocyanins in our mortiño berries share with profiles described by Kalt et al. (*36*) for *V. myrtillus*, *V. angustifolium*, *V. corymbosum* and *V. myrtilloides* are the delphinidin and cyanidin glycosides, but mortiño lacked petunidin, peonidin and malvidin glycosides and the five acetylated anthocyanin glycosides. The same was observed when comparing the profile of mortiño anthocyanins with those of the Finnish bilberry (*V. myrtillus*, **Figure 4**). The results suggest that, in the flavonoid biosynthetic pathway, the process where cyanidin and delphinidin are transformed to peonidin, petunidin and malvidin under the action of a methyltransferase does not take place (*37*). This could be a consequence of different factors such as genetic, geographical and/or agronomic practices. In the case of anthocyanins, it has been proven that environmental factors affect synthesis of the compounds. For example, the difference between day and night temperatures (*38*), sun irradiation (*39*), light intensity (*40*) and quality (wavelength) (*41*) have been shown to have an effect on the metabolism of anthocyanins in fruits and berries.

Jaakola et al. (*37*) reported that direct sunlight activated the biosynthesis of flavonoids in leaves and fruits of bilberry (*V. myrtillus* L.), providing further evidence that the flavonols quercetin, cyanidin-3-*O*-glucosides and hydroxycinnamic acids are part of the defense mechanism against solar radiation. Mortiño berries displayed a phenolic profile (predominantly quercetin, hydroxycinnamic acids and cyanidin-3-glucosides) characteristic of a wild berry grown under high solar radiation. Despite the very different profile and distribution of phenolic compounds, mortiño berries have a higher TEAC value (1200 mg Trolox/100 g FW) than *V. corymbosum* L. (200−675 mg Trolox/100 g FW) and *V. ashei* Reade (500−950 mg Trolox/100 g FW).

LITERATURE CITED

(1) Neto, C. Cranberry and blueberry: Evidence for protective effects against cancer and vascular diseases. *Mol. Nutr. Food Res.* **2007**, *51*, 652–664.

(2) Shukitt-Hale, B.; Lau, F. C.; Joseph, J. A. Berry fruit supplementation and the aging brain. *J. Agric. Food Chem.* **2008**, *56*, 636–641.

(3) Bagchi, D.; Roy, S.; Patel, V.; He, G. L.; Khanna, S.; Ojha, N.; Phillips, C.; Ghosh, S.; Bagchi, M.; Sen, C. K. Safety and whole-body antioxidant potential of a novel anthocyanin-rich formulation of edible berries. *Mol. Cell. Biochem.* **2006**, *281*, 197–209.

(4) Camire, M. E.; Dougherty, M. P.; Briggs, J. L. Functionality of fruit powders in extruded corn breakfast cereals. *Food Chem.* **2007**, *101*, 765–770.

(5) Ohr, L. M. Brain food. *Food Technol.* **2008**, *62*, 65 ff.

(6) 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.

(7) Taruscio, T. G.; Barney, D. L.; Exon, J. Content and profile of flavanoid and phenolic acid compounds in conjunction with the antioxidant capacity for a variety of northwest *Vaccinium* berries. *J. Agric. Food Chem.* **2004**, *52*, 3169–3176.

(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 chromato graphy/mass spectrometry. *J. Sci. Food Agric.* **2005**, *85*, 2149–2158.

(9) Rimando, A. M.; Kalt, W.; Magee, J. B.; Dewey, J.; Ballington, J. R. Resveratrol, pterostilbene, and piceatannol in *Vaccinium* berries. *J. Agric. Food Chem.* **2004**, *52*, 4713–4719.

(10) Prior, R. L.; Cao, G.; Martin, A.; Sofic, E.; McEwen, J.; O'Brien, C.; Lischner, N.; Ehlenfeldt, M. K.; Kalt, W.; Krewer, G.; Mainland, C. M. Antioxidant capacity as influenced by total phenolic and anthocyanin content, maturity, and variety of *Vaccinium* species. *J. Agric. Food Chem.* **1998**, *46*, 2686–2693.

(11) He, F.; Pan, Q. H.; Shi, Y.; Duan, C. Q. Biosynthesis and genetic regulation of proanthocyanidins in plants. *Molecules* **2008**, *13*, 2674–2703.

(12) Heck, C. I.; Schmalko, M.; Mejia, E. G. d. Effect of growing and drying conditions on the phenolic composition of mate teas (*Ilex paraguariensis*). *J. Agric. Food Chem.* **2008**, *56*, 8394–8403.

(13) Wang, S. Y.; Chen, C. T.; Sciarappa, W.; Wang, C. Y.; Camp, M. J. Fruit quality, antioxidant capacity, and flavonoid content of organically and conventionally grown blueberries. *J. Agric. Food Chem.* **2008**, *56*, 5788–5794.

(14) Popenoe, H.; King, S.; León, J.; Kalinowski, L.; Vietmeyer, N.; Dafforn, M. Mortiño. In *Lost Crops of the Incas. Little known plants of the Andes with promise for worldwide cultivation*; National Academy Press: Washington, DC, 1989; p 428.

(15) Horwits, W. *Official Methods of Analysis*; AOAC International: Gaithersburg, MD, 2000.

(16) Food energy - methods of analysis and conversion factors; *FAO Food Nutr.* 2003; p 87.

(17) Asp, N. G.; Johansson, C. G.; Hallmer, H.; Siljestroem, M. Rapid enzymatic assay of insoluble and soluble dietary fiber. *J. Agric. Food Chem.* **1983**, *31*, 476–482.

(18) Pérez, A. G.; Olías, R.; Espada, J.; Olías, J. M.; Sanz, C. Rapid determination of sugars, nonvolatile acids, and ascorbic acid in strawberry and other fruits. *J. Agric. Food Chem.* **1997**, *45*, 3545–3549.

(19) Abdulla, M. *Inorganic elements in prepared meals in Sweden*; University of Lund: Lund, 1986.

(20) Van Niekerk, P., Determination of vitamins. In *HPLC in food analysis*; Macrae, R., Ed.; Academic Press Ltd.: London, U.K., 1988; p xiv + 502 pp.

(21) Pettersson, A.; Jonsson, L. Separation of cis-trans isomers of alpha- and beta-carotene by adsorption HPLC and identification with diode array detection. *J. Micronutr. Anal.* **1990**, *8*, 23–41.

(22) Larrauri, J. A.; Rupérez, P.; Saura-Calixto, F. Mango peel fibres with antioxidant activity. *Z. Lebensm.-Unters. -Forsch. A* **1997**, *205*, 39–42.

(23) Folin, O.; Ciocalteu, V. On tyrosine and tryptophane determinations in proteins. *J. Biol. Chem.* **1927**, 627–650.

(24) Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biol. Med.* **1999**, *26*, 1231–1237.

(25) Määttä, K.; Kamal-Eldin, A.; Törrönen, R. High-performance liquid chromatography (HPLC) analysis of phenolic compounds in berries with diode array and electrospray ionization mass spectrometric (MS) detection: *Ribes* species. *J. Agric. Food Chem.* **2003**, *51*, 6736–6744.

(26) Arnemo, M.; Becker, W.; Bergström, L.; Barbieri, H. E.; Munoz, M.; Ohlander, E.-M.; Torelm, I., Livsmedelstabell. In *Energi och näringsämnen 2002*; Livsmedelsverket: Uppsala, 2002; p 199.

(27) *Dietary Reference Intakes for Water, Potassium, Sodium, Chloride, and Sulfate*; National Academies Press: Washington, DC, 2004; pp 173−246.

(28) Vasco, C.; Ruales, J.; Kamal-Eldin, A. Total phenolic compounds and antioxidant capacities of major fruits from Ecuador. *Food Chem.* **2008**, *111*, 816–823.
(29) Sellappan, S.; Akoh, C. C.; Krewer, G. Phenolic compounds and antioxidant capacity of Georgia-grown blueberries and blackberries. *J. Agric. Food Chem.* **2002**, *50*, 2432–2438.
(30) 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*, 8485–8491.
(31) Termentzi, A.; Kefalas, P.; Kokkalou, E. LC-DAD-MS (ESI+) analysis of the phenolic content of *Sorbus domestica* fruits in relation to their maturity stage. *Food Chem.* **2008**, *106*, 1234–1245.
(32) Fang, N. B.; Yu, S. G.; Prior, R. L. LC/MS/MS characterization of phenolic constituents in dried plums. *J. Agric. Food Chem.* **2002**, *50*, 3579–3585.
(33) Riihinen, K.; Jaakola, L.; Kärenlampi, S.; Hohtola, A. Organ-specific distribution of phenolic compounds in bilberry (*Vaccinium myrtillus*) and "northblue" blueberry (*Vaccinium corymbosum x V. angustifolium*). *Food Chem.* **2008**, *110*, 156–160.
(34) Lätti, A. K.; Riihinen, K. R.; Kainulainen, P. S. Analysis of anthocyanin variation in wild populations of bilberry (*Vaccinium myrtillus* L.) in Finland. *J. Agric. Food Chem.* **2008**, *56*, 190–196.
(35) Vasco, C.; Riihinen, K.; Ruales, J.; Kamal-Eldin, A. Phenolic Compounds in *Rosaceae* Fruits from Ecuador. *J. Agric. Food Chem.* **2009**, *57*, 1204–1212.
(36) Kalt, W.; McDonald, J. E.; Ricker, R. D.; Lu, X. Anthocyanin content and profile within and among blueberry species. *Can. J. Plant Sci.* **1999**, *79*, 617–623.
(37) Jaakola, L.; Määttä-Riihinen, K.; Kärenlampi, S.; Hohtola, A. Activation of flavonoid biosynthesis by solar radiation in bilberry (*Vaccinium myrtillus* L.) leaves. *Planta* **2004**, *218*, 721–728.
(38) Dela, G.; Or, E.; Ovadia, R.; Nissim-Levi, A.; Weiss, D.; Oren-Shamir, M. Changes in anthocyanin concentration and composition in "Jaguar" rose flowers due to transient high-temperature conditions. *Plant Sci.* **2003**, *164*, 333–340.
(39) Albert, N. W.; Lewis, D. H.; Zhang, H.; Irving, L. J.; Jameson, P. E.; Davies, K. M. Light-induced vegetative anthocyanin pigmentation in Petunia. *J. Exp. Bot.* **2009**, *60*, 2191–2202.
(40) Cortell, J. M.; Kennedy, J. A. Effect of shading on accumulation of flavonoid compounds in (*Vitis vinifera* L.) Pinot Noir fruit and extraction in a model system. *J. Agric. Food Chem.* **2006**, *54*, 8510–8520.
(41) Dussi, M. C.; Sugar, D.; Wrolstad, R. E. Characterizing and quantifying anthocyanins in red pears and the effect of light quality on fruit color. *J. Am. Soc. Hortic. Sci.* **1995**, *120*, 785–789.

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