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Phenolic Acid Profiles in Some Small Berries

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The composition of phenolic acids in several small berries grown in Northeastern Poland, namely, low-bush blueberries, black mulberries, European juneberries, black currants, fruits of blue-berried honeysuckle, and blackberries, was determined by gas chromatography (GC) and mass spectrometry (MS). The total content of phenolic acids, identified by GC-MS, ranged from 2845.8 ± 141.0 (black mulberries) to 5418.2 ± 228.0 (blue-berried honeysuckle). Twenty phenolic acids were identified in the berries. Of these, hydroxycaffeic, *m*- and *p*-coumaric, and 3,4-dimethoxycinnamic acids were the major phenolic acids in blackberries and blueberries, *m*-coumaric acid was the major phenolic acid in blue-berried honeysuckle and black currant fruits, while salicylic, caffeic, and *m*- and *p*-coumaric acids were the predominant phenolic acids in European juneberries. Syringic and veratric acids were detected only in blueberries, while *p*-hydroxybenzoic and sinapic acids were present only in black currants and *o*-coumaric acid was present in blueberries and black mulberries. The phenolic acids liberated from esters and glycosidic bonds were the major fractions of phenolic acids in the berries.

KEYWORDS: Phenolics acids; low-bush blueberry (*Vaccinium myrtillus*); black mulberry (*Morus nigra*); blue-berried honeysuckle (*Lonicera caerulea*); black currant (*Ribes nigrum*); blackberry (*Rubus plicatus*); European juneberry (*Amelanchier ovalis*)

INTRODUCTION

Small berries constitute one of the important sources of potential health promoting phytochemicals. These fruits are rich sources of phenolic compounds such as phenolic acids as well as anthocyanins, proanthocyanidins, and other flavonoids, which display potential health promoting effects (*1*−*9*). For example, over 180 *Vaccinium*-based pharmaceuticals are available commercially (*10*).

The content of phenolics in berries is affected by the degree of maturity at harvest, genetic differences (cultivar), preharvest environmental conditions, postharvest storage conditions, and processing (*9*, *11*−*16*). Phenolic acids constitute about one-third of the dietary phenols, and they are present in plants in free and bound forms. Bound phenolics may be linked to various plant components through ester, ether, or acetal bonds (*17*). Clifford (*18*) estimated that daily consumption of phenolic acids ranged from 25 mg to 1 g. An increasing interest in determining the antioxidant activities exhibited by phenolic acids and their derivates should also be noted (*19*−*22*).

Published data on phenolic acid profiles of small berries are still incomplete. Therefore, the purpose of this study was to determine the composition of free and bound phenolic acids in some small berries grown in Northeastern Poland using established analytical methodologies.

MATERIAL AND METHODS

Materials. Berries of blueberries (*Vaccinium myrtillus*), black mulberry (*Morus nigra*), blue-berried honeysuckle (*Lonicera caerulea* var. *camtschatica Sevast*), European juneberry (*Amelanchier ovalis*), blackberries (*Rubus plicatus*), and black currant (*Ribes nigrum*) were harvested near Olsztyn, Poland, in 2002. All berries were picked at the commercially ripe stage. The berries were cleaned to remove damaged, diseased, or pest-infested fruits, stems, and leaves and then stored in polyethylene bags at −20 °C (up to 1 month) until analysis. Before analysis, frozen berries were crushed in a food processor.

Chemicals. Caffeic, gallic, gentisic, ferulic, *p*-hydroxybenzoic, protocatechuic, salicylic, sinapic, syringic, vanillic, and veratric acids, as well as (+)-catechin, sodium bicarbonate, sodium hydroxide, diethyl ether, methanol, and N,O-bis(trimethylsilyl)acetamide were purchased from Sigma Chemical Co. (Sigma-Aldrich Sp. zoo; Gliwice, Poland), while *o*-, *m*-, and *p*-coumaric acids were obtained from Fluka (Sigma-Aldrich Sp. zoo; Gliwice, Poland).

Preparation of Crude Phenolic Extract. Soluble phenolics were extracted six times from crushed berries into aqueous 80% (vol/vol) methanol (at a ratio of 1:1, wt/vol) at room temperature for 1 h using an orbital shaker at 250 rpm. The mixture was centrifuged at 1750*g* for 10 min, and the supernatants were collected, combined, evaporated to near dryness under vacuum at ≤40 °C, and lyophilized.

Fractionation of Phenolic Acids. Phenolic acids present in crude extract were fractionated into free and bound forms according to the procedure described by Kozłowska et al. (*23*) and Zadernowski (*24*, *25*). A 0.5 g sample of dried crude phenolic extract was suspended in 50 mL of triply distilled water, acidified to pH 2 using 6 M HCl, and extracted five times with diethyl ether (1:1, vol/vol) at room temperature. The ether extracts of phenolic acids (referred to as free phenolic

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acids) were combined and evaporated to dryness under vacuum at ≤40 °C. The water phase was adjusted to pH 7 with 2 M NaOH and then evaporated to almost dryness under vacuum at ≤40 °C. The residue was treated with 20 mL of 4 M NaOH under nitrogen for 4 h at room temperature. The reaction mixture was then acidified with 6 M HCl to pH 2 and extracted with diethyl ether as described above. The ether extracts of phenolic acids are referred to as phenolic acids liberated from ester bonds. Following this, the water phase was again adjusted to pH 7 with 2 M NaOH and then evaporated to almost dryness under vacuum at ≤40 °C. The residue was heated with 50 mL of 2 M HCl for 30 min at 95 °C, cooled to room temperature, and extracted with diethyl ether as described above. These ether extracts of phenolic acids are referred to as phenolic acids liberated from glycosidic bonds.

Purification of Phenolic Acids Fractions. Each of the residues of phenolic acid fractions, obtained as described above, was dissolved in 50 mL of 5% (wt/vol) $NaHCO_3$ (pH 8) and extracted five times with diethyl ether to remove residual fatty material. The water phase was then acidified with 6 M HCl to pH 2 and extracted with diethyl ether as described above. The dry residues of phenolic acids were dissolved in 5 mL of 80% (vol/vol) methanol.

Formation of Trimethylsilyl Derivatives. To 0.5 mL of methanolic solution of purified phenolic acids in the reaction vial 20−50 μL of N,O-bis(trimethylsilyl)acetamide was added, depending on the phenolic acid concentrations. The vial was then tightly closed and left at room temperature for 24 h.

Gas Chromatography−Mass Spectrometry (GC-MS) Identification of Phenolic Acids. The trimethylsilyl derivatives of phenolic acids were identified using GC-MS methodology as described by Zadernowski (*24*, *25*), Horman and Viani (*26*), Tin and White (*27*), and Xing and White (*28*). GC-MS analysis was carried out on a Hewlett-Packard 5890 Series II gas chromatograph interfaced with a MS Hewlett-Packard 5970 mass selective detector (Kennett Square, PA). Separations were performed using a 30 m × 0.25 mm (i.d.) SPB-1 silica-fused capillary column coated with 0.25 μm film of poly(dimethylsiloxane) as the stationary phase (Supelco Inc., Bellefonte, PA). Helium was used as the carrier gas at an average flow rate of 28 cm^3 per min. The injector and the transfer line temperature were kept at 240 °C. The oven temperature program used was 120−260 °C at a rate of 20 °C per min. Initial and final temperatures were held for 2 and 10 min, respectively. The injections were carried out in a split mode with a split ratio of 20:1. The mass spectrometer was operated with an ionization voltage of 235 eV and an electron multiplier voltage of 1700 V and was scanned from 50 to 500 *m*/*z* at 0.8 s per scan. The volume of injected samples ranged from 1 to 2 μL, depending on the sample. Caffeic, *o*-, *m*-, and *p*-coumaric, gallic, gentisic, ferulic, *p*-hydroxybenzoic, protocatechuic, salicylic, sinapic, syringic, vanillic, and veratric acids were identified by using mass spectra of standard derivatives. The remaining phenolic acids were identified using the mass spectra library provided by the GC-MS supplier. **Figure 1** shows typical GC chromatograms of free and bound phenolic acids isolated from European juneberries.

Quantitation of Phenolic Acids. The phenolic acids were quantified as described by Zadernowski (*24*, *25*) using a Hewlett-Packard 5890 Series II gas chromatograph equipped with a flame ionization detector. Separations of trimethylsilyl derivatives of phenolic acids were performed as described in the previous paragraph. N-tetracosane was used as an internal standard. The contents of the phenolic acids are expressed as mg per kg of fruit on a dry weight basis.

Chemical Analysis. The total phenols (TPH) content in crude extracts was estimated by the Folin−Ciocalteau assay (*9*) and expressed in mg (+)-catechin equivalents per kg of berries on a dry matter (dm) basis. The moisture content was measured by drying the ground berries at 105 °C until a constant weight was obtained (*29*).

Data Treatment. The results presented in the tables are mean values (n = 6 and n = 3 for **Table 1** and n = 3 for **Tables 2−5**) ± SD (standard deviation). Statistical analysis of data (two-sample *t*-test) was performed using SigmaStat v.3.0 (SSPS, Chicago, IL). Differences at $P \leq 0.05$ were considered to be significant.

RESULTS AND DISCUSSION

TPH. An 80% (vol/vol) aqueous methanol−water is commonly used for the extraction of phenolic acids and their derivatives from plant materials (*24*, *25*, *30−32*); therefore, we selected this solvent for extraction of phenolics from berries. **Table 1** shows the moisture and TPH contents in fruits of six small berries grown in Northeastern Poland. In the literature, the reported TPH values are calculated per fresh weight of berries or per dm of berries. Therefore, moisture contents are provided here in order to aid the readers comparing our results with those published in the literature. TPH in the fruits of Polish berries ranged from 9907 ± 470 in black currants to over 23000 mg of (+)-catechin equivalents per kg of berries (dm) in European juneberries, blueberries, and blackberries (**Table 1**).

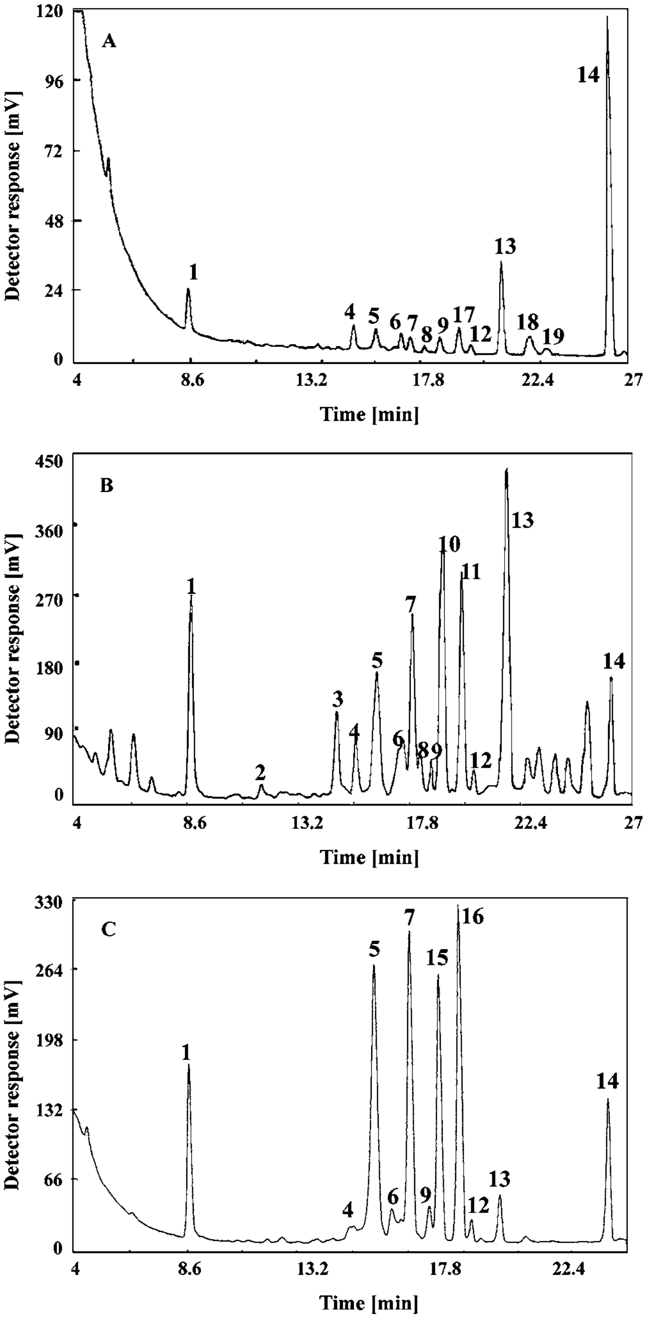

Figure 1. GC chromatograms of various fractions of phenolics acids isolated from European juneberries: (**A**) free phenolic acids, (**B**) phenolic acids liberated from ester bonds, and (**C**) phenolic acids liberated from glycosidic bonds, where 1 = salicylic, 2 = *p*-hydroxyphenyl-acetic, 4 = protocatechuic, 5 = *m*-coumaric, 6 = *p*-hydroxyphenyl-lactic, 7 = *p*-coumaric, 8 = hydroxycaffeic, 9 = gallic, 10 = gentisic, 12 = ferulic, and 13 = caffeic acids, while 14 = internal standard and 3, 11, 15–19 = unknowns.

Table 1. TPH and Contributions of Phenolic Acid Fractions in Six Small Berries

sample	moisture (%)	TPH (mg/kg dm[a])	free phenolic acids (%)[b]	phenolic acids liberated from: esters (%)[b]	phenolic acids liberated from: glycosides (%)[b]
blueberry	81.2 ± 3.6	23714 ± 560^{a}	2.6 ± 0.1	40.7 ± 2.4	56.7 ± 1.9
black mulberry	80.9 ± 2.7	11546 ± 530	4.2 ± 0.3	58.5 ± 3.9^{a}	37.3 ± 2.6^{a}
black currants	79.0 ± 0.8	9907 ± 470	1.7 ± 0.1^{a}	$65.4 \pm 4.7^{a,b}$	$32.9 \pm 2.4^{a,b}$
blue-berried honeysuckle	86.0 ± 4.9	21279 ± 890^{a}	1.7 ± 0.1^{a}	$62.3 \pm 2.9^{a,b,c}$	$36.0 \pm 1.3^{a,b,c}$
European juneberry	77.4 ± 1.1	23154 ± 510^{a}	2.1 ± 0.2	$57.6 \pm 3.0^{a,c,d}$	$40.3 \pm 2.9^{a,c,d}$
blackberries	80.5 ± 8.5	23000 ± 1210^{a}	3.3 ± 0.3	$53.1 \pm 3.8^{a,d}$	$43.6 \pm 3.6^{a,d}$

[a] mg (+)-catechin equivalents per kg of dm of berries. [b] Percent of the total phenolic acids as determined by GC-MS methodology; values for the same column marked by the same letter are not significantly different (TPH, $n = 6$; *t*-test; $P > 0.05$; phenolic acids, $n = 3$; *t*-test; $P > 0.05$).

Table 2. Total Phenolic Acid Contents in Six Polish Berries (mg Per kg of dm of Berries)

phenolic acid	blueberries	black mulberries	black currants	blue-berried honeysuckle	European juneberries	blackberries
			hydroxybenzoic acid derivatives (HBA)			
gentisic[b]	152.1 ± 10.9^{a}	114.9 ± 10.8	159.3 ± 14.9^{a}	153.5 ± 10.9^{a}	229.4 ± 10.8	136.6 ± 10.8^{a}
gallic[b]	93.6 ± 9.0^{a}	27.3 ± 5.0	72.3 ± 6.1	44.3 ± 2.6	105.9 ± 15.0	89.0 ± 5.0^{a}
p-hydroxybenzoic[b]	*a*		39.3 ± 5.0			
o-pyrocatechuic	1.4 ± 0.1		4.3 ± 0.3	28.6 ± 1.2		0.2 ± 0.1
protocatechuic[b]	114.0 ± 10.0^{a}	121.8 ± 10.5^{a}	79.6 ± 5.0	$144.4 \pm 10.0^{b,c}$	163.0 ± 18.5^{b}	$129.5 \pm 10.5^{a,c}$
salicylic[b]	488.5 ± 40.0^{a}	88.5 ± 9.0	512.1 ± 50.9^{a}	1234.9 ± 140.0	573.5 ± 81.0^{a}	524.1 ± 79.0^{a}
syringic[b]	41.6 ± 3.3					
vanillic[b]	111.7 ± 10.2	6.5 ± 1.2	48.3 ± 5.0^{a}	21.1 ± 2.8		45.1 ± 5.3^{a}
veratric[b]	7.6 ± 0.6					
			hydroxycinnamic acid derivatives (HCA)			
caffeic[b]	117.2 ± 8.2^{a}	574.5 ± 43.0^{b}	217.6 ± 14.0	598.2 ± 35.9^{b}	1027.6 ± 73.8	105.5 ± 4.0^{a}
m-coumaric[b]	474.0 ± 45.0^{a}	285.5 ± 25.1	1872.9 ± 145.0^{b}	2014.5 ± 145.0	1016.4 ± 99.1	596.6 ± 75.1^{a}
o-coumaric[b]	212.7 ± 15.6	7.2 ± 0.7				
p-coumaric[b]	761.8 ± 60.7	1448.3 ± 130.0	316.5 ± 30.7	987.1 ± 100.0^{a}	1070.1 ± 110.0^{a}	421.2 ± 50.0
3,4-dimethoxycinnamic	725.2 ± 50.8	33.9 ± 11.3^{a}	7.4 ± 0.8	44.2 ± 5.0^{a}		501.9 ± 71.3
ferulic[b]	34.1 ± 1.5^{a}	78.3 ± 7.5	57.5 ± 4.9^{b}	36.9 ± 2.5^{a}	64.1 ± 7.2^{b}	55.3 ± 7.5^{b}
hydroxycaffeic	723.8 ± 50.6^{a}	14.3 ± 1.6^{b}	16.2 ± 1.6^{b}	51.9 ± 6.0	35.3 ± 4.6	627.6 ± 75.0^{a}
sinapic[b]			36.7 ± 3.0			
			other phenolic acids (other PA)			
p-hydroxyphenyl-acetic	16.4 ± 1.7^{a}	21.6 ± 1.2^{b}	16.9 ± 1.7^{a}	10.3 ± 0.1	24.5 ± 1.7^{b}	
p-hydroxyphenyl-lactic		23.2 ± 2.8^{a}	27.2 ± 3.7^{a}	48.3 ± 5.0	22.4 ± 2.8^{a}	22.6 ± 3.8^{a}
totalHBA	1010.5 ± 45.0^{a}	359.0 ± 18.3	915.2 ± 54.0^{a}	1626.8 ± 141.0	1071.8 ± 85.0^{a}	924.5 ± 81.0^{a}
total HCA	3048.8 ± 106.0^{a}	2442.0 ± 140.0^{b}	2524.8 ± 149.0^{b}	3732.8 ± 180.0	3213.5 ± 166.0^{a}	2308.1 ± 138.0^{b}
total other PA	16.4 ± 1.7^{a}	44.8 ± 3.1^{b}	44.1 ± 4.1^{b}	58.6 ± 5.0	46.9 ± 3.3^{b}	22.6 ± 3.8^{a}
total	4075.7 ± 115.0^{a}	2845.8 ± 141.0	3484.1 ± 158.6^{b}	5418.2 ± 229.0	4332.2 ± 186.0^{a}	3255.2 ± 160.0^{b}

[a] Blank cells, not detected. [b] Identified using the mass spectrum of the standard derivative; values in each row marked by the same superscript letter are not significantly different ($n = 3$; *t*-test; $P > 0.05$).

TPH values for blueberries and blackberries are within the range of previously published results (*33*, *34*), but those for black currants and blue-berried honeysuckle were below values reported in the literature (*1*, *35*, *36*). Numerous factors, such as varietals and regional differences (*37*), the degree of berry ripeness (*38*), harvest time, as well as the analytical procedure used for extraction and quantification of phenolics (*9*, *17*, *39*) might contribute to these differences.

Total Phenolic Acids. Many analytical procedures have been employed for the determination of phenolic compounds in plant materials (*39*), but those currently used for the determination of phenolics in extracts from berries only target major flavonoids and/or phenolic acids and their conjugates (*33*, *40*). This makes the comparison of our results with those reported in the literature cumbersome.

The total content of phenolic acids in Polish berries ranged from 2845.8 ± 141.0 (black mulberries) to 5418.2 ± 228.0 mg per kg, dm, (blue-berried honeysuckle) (**Table 2**). These values are up to 2−3 times higher than those reported for sea buckthorn berries (*41*). Hydroxycinnamic acids constituted from 68.9 (blue-berried honeysuckle) to 85.8% (black mulberries) of the total phenolic acids present in the berries. The amounts of hydroxycinnamic acids found in fruits of blueberries, blue-berried honeysuckle, and black currants are comparable to those reported in the literature (*35*, *40*, *42*, *43*). Twenty phenolic acids were identified in the berries studied (**Table 2**). Of these, syringic and veratric acids were only found in blueberries, *p*-hydroxybenzoic and sinapic acids were only found in black currants, and *o*-coumaric acid was only found in blueberries and black mulberries. Chlorogenic acid (5-*O*-caffeolquinic acid; 5-CQA) and its derivatives were not identified in this study because these acids are unstable under alkaline conditions used here and rapidly hydrolyze to caffeic acid (*39*). In addition, the blueberry and blackberry extracts of phenolic acids contained 870.2 ± 75.0 and 537.5 ± 50.0 mg of quinic acid per kg, dm, respectively.

Seventeen phenolic acids were identified in wild blueberries grown in Northeastern Poland (**Table 2**). Sellappan et al. (*33*) detected only five phenolic acids in both rabbiteye blueberries (*Vaccinium ashei* Raede) and southern higbush blueberries (*Vaccinium corymbosum* L.), namely, gallic, caffeic, *p*-coumaric, ferulic, and ellagic acids. Similarly, Häkkinen et al. (*2*) identified

Table 3. Free Phenolic Acids Content in Six Polish Small Berries (mg Per kg of dm of Berries)

phenolic acid	blueberries	black mulberries	black currants	blue-berried honeysuckle	European juneberries	blackberries
hydroxybenzoic acid derivatives (HBA)						
gentisic	12.2 ± 1.0	0.3 ± 0.1	[a]	1.5 ± 0.2		3.2 ± 0.1
gallic	3.4 ± 0.3^{a}	3.0 ± 1.3^{a}		0.1 ± 0.0	7.2 ± 4.2	0.3 ± 0.1
o-pyrocatechuic	1.4 ± 0.1^{a}		1.6 ± 0.1^{a}			0.2 ± 0.1
protocatechuic	3.7 ± 0.3^{a}	4.3 ± 0.5^{a,b}	7.1 ± 0.3	2.3 ± 0.3	10.1 ± 0.5	5.0 ± 0.5^{b}
salicylic	9.8 ± 0.6^{a,b}	0.5 ± 0.1	5.0 ± 0.3	9.0 ± 0.6^{a}	11.1 ± 1.0^{b}	14.0 ± 1.1
syringic	5.1 ± 0.3					
vanillic	25.8 ± 2.1		0.2 ± 0.0			6.6 ± 0.7
hydroxycinnamic acid derivatives (HCA)						
caffeic	17.4 ± 1.0	4.9 ± 1.0	10.6 ± 0.6	22.4 ± 2.8^{a}	44.2 ± 3.5	24.3 ± 1.0^{a}
m-coumaric	4.4 ± 0.3^{a}	5.4 ± 0.2^{b,c}	11.8 ± 1.3	6.4 ± 0.4^{b,d}	6.8 ± 4.0^{a,c,d}	0.6 ± 0.0
o-coumaric	1.0 ± 0.2					
p-coumaric	6.4 ± 0.6	21.9 ± 2.6^{a}	3.6 ± 0.2	23.5 ± 1.2^{a}	5.4 ± 0.6	0.5 ± 0.1
ferulic	13.5 ± 1.1	66.2 ± 7.1	8.1 ± 0.8	20.7 ± 2.1	2.7 ± 0.3	39.0 ± 7.1
other phenolic acids (other PA)						
p-hydroxyphenyl-acetic			9.7 ± 0.7	0.9 ± 0.2		
p-hydroxyphenyl-lactic		7.6. ± 0.8	3.0 ± 0.4	0.5 ± 0.1^{a}	1.0 ± 0.1^{b}	1.0 ± 1.0^{a,b}
total HBA	61.4 ± 2.5	8.1 ± 1.4	13.9 ± 0.4^{a}	12.9 ± 0.7^{a}	28.4 ± 4.3^{b}	29.3 ± 1.4^{b}
total HCA	43.3 ± 1.6	102.9 ± 7.6	34.1 ± 1.7	78.4 ± 3.7^{a}	63.1 ± 5.4^{b}	76.7 ± 7.3^{a,b}
total other PA		7.6 ± 0.8	12.7 ± 0.8	1.4 ± 0.2^{a}	1.0 ± 0.1^{a}	1.0 ± 1.0^{a}
total	104.6 ± 3.0^{a,b}	118.6 ± 7.8^{a}	60.7 ± 1.9	92.7 ± 3.8^{c}	92.5 ± 6.9^{b,c}	107.0 ± 7.5^{a,b}

[a] Blank cells, not detected; values in each row marked by the same superscript letter are not significantly different ($n = 3$; *t*-test; $P > 0.05$).

only *p*-coumaric, caffeic, ferulic, and ellagic acids in Northcountry and Northblue (*Vaccinum corymbosum*) blueberry cultivars. Hydroxycaffeic, *m*- and *p*-coumaric, salicylic, and 3,4-dimethoxycinnamic were the major phenolic acids found in Polish blueberries, and these acids comprised 17.8, 11.6, 18.7, 12.0, and 17.8% of the total phenolic acids present in these berries, respectively (**Table 2**). On the other hand, ferulic acid was reported to be the principal phenolic acid in blueberry cultivars Northcountry and Northblue (*2*), while in blueberry cultivars Clon 908, Heerma I, and Heerma II it was caffeic acid (*44*), and in blueberry cultivars Coville and Sierra, 5-CQA was predominant (*45*, *46*). Moreover, Taruscio et al. (*47*) reported that chlorogenic acid was the major phenolic acid in highbush (Bluecrop, Bluejay, and Jersey) and half-highbush (Northblue, Northcountry, and Northsky) blueberries species, while *p*-coumaric and caffeic acids were the major phenolic acids found in wild blueberries (*Vaccinium ovalifolium* Smith) grown within the Northwestern United States.

Fourteen phenolic acids were identified in blackberries. The major phenolic acids, namely, *m*- and *p*-coumaric, 3,4 -dimethoxycinnamic, and hydroxycaffeic acids constituted 18.3, 12.9, 15.4, and 16.1% of the total phenolic acids present in berries, respectively (**Table 2**). Sellapan et al. (*33*) identified only five phenolic acids, namely, gallic, caffeic, ferulic, *p*-coumaric, and ellagic acids in Georgia-grown blackberries. Of these, ellagic acid was the predominant phenolic acid in these berries.

m-Coumaric acid was found to be the predominant phenolic acid in blue-berried honeysuckle and black currant fruits. It comprised 37.2 and 53.8% of the total phenolic acids present in these fruits, respectively. Significant quantities of salicylic, *p*-coumaric, and caffeic acids were also detected (**Table 2**). In addition, up to 11 minor phenolic acids were also identified in these berries. On the other hand, Chaovanalikit et al. (*43*) detected only 5-CQA and neochlorogenic (3-*O*-caffeoylquinic acid; 3-CQA) acids and one unknown hydroxycinnamic acid derivative in the fruits of 10 blue-berried honeysuckle genotypes grown in Corvallis, Oregon. Furthermore, Häkkinen et al. (*2*) reported the presence of caffeic, ferulic, *p*-hydroxybenzoic, and ellagic acids in Finnish black currants. These phenolic acids comprised 24.4% of the total content of all identified phenolics in these berries. In addition, a number of phenolic acids and their derivatives were identified in black currant seeds, namely, caffeic acid, ferulic acid, *p*-coumaric acid, protocatechuic acid, gallic acid, *p*-hydroxybenzoic acid, 1-cinnamoyl-β-D-glucoside, and 1-*p*-coumaroyl-β-D-glucoside (*48*).

Caffeic, salicylic, and *m*- and *p*-coumaric acids were the major phenolic acids of European juneberries comprising 85.1% of the total phenolic acids present in the fruit. On the other hand, *p*-coumaric acid was the predominant phenolic acid in black mulberries. Significant amounts of caffeic and *m*-coumaric acids in black mulberries and gentisic and protocatechuic acids in European juneberries were also found.

Free Phenolic Acids. Free phenolic acids were the minor fraction of phenolic acids constituting only from 1.7 (black currants) to 4.2% (black mulberry) of the total phenolic acids present in these berries. Hydroxybenzoic acid derivatives were found to be the major phenolic acids in blueberries, while hydroxycinnamic acid derivatives dominated in the other berries. Ten to thirteen free phenolic acids were only identified in this fraction (**Table 3**). Of these, caffeic, gentisic, ferulic, and vanillic acids were the major phenolic acids in blueberry, while caffeic, *m*-coumaric, ferulic, and *p*-hydroxyphenyl-lactic acids were in black currants and ferulic acid was in black mulberries. Furthermore, caffeic acid was the principal phenolic acid in European juneberries; caffeic and ferulic acids dominated in blackberries, while caffeic, ferulic, and *p*-coumaric acids were the major phenolic acids in blue-berried honeysuckle fruits. Syringic and *o*-coumaric acids were only found in blueberries, vanillic acid was present only in blueberries and black currants, while *p*-hydroxyphenyl-lactic acid was found only in black currants and fruits of blue-berried honeysuckle. The levels of individual phenolic acids, however, did not exceed the taste thresholds reported in the literature (*49*). Thus, the fraction of free phenolic acids may not have any significant contribution to the berries flavor.

Bound Phenolic Acids. Phenolic acids liberated from soluble esters were the predominant phenolic acids in the berries. This fraction comprised from 55.5 (blackberries) to 69.7% (blue-berried honeysuckle) of the total phenolic acids present in the berries. Hydroxycinnamic acids constituted from 67.3 (blackberries) to 79.3% (black mulberries) of phenolic acids identified in this fraction. Up to 15 phenolic acids have been detected in

Table 4. Content of Phenolic Acids Liberated from Esters in Six Polish Small Berries (mg Per kg of dm of Berries)

phenolic acid	blueberries	black mulberries	black currants	blue-berried honeysuckle	European juneberries	blackberries
hydroxybenzoic acid derivatives (HBA)						
gentisic	97.7 ± 5.8^{a}	110.0 ± 9.8^{a}	159.3 ± 14.9	116.8 ± 15.8^{a}	229.4 ± 10.8	101.2 ± 19.8^{a}
gallic	1.6 ± 0.1	20.6 ± 2.1	[a]	43.8 ± 3.0	53.3 ± 2.9	12.1 ± 2.1
p-hydroxybenzoic			26.3 ± 3.0			
o-pyrocatechuic				22.5 ± 1.1		
protocatechuic	86.6 ± 5.7^{a}	104.7 ± 15.7^{a,b,c}	72.5 ± 4.9	105.2 ± 11.7^{a,b,c}	126.8 ± 5.7^{c}	95.5 ± 5.7^{a,b}
salicylic	227.1 ± 18.0	75.9 ± 8.0	282.1 ± 25.8	824.8 ± 78.0	340.0 ± 26.0^{a}	309.1 ± 28.0^{a}
syringic	35.8 ± 3.0					
vanillic	85.5 ± 5.8	6.5 ± 1.2	35.3 ± 5.8^{a}	10.2 ± 1.3		37.9 ± 11.3^{a}
hydroxycinnamic acid derivatives (HCA)						
caffeic	18.5 ± 2.0	566.9 ± 43.0^{a}	107.0 ± 12.0	536.6 ± 35.7^{a}	907.9 ± 48.2	29.7 ± 3.7
m-coumaric	398.0 ± 20.6^{a}	191.0 ± 20.0	1262.2 ± 120.6^{b}	1402.1 ± 20.6^{b}	354.0 ± 37.0^{a}	504.4 ± 57.0
o-coumaric	206.4 ± 17.9	1.2 ± 0.9				
p-coumaric	744.3 ± 60.0^{a}	517.5 ± 51.0^{b}	239.5 ± 21.0	631.7 ± 67.9^{b}	377.0 ± 21.0^{c}	399.6 ± 50.0^{b,c}
3,4-dimethoxycinnamic	183.7 ± 11.3	33.9 ± 11.3^{a}		29.9 ± 2.0^{a}		
ferulic	8.0 ± 0.6	0.6 ± 0.1	26.8 ± 1.1	13.1 ± 1.6^{a}	35.9 ± 2.1	13.7 ± 2.1^{a}
hydroxycaffeic	202.9 ± 10.3^{a}	8.8 ± 0.4	16.2 ± 1.6		30.3 ± 1.4	215.0 ± 29.4^{a}
sinapic			21.3 ± 2.5			
other phenolic acids (other PA)						
p-hydroxyphenyl-acetic	16.4 ± 1.7^{a}	15.8 ± 0.7^{a}	7.2 ± 0.7	9.4 ± 0.6	24.5 ± 1.7	
p-hydroxyphenyl-lactic		11.9 ± 1.8	22.3 ± 1.3	29.2 ± 1.9	17.2 ± 1.8	8.8 ± 0.9
total HBA	534.3 ± 20.1^{a}	317.7 ± 20.3	575.5 ± 31.0^{a}	1123.3 ± 81.0	749.5 ± 29.0	555.8 ± 36.6^{a}
total HCA	1761.8 ± 67.7^{a}	1319.9 ± 71.0^{b}	1673.0 ± 123.0^{a}	2613.4 ± 79.0	1705.1 ± 64.0^{a}	1162.4 ± 81.4^{b}
total other PA	16.4 ± 1.7	27.7 ± 1.9^{a}	29.5 ± 1.5^{a}	38.6 ± 2.0^{b}	41.7 ± 2.5^{b}	8.8 ± 0.9
total	2312.5 ± 71.0^{a}	1665.3 ± 74.0^{b}	2278.0 ± 127.0^{a,c}	3775.3 ± 113.0	2496.3 ± 71.0^{c}	1727.0 ± 89.0^{b}

[a] Blank cells, not detected; values in each row marked by the same superscript letter are not significantly different ($n = 3$; *t*-test; $P > 0.05$).

Table 5. Content of Phenolic Acids Liberated from Glycosides in Six Polish Small Berries (mg Per kg of dm of Berries)

phenolic acid	blueberries	black mulberries	black currants	blue-berried honeysuckle	European juneberry	blackberries
hydroxybenzoic acid derivatives (HBA)						
gentisic	42.2 ± 3.9^{a}	4.6 ± 0.4	[a]	35.2 ± 3.8^{a,b}		32.2 ± 4.4^{b}
gallic	88.6 ± 6.1^{a}	3.7 ± 0.4	72.3 ± 6.1^{b}	0.4 ± 0.1	45.4 ± 3.4	76.6 ± 7.4^{a,b}
p-hydroxybenzoic			13.0 ± 0.1			
o-pyrocatechuic			2.7 ± 0.1	6.1 ± 0.8		
protocatechuic	23.7 ± 2.0^{a}	12.8 ± 2.8		36.9 ± 4.0	26.1 ± 2.1^{a,b}	29.0 ± 2.1^{b}
salicylic	251.6 ± 20.0^{a}	12.1 ± 2.0	225.0 ± 23.9	401.1 ± 20.0	222.4 ± 21.0^{a}	201.0 ± 29.0^{a}
syringic	0.7 ± 0.1					
vanillic	0.4 ± 0.1		12.8 ± 0.9^{a}	10.9 ± 2.0^{a}		0.6 ± 0.2
veratric	7.6 ± 0.6					
hydroxycinnamic acid derivatives (HCA)						
caffeic	81.3 ± 7.8^{a,b}	2.7 ± 0.1	100.0 ± 10.0^{a}	39.2 ± 4.1	75.5 ± 5.3^{b}	51.5 ± 7.4
m-coumaric	71.6 ± 6.5	89.1 ± 7.5^{a}	598.9 ± 60.5^{b}	606.0 ± 45.5^{b}	655.6 ± 85.0^{b}	91.6 ± 8.5^{a}
o-coumaric	5.3 ± 0.2	6.0 ± 0.3				
p-coumaric	11.1 ± 0.9	908.9 ± 51.0	73.4 ± 5.9	331.9 ± 25.2	687.7 ± 51.0	21.1 ± 2.9
3,4-dihydroxycinnamic	81.2 ± 7.8			3.2 ± 0.4		51.2 ± 7.4
3,4-dimethoxycinnamic	541.5 ± 44.0^{a}		7.4 ± 0.8	14.3 ± 1.8		501.9 ± 71.3^{a}
ferulic	12.6 ± 1.5^{a}	11.5 ± 1.5^{a}	22.6 ± 2.0^{b}	3.1 ± 1.5^{c}	25.5 ± 2.0^{b}	2.6 ± 0.5^{c}
hydroxycaffeic	520.3 ± 44.2	1.0 ± 0.2^{a}		46.5 ± 4.2	1.0 ± 0.1^{a}	400.3 ± 56.2
sinapic			15.4 ± 2.5			
other phenolic acids (other PA)						
p-hydroxyphenyl-acetic		5.8 ± 0.7				
p-hydroxyphenyl-lactic		3.7 ± 0.2^{a}	1.9 ± 0.2	18.6. ± 1.4	4.2 ± 0.5^{a}	12.8 ± 2.0
total HBA	414.8 ± 21.4	33.2 ± 3.5	325.8 ± 24.8^{a}	526.2 ± 20.8	293.9 ± 21.4^{a}	339.4 ± 30.3^{a}
total HCA	1243.7 ± 63.2^{a}	1019.2 ± 51.6^{b}	817.7 ± 61.6	1041.0 ± 52.4^{b}	1445.3 ± 99.3	1069.0 ± 91.5^{a,b}
total other PA		9.5 ± 0.7^{a}	1.9 ± 0.2	18.6 ± 1.4	4.2 ± 0.5	12.8 ± 2.0^{a}
total	1657.6 ± 64.0^{a}	1061.9 ± 52.0^{b}	1145.4 ± 66.0^{b}	1550.2 ± 56.0^{a,c}	1743.4 ± 102.0^{a}	1421.2 ± 96.0^{c}

[a] Blank cells, not detected; values in each row marked by the same superscript letter are not significantly different ($n = 3$; *t*-test; $P > 0.05$).

this fraction (**Table 4**). Of these, *m*- and *p*-coumaric acids were the major phenolic acids in blueberries and blackberries while *p*-coumaric and 3,4-dihydroxycinnamic acids dominated in black mulberries. Furthermore, *m*-coumaric acid was the principal phenolic acid of black currants and fruits of blue-berried honeysuckle, while 3,4-dimethoxycinnamic acid was unique to European juneberry. Syringic acid was only detected in blueberries, *o*-coumaric acid was only detected in blueberries and blackberries, but *o*-pyrocatechuic and sinapic acids were found only in black currants. Caffeic, ferulic, gallic, *p*-hydroxyphenyl acetic, *p*-hydroxyphenyl lactic, and vanillic acids were the minor phenolic acids in all of the berries.

Phenolic acids linked to sugars by glycosidic bonds comprised from 28.6 (blue-berried honeysuckle) to 43.6% (blackberries) of the total phenolic acids present in these berries. Hydroxycinnamic acids were the predominant class of phenolic acids comprising from 67.1 (blue-berried honeysuckle) to 96% (black mulberries) of phenolic acids found in this fraction. **Table 5** shows the phenolic acid profiles for this fraction. Sugar moieties of glycosides were not identified in this study. Hydroxycaffeic and 3,4-dimethoxycinnamic acids were the principal phenolic acids present in blueberries and blackberries, while *m*- and *o*-coumaric acids were the major phenolic acids in European juneberries. Moreover, *m*-coumaric acid was found to be the predominant phenolic acid in black currants and fruits of blue-berried honeysuckle, while *p*-coumaric acid dominated in black mulberries. In addition, *o*-coumaric acid was identified only in blueberries and blackberries, while *o*-pyrocatechuic acid was found only in black currants and blue-berried honeysuckle. Furthermore, *p*-hydroxybenzoic and sinapic acids were only detected in black currants, and syringic and veratric acids were unique to blueberries. The contents of ferulic, gentisic, protocatechuic, and vanillic acid did not exceed 50 mg per kg, dm of berries in all of the fruits studied.

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