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ARTICLES

Separation, Characterization, and Quantitation of Phenolic Acids in a Little-Known Blueberry (*Vaccinium arctostaphylos* L.) Fruit by HPLC-MS

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The aim of this study was the qualitative and quantitative determination of free, ester, glycoside, and ester-bound phenolic acids in the blueberry (*Vaccinium arctostaphylos* L.) fruit. A method for the determination of the profile of phenolic acids of four different phenolic fractions in the fruit was developed using high-performance liquid chromatography−mass spectrometry (HPLC-MS). Thirteen compounds (gallic, protocatechuic, *p*-hydroxybenzoic, *m*-hydroxybenzoic, gentisic, chlorogenic, *p*-coumaric, caffeic, ferulic, syringic, sinapic, salicylic, and *trans*-cinnamic acids) were identified and quantified in the berry. These experimental results showed that the predominant phenolic acid in the fruit of *V. arctostaphylos* is caffeic acid in free and insoluble ester-bound forms and *p*-coumaric acid in soluble ester and glycoside forms. Seven phenolic acids were identified as hydroxybenzoic acid derivatives (HBAs) and four as hydroxycinnamic acid derivatives (HCAs). Total content of HBAs and HCAs in the four phenolic fractions constituted 30.1 and 69.9% of the free, 27.9 and 72.1% of the ester, 24.7 and 75.3% of the glycoside, and 51.7 and 48.3% of the ester-bound forms, respectively. Total phenolics as the sum of individual phenolic acids identified is 698.5 ng/g of fresh weight (fw) for the free, 3399.2 ng/g of fw for the ester, 3522.1 ng/g of fw for the glycoside, and 3671.6 ng/g of fw for the ester-bound phenolic fractions. The present results were compared with reported levels of phenolic acids in the fruits of different *Vaccinium* species. These data suggest that the fruit can be considered as a potentially good dietary source of phenolic acids.

KEYWORDS: Blueberry; fruit; *Vaccinium arctostaphylos*; phenolic acids; HPLC-MS

INTRODUCTION

As non-nutrient biologically active compounds, phenolics are naturally occurring secondary plant metabolites widely distributed in the plant kingdom. They are also present in many foods and beverages of plant origin, that is, in fruits, vegetables, tea, red wine (*1*, *2*), nuts, seeds, flowers, and barks (*2*). Most of these compounds are an integral part of the human diet, and they are also taken as medicinal preparations (*2*). The importance of these compounds as health-promoting and disease-preventing substances is now being recognized through scientific investigations. Many of the health protective effects of phenolic compounds have been ascribed to their antioxidant, antimutagenic, anticarcinogenic, antiinflammatory, antimicrobial, and other biological properties (*3*−*11*). In addition, at the cellular level, they also affect several stages of signal transduction, including cell surface and intracellular receptors, intracellular mediator, kinases, the cell cycle, DNA replication-related enzymes, and gene expression (*12*).

Interest in the role of phenolic antioxidants in human health has prompted research into the separation and characterization of active phenolic components in various plant-derived foods (*1*, *13*−*16*). A number of traditional separation techniques such as paper, thin-layer, and column chromatography, with various solvent systems and spray reagents, have been described as having the ability to separate and identify phenolic compounds (*17*−*19*). More recently, gas−liquid chromatography (GLC), high-performance liquid chromatography (HPLC), and capillary electrophoresis (CE) methods have been developed (*16*, *19*−*30*). Of these, in contrast to GLC methods, HPLC coupled with

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a photodiode array detector or mass spectrometer does not require derivatization prior to the quantitative analysis. However, the ultraviolet−visible (UV−vis) spectrum does not supply sufficient identifying power (*19*, *22*, *26*). For this reason, HPLC combined with mass spectrometry (HPLC-MS) remains the method of choice for separating and characterizing of fruit phenolics.

The phenolics of the fruit of the *Vaccinium* species have received considerable attention. In bilberry (*V. myrtillus*) fruit, Azar et al. (*31*), for example, identified 12 phenolic acids and three flavonol glycosides by both TLC and HPLC analyses. During the past decade, with improving separation, characterization, and quantitation techniques, a number of phenolic compounds of blueberry fruits and their antioxidant capacity of different species from various countries have been added to the literature given here selectively (*14*, *15*, *32*−*37*).

In view of the growing interest in these compounds, there is a need to identify these important compounds in fruits and vegetables and their products. Some of these compounds are present in many fruits, but others are specific to a particular kind of fruit or vegetable. Furthermore, within the same fruit type, the growing season, variety, environment and climatic conditions, plant disease, soil type, geographic location, and even maturity seem to influence the concentration of phenolic compounds (*1*, *2*).

The wild blueberries (*Vaccinium* spp.) of the Turkish flora are represented by four species, namely, *V. vitis-idea*, *V. uliginosum*, *V. myrtillus*, and *V. arctostaphylos*, and are a significant element of the native flora of northeastern Anatolia (Turkey) (*38*). The succulent fruits of all these Turkish species can be eaten, those of *V. arctostaphylos* being especially prized (*38*), although the fruits of this species have been harvested in significant quantities in the region over the years, for both commercial and home uses. The species has also been described in the Caucasus, western and southern Transcaucasus, and the Balkans of Asia and Asia Minor (central Asiatic Turkey) (*39*).

Although the phenolic compositions of three other blueberry (*V. vitis-idea*, *V. uliginosum*, and *V. myrtillus*) fruits have been reported in different geographical regions outside Turkey (*15*, *16*, *36*−*41*), no satisfactory reports of Turkish blueberry phenolic acid compositions are available. The present study provides preliminary data on the phenolic acid profile of a little-known blueberry (*V. arctostaphylos*) fruit found in northeastern Anatolia. The study forms part of our ongoing investigation into the chemical and nutrient composition of Turkish blueberries, which are an emerging interest among food technologists. The objective of this study was to separate and characterize the phenolic acid profiles in four phenolic fractions extracted and isolated from blueberry (*V. arctostaphylos*) fruit using HPLC-MS.

MATERIALS AND METHODS

Plant Materials. Blueberry (*V. arctostaphylos* L.) fruits, average diameter of 9.2 ± 0.3 mm, were randomly harvested at midmorning from different parts of bushes (120) from their single genotypes of six bulk populations, at an altitute of between 600 and 650 m above sea level, in the native habitat of the species near the cities of Giresun, Trabzon, Rize, and Artvin, in northeastern Anatolia (Turkey), in August 2001 and 2002. The harvested fruits were maintained in an ice box container at below 5 °C until arrival at the laboratory (1.5 h). In the laboratory, the blueberry samples were immersed in liquid nitrogen for several minutes and then stored for several days in a medical freezer at −40 °C until extraction.

Chemicals and Reagents. Standards of 3,5-dihydroxybenzoic, gallic, protocatechuic, *p*-hydroxybenzoic, *m*-hydroxybenzoic, gentisic, *o*-coumaric, *p*-coumaric, caffeic, ferulic, syringic, sinapic, chlorogenic (5-caffeoylquinic), salicylic, *trans*-cinnamic, and *m*-coumaric acids (internal standard) were purchased from Sigma-Aldrich Fine Chemicals (St. Louis, MO). Formic acid and methanol for HPLC were purchased from Merck (Darmstadt, Germany), and deionized water was prepared using Simplicity 185 (Millipore, Bedford, MA).

Extraction of Phenolic Acids from Blueberry Fruit. Phenolic acids were isolated from the extract according to a previously described method (*40*, *41*). Natural blueberry fruit (160 g) was treated with liquid N_2 and ground in 80% methanol (300 mL) including an antioxidant, 2,6-di-*tert*-butyl-*β*-cresol (DBC) with an electrical high-speed blender. The homogenate was filtered, and the slurry was concentrated under vacuo in a rotary evaporator. An aliquot (30 mL) of the concentrate was adjusted to pH 2 (6 M HCl), and free phenolic acids were extracted 5 times into 100 mL of diethyl ether using a separatory funnel. The ether extract was evaporated to dryness under vacuum at room temperature. The aqueous phase was first treated by alkaline hydrolysis (2 M NaOH) for 4 h under a nitrogen atmosphere at room temperature. After acidification to pH 2 using 6 M HCl, phenolic acids released from soluble esters were extracted from the hydrolysate five times into 120 mL of diethyl ether using a separatory funnel. To the second half of the aqueous concentrates was added 6 M HCl, and the medium was placed under a nitrogen atmosphere and hydrolyzed for 1 h in a boiling water bath. Phenolic acids released from soluble glycosides were separated from the hydrolysate five times into 90 mL of diethyl ether. The solid residue gained after centrifugation of the first homogenate was dissolved in 2 M NaOH for 4 h under a nitrogen atmosphere at room temperature. After acidification to pH 2 using 6 M HCl, phenolic acids released from methanol-insoluble ester-bound phenolic acids were extracted from the hydrolysate four times into 130 mL of diethyl ether using a separatory funnel.

Preparation of Calibration Standards. Standard solutions each containing 15 target compounds, that is, 3,5-dihydroxybenzoic, gallic, protocatechuic, *p*-hydroxybenzoic, *m*-hydroxybenzoic, gentisic, *o*-coumaric, *p*-coumaric, caffeic, ferulic, syringic, sinapic, chlorogenic, salicylic, and *trans*-cinnamic acids, were first prepared in methanol at the concentration of 10^{-3} mol L^{-1} and were gradually diluted to the working concentrations of 5×10^{-5}, 10^{-5}, 5×10^{-6}, 10^{-6}, 5×10^{-7}, and 10^{-7} mol L^{-1}. An internal standard of *m*-coumaric acid was added to set up a resulting concentration of 10^{-5} mol L^{-1} in all standard solutions. Ten microliters of standard solutions was injected onto the column when calibration points and range of calibration curves were determined.

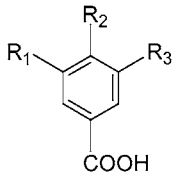
HPLC-MS Instrumentation and Conditions. HPLC-MS analyses were carried out on an Alliance 2690 separations module (Waters, Milford, MA) linked simultaneously to a PDA 996 (Waters) and a ZMD 2000 single-quadrupole mass spectrometer equipped with an electrospray interface (Micromass, Manchester, U.K.). Data were processed by MassLynx software (Data Handling System for Windows, version 4.0, Micromass, Altrincham, U.K.).

Dried fractions were dissolved in 10 mL of 10% acetonitrile, filtered through microspin filter tubes (nylon, 0.45 μm, Alltech Associates Inc., Deerfield, IL), and 10 μL was injected on the reversed phase column (Luna Phenyl-Hexyl, 5 μm, 250 × 2 mm, Phenomenex, Torrance, CA). The column thermostat was set at 35 °C. Solvent A consisted of 5 mM formic acid, and solvent B consisted of methanol. At a flow rate of 200 μL min^{-1}, the following binary gradient with linear interpolation was used: 0 min, 2% B; 5 min, 2% B; 15 min, 15% B; 35 min, 30% B; 40 min, 35% B; 50 min, 70% B. At the end of the gradient the column was washed with 100% methanol and equilibrated to initial conditions for 15 min. Using postcolumn splitting (1:1), effluent was simultaneously introduced into the PDA detector (scanning range, 210−600 nm; resolution, 1.2 nm) and an electrospray source (source block temperature, 100 °C; desolvation temperature, 250 °C; capillary voltage, 2.7 kV; cone voltage, 35 V). Nitrogen was used both as nebulizing (550 L h^{-1}) and as drying gas (50 L h^{-1}). Electrospray ionization−mass spectrometry (ESI-MS) data were acquired in negative mode, and quantitation was done by selective ion monitoring of pseudo-molecular ions of $[M - H]^-$. Particular monitored ions, retention windows, and dwell times are listed in **Table 1**.

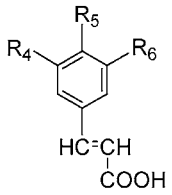
Table 1. Retention Times, Pseudo-molecular Ions, Dwell Times, Retention Windows, and Limits of Detection (LOD) of Phenolic Acids

compound	retention time (min)	$[M-H]^-$ [a]	dwell time (s)	retention window (min)	LOD[b] (pmol injected)
gallic acid	8.57 ± 0.03	168.9	1.49	2–19	0.25
3,5-dihydroxybenzoic acid	16.08 ± 0.03	152.9	1.49		0.25
protocatechuic acid	16.95 ± 0.06	152.9	1.49		0.25
p-hydroxybenzoic acid	21.84 ± 0.06	136.9	0.32	19–35	0.75
chlorogenic acid	23.96 ± 0.03	353.0	0.32		0.5
gentisic acid	24.44 ± 0.06	152.9	0.32		0.75
caffeic acid	24.75 ± 0.05	178.9	0.32		0.5
m-hydroxybenzoic acid	24.95 ± 0.07	136.9	0.32		0.75
syringic acid	25.87 ± 0.03	197.0	0.32		0.5
p-coumaric acid	29.79 ± 0.03	162.9	0.32		0.75
ferulic acid	32.01 ± 0.05	192.9	0.32		0.5
sinapic acid	32.04 ± 0.02	223.0	0.32		0.5
m-coumaric acid	32.90 ± 0.04	162.9	0.32		0.25
o-coumaric acid	36.10 ± 0.01	162.9	0.5	35–50	0.75
salicylic acid	38.58 ± 0.02	136.9	0.5		0.5
trans-cinnamic acid	45.12 ± 0.03	146.9	0.5		2.0

[a] Deprotonated pseudo-molecular ion. [b] Signal peak height 3 times the average baseline noise, measured peak to peak.



benzoic acid derivates	R_1	R_2	R_3
p-hydroxybenzoic acid	H	OH	H
protocatechuic acid	OH	OH	H
gallic acid	OH	OH	OH
syringic acid	OCH_3	OH	OCH_3
3,5-dihydroxybenzoic acid	OH	H	OH



trans-cinnamic acid derivates	R_4	R_5	R_6
m-coumaric acid	OH	H	H
p-coumaric acid	H	OH	H
caffeic acid	H	OH	OH
ferulic acid	H	OH	OCH_3
sinapic acid	OCH_3	OH	OCH_3

Figure 1. Chemical structures of selected phenolic acids.

Identification and Quantitation. The phenolic acids in the fruits of blueberry (*V. arctostaphylos*) were identified by matching retention times and mass spectral data with the calibration standards. The determination of endogenous levels was performed according to the method of internal standardization using *m*-coumaric acid at a concentration of 10^{-5} mol L^{-1}. The quantitation of the phenolic compounds was based on the ratio of peak area of the compound of interest over the peak area of the internal standard. The calibration standards and blueberry fruit samples were run in triplicate. A linear dynamic range of 1−500 pmol injected was found for all standards (except *trans*-cinnamic acid), with detection limits ranging from 0.25 to 2 pmol injected (**Table 1**).

RESULTS

The structures of selected phenolic compounds of interest to this investigation are shown in **Figure 1**. The HPLC-MS conditions were optimized to obtain an acceptable compromise between separation effectiveness and limits of detection. The resulting separation is visualized in **Figure 2a**, which shows the sum of HPLC-ESI-MS chromatograms of the mixture of calibration standards. Eleven simple phenolic acids, *trans*-cinnamic acid, and chlorogenic acid (as a representative of phenolic esters) were detected and quantified in the blueberry (*V. arctostaphylos*) fruit.

Eleven free phenolic acids identified in the investigated fruit were gallic, protocatechuic, *p*-hydroxybenzoic, *m*-hydroxybenzoic, gentisic, *p*-coumaric, caffeic, ferulic, syringic, sinapic, and salicylic acids (**Table 2**). Typical HPLC-ESI-MS chromatograms of the fraction of free phenolic acids are visualized in **Figure 2b**. Nine phenolic acids quantified after liberation from soluble ester fraction were gallic, protocatechuic, *p*-hydroxybenzoic, syringic, salicylic, *p*-coumaric, caffeic, ferulic, and sinapic acids (**Table 2**). Neither *trans*-cinnamic nor chlorogenic acid was found in this fraction. The soluble glycoside fraction resulted in eight phenolic acids identified as protocatechuic, *p*-hydroxybenzoic, *m*-hydroxybenzoic, syringic, *p*-coumaric, caffeic, ferulic, and sinapic acids (**Table 2**). No detectable levels of gallic, gentisic, salicylic, and *trans*-cinnamic acids were found in this fraction. With the exception of gentisic acid, 10 phenolic acids determined in the insoluble ester-bound phenolic fraction were gallic, protocatechuic, *p*-hydroxybenzoic, *m*-hydroxybenzoic, syringic, salicylic, *p*-coumaric, caffeic, ferulic, and sinapic acids. In contrast to the first three fractions, *trans*-cinnamic acid

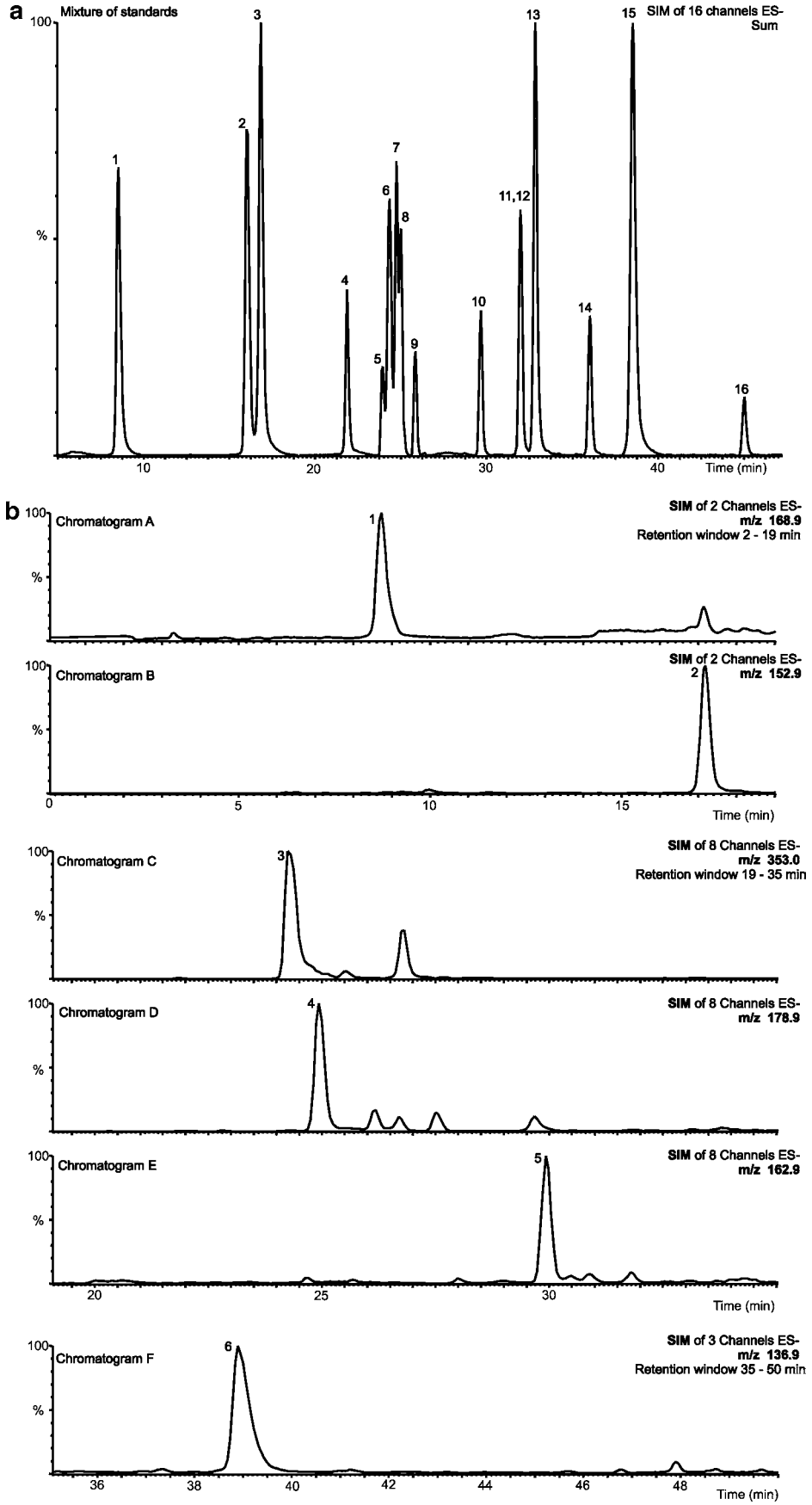


Figure 2. (**a**) HPLC-ESI-MS chromatogram of the mixture of gallic acid (1), 3,5-dihydroxybenzoic acid (2), protocatechuic acid (3), *p*-hydroxybenzoic acid (4), chlorogenic acid (5), gentisic acid (6), caffeic acid (7), *m*-hydroxybenzoic acid (8), syringic acid (9), *p*-coumaric acid (10), ferulic acid (11), sinapic acid (12), *m*-coumaric acid (13), *o*-coumaric acid (14), salicylic acid (15), and *trans*-cinnamic acid (16). (**b**) Typical HPLC-ESI-MS chromatograms (A–F) of free phenolic acids in *V. arctostaphylos* fruit: gallic acid (1), protocatechuic acid (2), chlorogenic acid (3), caffeic acid (4), *p*-coumaric acid (5), and salicylic acid (6).

Table 2. Phenolic Acids Content (Nanograms per Gram of Fresh Weight) in Blueberry (*V. arctostaphylos* L.) Fruit Collected from Northeastern Anatolia (Turkey)[a]

	phenolic fraction				
phenolic acid	free	esters	glycoside	ester-bound	total[b]
gallic acid	2.8 ± 0.3	3.0 ± 0.2	ND[c]	73.0 ± 8.1	78.8
protocatechuic acid	56.1 ± 10.8	451.0 ± 14.8	481.5 ± 22.4	467.8 ± 12.7	1456.4
p-hydroxybenzoic acid	23.1 ± 2.5	60.3 ± 5.4	81.2 ± 9.4	568.0 ± 18.7	732.6
m-hydroxybenzoic acid	10.8 ± 0.6	ND	157.0 ± 4.5	37.1 ± 4.2	204.9
gentisic acid	1.8 ± 0.2	ND	ND	ND	1.8
syringic acid	54.8 ± 1.2	433.5 ± 16.0	151.8 ± 8.5	710.3 ± 25.6	1350.4
salicylic acid	60.7 ± 7.1	1.5 ± 0.5	ND	42.5 ± 5.7	104.7
p-coumaric acid	162.7 ± 12.9	1284.2 ± 17.5	1354.1 ± 15.2	596.6 ± 22.3	3397.6
caffeic acid	310.6 ± 21.3	1094.9 ± 12.4	1193.8 ± 17.3	1075.0 ± 5.4	3674.3
ferulic acid	14.2 ± 1.8	69.9 ± 5.1	72.4 ± 7.0	53.7 ± 10.6	210.2
sinapic acid	0.9 ± 0.2	0.9 ± 0.0	30.3 ± 0.9	41.0 ± 8.4	73.1
trans-cinnamic acid	ND	ND	ND	6.6 ± 1.8	6.6
$\Sigma_{benzoics}$	210.1	949.3	871.5	1898.7	3929.6
$\Sigma_{cinnamics}$	488.4	2449.9	2650.6	1772.9	7361.8
$\Sigma_{benzoics}$ (%)	30.1	27.9	24.7	51.7	34.8
$\Sigma_{cinnamics}$ (%)	69.9	72.1	75.3	48.3	65.2
total[d]	698.5	3399.2	3522.1	3671.6	11291.4

[a] Values are means ± SD ($n = 3$). [b] Total is sum of each phenolic acid of four phenolic fractions. [c] Not detected. [d] Total is sum of individual phenolic acids identified in each phenolic fraction.

was detected only in ester-bound fraction (**Table 2**). In addition, an ester of caffeic and quinic acid (chlorogenic acid) was determined in both free and glycoside forms in quantities of 193.7 and 211.2 ng/g of fresh weight (fw), respectively.

To sum up, the most abundant phenolic acids in the fruit were caffeic acid in free (310.6 ng/g of fw) and ester-bound (1075.0 ng/g of fw) forms and *p*-coumaric acid in ester (1284.2 ng/g of fw) and glycoside (1354.1 ng/g of fw) forms. Total phenolics as the sum of individual phenolic acids identified was 698.5 ng/g of fw for the free, 3399.2 ng/g of fw for the ester, 3522.1 ng/g of fw for the glycoside, and 3671.6 ng/g of fw for the ester-bound phenolic fractions (**Table 2**).

DISCUSSION

Two families of phenolic acids are commonly found in plants, that is, a range of substituted benzoic acid derivatives (salicylic, gallic, vanillic, *p*-hydroxybenzoic, syringic, and protocatechuic) and those derived from cinnamic acid (*p*-coumaric, ferulic, caffeic, and sinapic). Both types of phenolic acids usually occur in conjugated forms, and the levels of free forms in living plant tissue are strictly controlled. It should be mentioned that phenolic acids readily convert from one kind to another during different physiological processes (*42*).

Our continual studies on fruit phenolics have necessitated analyses of both free and conjugated phenolic acids in *Vaccinium* berries distributed in Turkey. In this respect, the first goal of our investigation was focused specifically on the isolation and analysis of free, ester, glycoside, and ester-bound phenolic fractions of *V. arctostaphylos* fruit, the especially prized one among all Turkish *Vaccinium* berries, which can be used both for commercial and for home uses (*38*).

To the best of our knowledge, this is the first study showing the comprehensive composition of phenolic acids and their conjugates in the fruit of *V. arctostaphylos*. The blueberry fruits analyzed in the present study resulted in seven hydroxybenzoic acid derivatives (HBAs) represented by gallic, protocatechuic, *p*-hydroxybenzoic, *m*-hydroxybenzoic, gentisic, syringic, and salicylic acids and four hydroxycinnamic acid derivatives (HCAs) represented by *p*-coumaric, caffeic, ferulic, and sinapic acids. The comparative analyses of phenolic acids in the four phenolic fractions have revealed lower amounts of free phenolic acids when compared to the other three fractions. It has been reported that phenolic acids in free forms are very rarely present in plants, whereas the majority of phenolic acids are present in bound form (*16*, *43*).

Previous studies on fruit phenolics in *Vaccinium* berries have shown that fruits of different species or cultivars/forms are represented by specific phenolic acid profiles. Caffeic acid was found to be the predominant phenolic acid in the fruits of three blueberry clones (Clon 908, Heerma I, and Heerma II) (*44*) and in alpine bilberry (*V. uliginosum* L.) (142 μg/g of fw) (*32*). Oval-leaf or Alaska blueberry (*V. ovalifolium* Smith) and wild cranberry (*V. oxycoccus* L.) fruits were characterized by an abundance of *p*-coumaric acid at concentrations of 23.9 and 101 μg/g of fw, respectively (*32*). Fruits of red huckleberry (*V. parvifolium* Smith) were specific in content of *p*-hydroxybenzoic acid (553 μg/g of fw), whereas caffeic and *p*-coumaric acids were in lower amounts (*32*). Hybrids of highbush blueberries (*V. corymbosum* L.) contained gallic acid at ∼3.4 mg/100 g of fw, caffeic acid at ∼3.2 mg/100 g of fw, *p*-coumaric acid at ∼5 mg/100 g of fw, and ferulic acid at ∼3.7 mg/100 g of fw, whereas no detectable level of *p*-hydroxybenzoic acid was determined (*37*). Häkkinen et al. (*14*) have reported that the most abundant phenolic acid in cranberry and blueberry (cvs. Northblue and Northcountry) fruits was ferulic acid. On the other hand, the major phenolic acid in lingonberry (*V. vitis-idea*) and bilberry fruits was *p*-coumaric acid (*14*). In cranberry (*V. macrocarpon* Ait. var. Early Black) fruit, only benzoic, *p*-coumaric, caffeic, ferulic, and sinapic acids have been identified by liquid chromatography (*46*). Later, in the fruits of the same species, Zheng and Shetty (*47*) identified and quantified bound gallic, chlorogenic, *p*-hydroxybenzoic, and *p*-coumaric acids. Recently, in cranberry (*V. macrocarpon*) fruit, *p*-coumaric acid was found to be the most abundant phenolic acid in free (21.6 μg/g of fw) and bound (232.2 μg/g of fw) forms (*16*).

The results obtained in the present study show that caffeic and *p*-coumaric acids are the major phenolic acids quantified in the Anatolian blueberry fruit at total concentrations of 3674.3 and 3397.6 ng/g of fw, respectively. It can be concluded that the phenolic acid concentrations are mostly lower in *V. arctostaphylos* in comparison to the above-mentioned berries. Noticeably, levels of caffeic, *p*-coumaric, and ferulic acids in

fruits of nine different *Vaccinium* species (*32*) were found to be higher in the present species. However, the content of particular phenolic acids such as caffeic acid was in good accordance with other berries as its content varied in a range between 1 and 500 μg/g of fw in different *Vaccinium* species reported (*33*). Our measurements were found to be within the range. Similarly, in fruits of different cultivars of rabbiteye (*V. asheii* Reade) and highbush blueberry hybrids (*37*) large quantitative and qualitative differences in phenolic acids content were also reported. In this case, the qualitative differences mean that some of the analytes were not detectable or, in other words, the concentrations did not reach the limits of detection. For that reason, it remains, of course, difficult to compare phenolic acid data of even very closely related species with the present results. Namely, it has been shown that fruit tissues are able to synthesize phenolic compounds being influenced by some factors either biotic or abiotic. When accessible data on *Vaccinium* fruit phenolics were evaluated, it was also postulated that differences vary among and within species or cultivars/forms/hybrids due to differences in fruit source, ripeness, and length of storage time, as well as differences in the procedures used for obtaining samples (*1*, *33*).

In conclusion, further studies are needed to determine levels of fruit phenolic acids or other phenolic compounds of *V. arctostaphylos*. Nevertheless, there are no satisfactory data to make any comparison of the phenolic acids profile of the present fruit with other blueberries (*V. myrtillus*, *V. vitis-idea*, and *V. uliginosum*) distributed in Turkey. The present information on the fruit phenolic acid composition of *V. arctostaphylos* may also be of use to consumers and food technologists introducing the fruit commercially. Furthermore, research on fruit phenolics of Turkish blueberries and their antioxidant properties may also be a desirable feature for selecting *Vaccinium* species with improved quality directed to reducing the risk of cancer and degenerative diseases.

ABBREVIATIONS USED

HBAs, hydroxybenzoic acid derivatives; HCAs, hydroxycinnamic acid derivatives; GLC, gas−liquid chromatography; UV−vis, ultraviolet−visible; CE, capillary electrophoresis, HPLC-MS, high-performance liquid chromatography with mass spectrometry, UV, ultraviolet; HPLC-ESI-MS, high-performance liquid chromatography−electrospray ionization−mass spectrometry; TLC, thin-layer chromatography.

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