JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Content and Profile of Flavanoid and Phenolic Acid Compounds in Conjunction with the Antioxidant Capacity for a Variety of Northwest *Vaccinium* Berries

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This investigation evaluated the content and profile of flavanoid and phenolic acid compounds present in nine *Vaccinium* species that included domestic blueberry cultivars and sample collections from undomesticated colonies. The study was focused in two areas of inquiry. The first involved extracting and analyzing the berries for total phenolics (TPH), total anthocyanins (ACY), and the antioxidant capacity. *Vaccinium* species differ in their polyphenolic content, and these high TPH and ACY levels are correlated to their antioxidant capacity. Second, berry extracts were analyzed by high-performance liquid chromatography equipped with photodiode array and mass spectrometric detectors to determine the content and profile of selected bioactive compounds. The flavanoid analytes of interest included the anthocyanidins, flavan-3-ols, and flavonol aglycons, as well as specific phenolic acid components. This semicomprehensive analysis begins to characterize the phytochemical profiles and illustrates the differences in the content of polyphenolic compounds present within these *Vaccinium* species.

KEYWORDS: Antioxidant capacity; anthocyanins; flavanoids; phenolic acids; *Vaccinium*

INTRODUCTION

Epidemiological and laboratory studies show a convincing link between the antioxidant properties of plant-derived polyphenolic compounds and their health-promoting and/or disease-preventing effects (*1*−*3*). The functionality of these phytochemical nutrients has been correlated to their capacity as antiradical scavengers (*4*, *5*) as well as inhibitors and/or activators of mammalian enzyme systems (*6*−*8*). A large volume of scientific evidence is beginning to promote the nutritional benefits of these plant-derived nutrients. Studies have shown that these phytochemicals can have positive effects on mammalian physiological systems such as enhancing red blood cell resistance to oxidative stress (*9*), inducing apoptosis of cancer cells (*10*), and inhibiting azoxymethane-induced colon carcinogenesis in rats (*11*).

Vaccinium berries have been shown to contain high levels of the flavanoid compounds (*12*−*14*). Every member of this extensively investigated group of polyphenolic compounds contains a common molecular structure that consists of the tricyclic $C_6-C_3-C_6$ "flavanoid skeleton". These flavanoid nutraceuticals include the natural red and blue pigments or anthocyanins, the proanthocyanidins or flavan-3-ols, which include the highly revered black and green tea catechins (*15*), and the flavonols, of which quercetin appears to be the primary constituent. Anthocyanins and flavonols typically occur in plants as glycosides, and these glycosides may also occur in acetylated forms. For example, the anthocyanidin, cyanidin, can occur in many glycosidic forms such as cyanidin 3-galactoside, cyanidin 3-glucoside, and cyanidin 3-arabinoside, as well as the acetylated forms, of which acetylated cyanidin-3-glucose is one example (*16*). Another important class of compounds is made up of the esters, glycosides, and amides of the hydroxycinnamic and hydroxybenzoic acids commonly referred to as phenolic acids. Some examples of phenolic acids that are found in fruits and beans include chlorogenic acid from blueberries (*17*) and caffeic acid from brewed coffee (*18*).

Considering the growing interest in the potential nutraceutical properties of fruits and berries, it seemed relevant to characterize the wild populations of *Vaccinium* species that grow within the northwestern United States. It also is important to compare and contrast these wild populations of *Vaccinium* berries with their domesticated highbush and half-highbush blueberry counterparts because considerable baseline data have been generated for the latter. Therefore, the scope of this study followed two areas of investigation pertaining to the differences in the polyphenolic contents of seven Northwest *Vaccinium* species and two domestic *Vaccinium* species. The first area of research examined the differences in the berries' total phenolic (TPH) and anthocyanin (ACY) contents as well as their antioxidant capacities as measured by commonly accepted spectrophotometric and fluorometric assays. The second line of investigation focused on expanding this initial component analysis by using high-

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10.1021/jf0307595 CCC: $27.50
Published on Web 04/27/2004

Table 1. *Vaccinium* Species Collections from Cultivated and Wild Colonies

common name	*Vaccinium* species	cultivar or designation	date collected	location of collection, state
half-highbush blueberry	*angustifolium* Aiton × *corymbosum* L.	Northblue	Aug 1, 2002	Sandpoint Research and Extension Center, ID
		Northcountry	Aug 1, 2002	
		Northsky	Aug 1, 2002	
highbush blueberry	*corymbosum* Linnaeus	Bluecrop	July 31, 2002	Sandpoint Research and Extension Center, ID
		Bluejay	July 31, 2002	
		Jersey	July 31, 2002	
cascade huckleberry	*deliciosum* Piper	VADE 019	Aug 17. 2001	Wenatchee NF,[b] WA
		VADE 021	Aug 24, 2001	Gifford Pinchot NF, WA
black- or thin-leaf huckleberry	*membranaceum* Douglas ex Hooker	VAME 098[a]	July 31, 2001	Kaniksu NF, ID
		VAME 103	Aug 18, 2001	Wenatchee NF, WA
		VAME 106	Aug 23, 2001	Gifford Pinchot NF, WA
oval-leaf or Alaska blueberry	*ovalifolium* Smith	VAOF 091[a]	July 17, 2001	Kaniksu NF, ID
		VAOF 093	Aug 17, 2001	Wenatchee NF, WA
		VAOF 102	Aug 23, 2001	Gifford Pinchot NF, WA
evergreen huckleberry	*ovatum* Pursh	VAOV 006	Nov 15, 2001	Olympic NF, WA
		VAOV 007	Nov 15, 2001	Olympic NF, WA
wild cranberry	*oxycoccus* Linnaeus	VAOX 001	Oct 16, 2001	Kaniksu NF, ID
		VAOX 002	Oct 16, 2001	Kaniksu NF, ID
red huckleberry	*parvifolium* Smith	VAPA 004	Aug 19, 2001	Olympic NF, WA
		VAPA 006	Aug 20, 2001	Olympic NF, WA
		VAPA 010	Aug 21, 2001	Olympic NF, WA
alpine bilberry	*uliginosum* Linnaeus	VAUG 016	Aug 8, 2002	Shoshone NF, WY
		VAUG 017	Aug 8, 2002	Shoshone NF, WY

[a] Samples of VAME 098 and VAOF 091 were collected in 2001 and 2002. [b] NF = National Forest.

pressure liquid chromatography (HPLC) equipped with either a photodiode array detector (DAD) or a mass spectrometric detector (MSD) to elucidate any differences in the content and profile of selected flavanoid and phenolic acid components present in the *Vaccinium* species.

MATERIALS AND METHODS

Chemicals. All of the flavan-3-ol and phenolic acid standards except chlorogenic acid [caffeic acid, *p*-coumaric acid, ferulic acid, *p*-hydroxybenzoic acid, gallic acid, (+) catechin, and (−)-epicatechin] as well as the flavonol standard, kaempferol, and diethyl dithiocarbamic acid (DETC) were obtained from Sigma Chemical Co. (St. Louis, MO). The flavonol standards quercetin dihydrate and myricetin as well as chlorogenic acid, trifluoroacetic acid (TFA), and Trolox were purchased from Aldrich (Milwaukee, WI). HPLC grade methanol and acetonitrile were purchased from Fisher Scientific (Boston, MA). Isorhamnetin was used as a flavonol HPLC internal standard and was obtained from Fluka (Milwaukee, WI). The anthocyanidin standards dephinidin, cyanidin, and malvidin were obtained from Indofine Chemical Co. (Somerville, NJ). All other chemicals were reagent grade products purchased from either J. T. Baker (Phillipsburg, NJ) or Fisher Scientific (Boston, MA).

Sampling Procedures. All of the *Vaccinium* fruit samples that were used in this study were collected from either cultivated or undomesticated colonies within the northwestern United States (**Table 1**). Highbush blueberry cultivars Bluecrop, Bluejay, and Jersey and half-highbush blueberry cultivars Northblue, Northcountry, and Northsky were harvested from mature (>10-year-old) bushes growing on silt loam soil at the University of Idaho Sandpoint Research and Extension Center. The plants were grown on the 2000 foot elevation site using commercial blueberry fertilization, pruning, and irrigation practices typical for the Pacific Northwest (*19*). Soil pH averaged ~5.5. Fruit was harvested when it developed a full-ripe, blue color, softened, and could easily be removed from the pedicels. No chemical or refractometer assays were used to determine ripeness. Wild fruits were collected over a wide geographic area from unmanaged, naturally occurring colonies. Elevations ranged from sea level to 2800 m, depending on species. The purpose was to sample from potentially diverse ecotypes. For each collection site, fruit was harvested from numerous genotypes to better represent a species average. Ripeness was determined on the basis of color, fruit softening, and ease of removal from the pedicels. Overripe fruits exhibiting shriveling were not harvested, nor were fruits showing evidence of pest or disease damage. Immediately after harvest, fruits were placed on ice in storage chests, transported to the laboratory, and stored frozen at −40 °C. Prior to analysis, the frozen berry samples were removed from cold storage and processed using a "freeze−fracture" technique. In short, samples were placed in a Robot Coupe food processor, and liquid nitrogen was added to maintain the frozen integrity of the samples during the grinding process. After macerating, the powdered berries were transferred to mason jars and returned to −40 °C cold storage.

Extraction for TPH, ACY, and Antioxidant Capacity. All of the berry samples were extracted and analyzed in triplicate, requiring the processing of multiple sample sets. Each sample set was extracted and analyzed for TPH and ACY within an 8 h period to minimize the loss of the bioactive components. Berry samples were extracted by modifying previously published methods (*13*, *20*, *21*) as described in the following. Frozen ground berries (5 g) were weighed into 50 mL Teflon centrifuge tubes, and 20 mL of the extraction solvent (acetone/water; 70:30) was added. The samples were ground for 1 min at room temperature with a Polytron homogenizer (Brinkmann Instruments, Westbury, NY). Berry mixtures were capped and centrifuged (DuPont Sorval RC-5, Newton, CT) at 3000*g* for 10 min and 0 °C. After clarification by centrifugation, supernatants were decanted into 200 mL Turbovap flasks, and the berry sample pellets were reprocessed two times with 10 mL of extraction solvent combining the supernatants. Samples were concentrated under nitrogen to 10 mL using a Zymark concentrator (Zymark Corp., Hicksville, NY), quantitatively transferred to 50 mL volumetric flasks, and brought to volume with HPLC grade water. Sample extracts were syringe filtered (0.45 μm Acrodisk GHP, Gelman) into 12 mL amber vials and sealed with Teflon-lined caps.

Total Phenolics. TPH was determined according to the Folin−Ciocalteu method as described by Slinkard and Singleton (*22*) and similar to that of Cliffe et al. (*23*). In short, berry extracts and standards were diluted with 2.5 mL of reagent water (1:10), and 0.5 mL of the Folin−Ciocalteu stock reagent was added with mixing. After 3 min, 1.0 mL of Na_2CO_3 reagent (75 g/L) was added with mixing, and the samples were incubated at room temperature for a total of 30 min. The absorbance was read at 765 nm using a Biomate 3 spectrophotometer (ThermoSpectronic, Rochester, NY). TPH results are expressed in milligrams of gallic acid equivalents per gram of frozen fruit (mg of GAE/g of FW).

Total Anthocyanins. ACY was determined according to the pH differential method similar to that of Moyer et al. (*13*). Berry extracts

were diluted 1:20 in pH 1.0 and 4.5 oxalic acid buffers and then measured at 520 and 700 nm using a Biomate 3 spectrophotometer. ACY was based on a cyanidin 3-glucoside (C3G) molar extinction coefficient of 29600 and a molecular weight of 449.2, and results were expressed as milligrams of C3G per gram of frozen fruit (mg of C3G/g of FW).

Antioxidant Capacity. Antioxidant capacity was measured using the oxygen radical absorbance capacity (ORAC) and ferric reducing ability of plasma (FRAP) assays as performed by the Linus Pauling Institute, Oregon State University. The ORAC assay was performed as described by Cao et al. (*24*) and adapted for use in a 96-well microplate fluorometer (model Cytofluor 4000, PerSeptive Biosystems, Framingham, MA). The FRAP assay (*25*) was adapted for use in a 96-well microplate spectrophotometer (ThermoMax, Molecular Devices, Foster City, CA). ORAC and FRAP assays were performed on representative frozen extracts and results are expressed as micromoles of Trolox equivalents per gram of fresh fruit (μmol of TE/g of FW).

Extraction and Analysis of Flavan-3-ols and Phenolic Acids. The acetone/water berry extracts, as described above, were analyzed before and after a hydrolysis step, described below, to determine the profile of flavonols and phenolic acids. Berry extracts and controls were subjected to an acid hydrolysis similar to that of Häkkinen et al. (*26*) by transferring 1.8 mL of berry extract into 13 × 100 mm borosilicate test tubes followed by the addition of 0.2 mL of 6 M HCl (final concentration of 0.6 M). The tubes were mixed, sealed with Parafilm, and heated to 85 °C for 30 min using an AIM500 digestion block (A. I. Scientific, Scarborough, Australia). Samples were transferred to amber autosampler vials for HPLC-MSD analysis.

Berry extracts were quantified using methods adapted from Baba et al. (*27*) and Arts and Hollman (*28*) to screen for flavan-3-ol and phenolic acid analytes. The HPLC apparatus consisted of an Agilent 1100 series pumping system, autosampler, and column oven, equipped with a Waters XTerra MS C_{18} column (3.9 × 150 mm, 5 μm) and guard column (3.9 × 20 mm, 5 μm). This HPLC system was connected to an Agilent G1946D SL model MSD. Samples and standards were injected (50 μL) and subjected to a gradient elution program, which consisted of 0.1% acetic acid in water (solution A) and acetonitrile/methanol (1:1; solution B) as follows: starting conditions 90% A/10% B, 20 min linear gradient to 10% A/90% B, column wash 10% A/90% B for 5 min, and 9 min equilibration prior to the next injection. Flow rate was 0.75 mL/min and the column temperature, 30 °C. The conditions for electrospray ionization in positive mode were as follows: capillary voltage, 3500 V; fragmentor, 100 V; nebulizing pressure, 30 psi; drying gas temperature, 350 °C; and flow, 13 L/min. The analytes were detected using selective ion monitoring (SIM) for the M + H adduct of the species, and the SIM *m*/*z* ratios as well as retention times were as follows: catechn, *m*/*z* 291 and 6.44 min; chlorogenic acid, *m*/*z* 355 and 6.55 min; *p*-hydroxybenzoic acid, *m*/*z* 139 and 6.91 min; caffeic acid, *m*/*z* 181 and 7.53 min; epicatechin, *m*/*z* 291 and 7.56 min; *p*-coumaric acid, *m*/*z* 165 and 9.19 min; and ferulic acid, *m*/*z* 195 and 9.62 min. Data were collected and analyzed on a Hewlett-Packard Vectra equipped with a Chemstation data handling system. Samples that contained high levels of chlorogenic acid were also analyzed by HPLC-DAD using the same chromatographic conditions and a wavelength of 320 nm.

Extraction and Analysis of Flavonols and Anthocyanins. Berry samples were extracted using a modification of the methods of Hertog et al. (*29*) and Nyman and Kumpulainen (30) that are described in the following. Frozen ground berries (5 g) were weighed into 50 mL Pyrex test tubes, and 20 mL of 62.5% methanol with DETC at 2 mg/mL (as an antioxidant) was added. The samples were then further ground for 1 min at room temperature with a Polytron homogenizer, and 5 mL of 6 N HCl was added after macerating. Samples were capped and refluxed at 90 °C for 90 min with regular mixing on the AIM500 digestion block. Hydrolyzed samples were allowed to cool and then brought up to 50 mL final volume with water. Samples (2 mL) were syringe filtered (0.45 μm nylon, Titan) into amber autosampler vials, isorhamnetin was added as an internal standard, and samples were analyzed by HPLC-DAD within 24 h.

Berry extracts were quantified using methods adapted from published techniques (*30*, *31*) to screen for flavonol and anthocyanin aglycons. The HPLC apparatus consisted of a Hewlett-Packard 1090 series II pumping system, an autosampler, an internal DAD, and a column oven equipped with a Waters XTerra MS C_{18} (3.9 × 150 mm, 5 μm) and guard column (3.9 × 20 mm, 5 μm). Flavonol components (quercetin, myricetin, and kaempferol) were identified and quantified with calibration standards, whereas only two anthocyanidins, dephinidin and cyanidin, were identified and quantified using calibration standards. We identified and quantified malividin with its calibration standard and determined that the response factors were similar to those of cyanidin, but the standard solution degraded markedly over the course of 1 week. The remaining anthocyanidins (peonidin and petunidin) were identified according to the procedure of Nyman and Kumpulainen (30) and quantified using cyanidin response factors. Samples and standards were injected (20 μL) and subjected to a gradient elution program, which consisted of 0.05% TFA in water at pH 2 (solution A) and 100% acetonitrile (solution B) as follows: starting conditions, 95% A/5% B for 2.0 min; 19 min linear gradient from 95% A/5% B to 50% A/50% B; column wash with a 2 min linear gradient to 10% A/90% B; held for 6 min; followed by an 8 min equilibration prior to the next injection. Flow rate was 1.0 mL/min, and the column temperature was 40 °C. The DAD was set to monitor 510 nm for the anthocyanidins and 365 nm for the flavonol aglycons.

RESULTS

Total Phenolics. The intra- and interspecies variations for the TPH results are presented in **Table 2** and ranged from 0.81 to 2.84 mg of GAE/g of FW. The red huckleberry (VAPA) exhibited the lowest TPH level (0.81 mg of GAE/g of FW), whereas the evergreen huckleberry (VAOV) and oval-leaf blueberry (VAOF) contained the highest levels of TPH (2.84 and 2.81 mg of GAE/g of FW, respectively). The two cultivated blueberry species exhibited similar TPH contents ranging from 1.54 to 1.15 mg of GAE/g of FW for half-highbush (VAAC) and highbush (VACM) blueberry species, respectively. The remaining four species exhibited similar levels of TPH ranging from 1.39 mg of GAE/g of FW for the wild cranberry (VAOX) to 1.7 mg of GAE/g of FW for the black huckleberry (VAME).

Total Anthocyanins. The ACY contents of the *Vaccinium* berries studied (**Table 2**) showed similar species differences when compared to the TPH levels. The red huckleberry and wild cranberry exhibited the lowest levels of ACY (0.11 and 0.31 mg of C3G/g of FW, respectively) as may be predicted by their redder pigmentation. The evergreen huckleberry and oval-leaf blueberry that contained the highest TPH contents also exhibited the greatest ACY levels with respect to all of the species studied (3.64 and 3.07 mg of C3G/g of FW, respectively). Similar to the TPH results, the other five species studied contained similar levels of ACY ranging from 0.82 to 1.13 mg of C3G/g of FW for the two cultivated blueberry species (VACM and VAAC) and from 1.24 to 1.69 mg of C3G/g of FW for the three remaining wild species (VADE, VAME, and VAUG).

Antioxidant Capacity. The relative antioxidant capacity as measured by the ORAC and FRAP assays mirrors the TPH and ACY intra- and interspecies differences discussed above and presented in **Table 2**. Total antioxidant capacity as measured by ORAC and expressed in Trolox equivalents (TE) ranged from a low of 7.3 μmol of TE/g of FW for red huckleberry to a high of 41.1 μmol of TE/g of FW for the evergreen huckleberry, whereas the FRAP values ranged from a low of 10 μmol of TE/g of FW to a high of 76 μmol of TE/g of FW for these two species, respectively.

Flavan-3-ol and Flavonol Content. The HPLC analytical results for the presence and quantity of catechin and epicatechin in the nine *Vaccinium* species are presented in **Table 3**. Three species of berries, black huckleberry, wild cranberry, and red

Table 2. Total Phenolics (TPH), Anthocyanins (ACY), Oxygen Radical Absorbance Capacity (ORAC), and Ferric Reducing Ability of Plasma (FRAP) Contents in Nine *Vaccinium* Species

cultivar or designation	TPH[a] (mg of GAE/g)	ACY[b] (mg of C3G/g)	ORAC[c] (μmol of TE/g)	FRAP[c] (μmol of TE/g)
***V. angustifolium* × *corymbosum* (VAAC)**				
Northblue	1.43	1.19	26.0	26.1
Northcountry	1.75	1.31	34.2	39.9
Northsky	1.44	0.89	31.3	30.5
mean ± SEM	**1.54 ± 0.11**	**1.13 ± 0.12**	**30.5 ± 2.4**	**32.2 ± 4.1**
***V. corymbosum* (VACM)**				
Bluecrop	1.18	0.74	22.1	20.2
Bluejay	1.20	0.88	20.7	25.5
Jersey	1.06	0.83	21.5	18.9
mean ± SEM	**1.15 ± 0.04**	**0.82 ± 0.04**	**21.4 ± 0.4**	**21.5 ± 2.0**
***V. deliciosum* (VADE)**				
VADE 019	1.43	1.34	13.3	30.2
VADE 021	1.40	1.37	15.8	30.1
mean ± SEM	**1.41 ± 0.01**	**1.35 ± 0.01**	**14.6 ± 1.2**	**30.2 ± 0.1**
***V. membranaceum* (VAME)**				
VAME 098	1.63	1.59	20.5	37.9
VAME 103	1.72	1.82	23.7	40.9
VAME 106	1.76	1.66	18.8	42.7
mean ± SEM	**1.70 ± 0.04**	**1.69 ± 0.07**	**21.0 ± 1.5**	**40.5 ± 1.4**
***V. ovalifolium* (VAOF)**				
VAOF 091	2.65	2.40	37.1	67.5
VAOF 093	3.19	3.95	47.1	95.1
VAOF 102	2.61	2.85	29.3	66.0
mean ± SEM	**2.81 ± 0.19**	**3.07 ± 0.46**	**37.8 ± 5.2**	**76.2 ± 9.5**
***V. ovatum* (VAOV)**				
VAOV 006	2.83	3.79	42.9	68.3
VAOV 007	2.85	3.49	39.2	72.2
mean ± SEM	**2.84 ± 0.10**	**3.64 ± 0.15**	**41.1 ± 1.9**	**70.2 ± 2.0**
***V. oxycoccus* (VAOX)**				
VAOX 001	1.44	0.31	14.2	27.7
VAOX 002	1.35	0.31	12.7	23.9
mean ± SEM	**1.39 ± 0.05**	**0.31 ± 0.01**	**13.5 ± 0.7**	**25.8 ± 1.9**
***V. parvifolium* (VAPA)**				
VAPA 004	0.93	0.12	11.0	12.8
VAPA 006	0.74	0.10	5.8	8.0
VAPA 010	0.76	0.10	5.0	9.4
mean ± SEM	**0.81 ± 0.06**	**0.11 ± 0.01**	**7.3 ± 1.9**	**10.0 ± 1.5**
***V. uliginosum* (VAUG)**				
VAUG 016	1.51	0.79	25.0	24.0
VAUG 017	1.71	1.70	33.6	28.2
mean ± SEM	**1.61 ± 0.10**	**1.24 ± 0.05**	**29.3 ± 4.3**	**26.1 ± 2.1**

[a] TPH results are expressed in gallic acid equivalents (GAE). [b] ACY results are expressed in cyanidin-3-glucose equivalents (C3G). [c] ORAC and FRAP results are expressed in Trolox equivalents (TE).

Table 3. Flavan-3-ol and Flavonol Content in Nine *Vaccinium* Species

cultivar or designation	μg/g of FW			
	catechin	epicatechin	myricetin	quercetin
***V. angustifolium* × *corymbosum* (VAAC)**				
Northblue	16.8	10.7	23.2	149.6
Northcountry	42.4	22.0	22.0	86.5
Northsky	22.5	11.0	14.1	71.3
mean ± SEM	**27.3 ± 7.8**	**14.6 ± 3.7**	**19.8 ± 2.9**	**102.5 ± 24.0**
***V. corymbosum* (VACM)**				
Bluecrop	58.3	22.6	14.7	81.0
Bluejay	45.9	25.2	13.9	86.9
Jersey	7.8	0.5	10.1	91.3
mean ± SEM	**37.3 ± 15.2**	**15.9 ± 8.0**	**12.9 ± 1.4**	**86.4 ± 3.0**
***V. deliciosum* (VADE)**				
VADE 019	<0.5	109.9	13.7	33.2
VADE 021	< 0.5	108.2	14.9	35.0
mean ± SEM	< 0.5	**109.1 ± 0.9**	**14.3 ± 0.6**	**34.1 ± 0.9**
***V. membranaceum* (VAME)**				
VAME 098	72.4	197.6	14.2	34.1
VAME 103	64.6	139.7	16.0	68.7
VAME 106	56.9	189.2	16.4	70.4
mean ± SEM	**64.6 ± 4.5**	**175.5 ± 18.1**	**15.5 ± 0.7**	**57.7 ± 11.8**
***V. ovalifolium* (VAOF)**				
VAOF 091	18.5	115.2	23.3	49.5
VAOF 093	6.3	60.3	37.3	47.8
VAOF 102	9.0	104.4	25.6	37.4
mean ± SEM	**11.3 ± 3.7**	**93.3 ± 16.8**	**28.7 ± 4.3**	**44.9 ± 3.8**
***V. ovatum* (VAOV)**				
VAOV 006	<0.5	76.1	10.0	82.9
VAOV 007	<0.5	62.3	10.6	85.3
mean ± SEM	<0.5	**69.2 ± 6.9**	**10.3 ± 0.3**	**84.1 ± 1.2**
***V. oxycoccus* (VAOX)**				
VAOX 001	82.3	119.4	30.5	218.7
VAOX 002	64.7	85.9	32.0	233.0
mean ± SEM	**73.5 ± 8.8**	**102.6 ± 16.7**	**31.3 ± 0.8**	**225.9 ± 7.2**
***V. parvifolium* (VAPA)**				
VAPA 004	40.2	169.2	<1.0	17.6
VAPA 006	30.2	114.3	<1.0	4.5
VAPA 010	12.5	96.2	<1.0	5.9
mean ± SEM	**27.6 ± 8.1**	**126.5 ± 21.9**	<1.0	**9.3 ± 4.2**
***V. uliginosum* (VAUG)**				
VAUG 016	18.8	82.9	210.1	188.0
VAUG 017	4.2	46.2	190.0	139.1
mean ± SEM	**11.5 ± 7.3**	**64.6 ± 18.4**	**200.1 ±10.1**	**163.6 ± 24.5**

huckleberry, contained by far the greatest concentration of catechin and epicatechin combined (catechins > 150 μg/g of FW). The black huckleberry had by far the highest level of catechins, 240 μg/g of FW, followed by wild cranberry and red huckleberry that contained 176 and 154 μg/g of FW, respectively. The predominant flavan-3-ol present in all of the species studied was epicatechin, and in two species, the evergreen and cascade huckleberries, catechin was not detected (<5 μg/g of FW).

The HPLC analytical results for the presence and quantity of the flavonols, quercetin and myricetin, are also presented in **Table 3**. The nine berry species were screened for kaempferol as part of the flavonol analysis, but no detectable quantities were present (<5 μg/g of FW). The predominant flavonol present in the *Vaccinium* species was quercetin. Two species of berry, the alpine bilberry followed by wild cranberry, had markedly higher levels of flavonols when compared to the other seven species. The wild cranberry contained the highest levels of quercetin (225.9 μg/g of FW), whereas the alpine bilberry also contained a significant amount of quercetin (163.6 μg/g of FW). Interestingly, the predominant flavonol present in the alpine bilberry species was myricetin (200.1 μg/g of FW) at levels nearly 5−10 times the amounts detected in the other *Vaccinium* berries. The remaining seven species all contained similar flavonol amounts ranging from 12.9 to 28.7 μg/g of FW for myricetin and from 34.1 to 102.5 μg/g of FW for quercetin with one exception. The red huckleberry contained the least amount of quercetin (9.3 μg/g of FW) and no detectable myricetin (<5.0 μg/g of FW).

Phenolic Acid Content. The HPLC analytical results for the presence and quantity of phenolic acids in berries are illustrated in **Table 4**. The most significant finding with respect to the phenolic acid contents of *Vaccinium* species was the quantity of the 5-*O*-caffeoyl ester of quinic acid, chlorogenic acid. The domesticated half-highbush and highbush blueberry species contained by far the highest levels of chlorogenic acid, exhibiting on average 1414 and 1261 μg/g of FW, respectively. The evergreen huckleberry also contained a significant amount of chlorogenic acid (466 μg/g of FW), whereas in the remaining six species of berries this compound was either found at <75 μg/g of FW or not present (<5 μg/g of FW). The red huckleberry contained a substantial amount benzoic acid in the form of *p*-hydroxybenzoic acid (553 μg/g of FW) compared to <15 μg/g of FW in the other eight species. The three remaining

Table 4. Phenolic Acid Content of Nine *Vaccinium* Species

cultivar or designation	μg/g of FW				
	caffeic	chlorogenic	*p*-coumaric	ferulic	*p*-hydroxybenzoic
			***V. angustifolium* × *corymbosum* (VAAC)**		
Northblue	186	1304	5.2	53.5	<1.0
Northcountry	139	1417	3.0	42.2	<1.0
Northsky	146	1521	5.2	52.0	<1.0
mean ± SEM	**157 ± 14.6**	**1414 ± 62.6**	**4.5 ± 0.8**	**49.3 ± 3.5**	<1.0
			***V. corymbosum* (VACM)**		
Bluecrop	223	1576	5.3	50.5	<1.0
Bluejay	172	1226	3.4	49.1	<1.0
Jersey	152	981	4.8	32.1	<1.0
mean ± SEM	**182 ± 21.2**	**1261 ± 173**	**4.5 ± 0.6**	**43.9 ± 5.9**	<1.0
			***V. deliciosum* (VADE)**		
VADE 019	36.4	67.6	29.0	27.2	5.9
VADE 021	46.2	57.5	4.3	18.0	8.0
mean ± SEM	**41.3 ± 4.9**	**72.4 ± 2.4**	**16.6 ± 12.3**	**22.6 ± 4.6**	**6.9 ± 1.1**
			***V. membranaceum* (VAME)**		
VAME 098	24.6	73.9	20.5	17.0	1.0
VAME 103	15.2	75.6	20.0	27.0	1.8
VAME 106	13.1	67.7	22.9	21.0	1.8
mean ± SEM	**17.6 ± 3.5**	**62.6 ± 5.1**	**21.1 ± 0.9**	**21.7 ± 2.9**	**1.5 ± 0.3**
			***V. ovalifolium* (VAOF)**		
VAOF 091	2.7	<1.0	15.1	18.6	1.0
VAOF 093	8.7	<1.0	24.4	19.9	2.8
VAOF 102	4.0	<1.0	32.3	15.4	1.1
mean ± SEM	**5.1 ± 1.8**	<1.0	**23.9 ± 5.0**	**17.9 ± 1.3**	**1.6 ± 0.6**
			***V. ovatum* (VAOV)**		
VAOV 006	4.5	465	29.3	118	11.6
VAOV 007	7.0	467	35.5	99.6	12.7
mean ± SEM	**5.8 ± 1.3**	**466 ± 0.8**	**32.4 ± 3.1**	**109 ± 9.4**	**12.1 ± 0.6**
			***V. oxycoccus* (VAOX)**		
VAOX 001	14.8	48.2	97.5	48.2	8.9
VAOX 002	10.5	53.6	105	69.3	6.7
mean ± SEM	**12.7 ± 2.1**	**50.9 ± 2.7**	**101 ± 3.7**	**58.7 ± 10.5**	**7.8 ± 1.1**
			***V. parvifolium* (VAPA)**		
VAPA 004	151	77.3	112	43.2	687
VAPA 006	143	53.2	75.6	36.1	407
VAPA 010	155	50.1	104	36.2	565
mean ± SEM	**150 ± 3.3**	**60.2 ± 8.6**	**97.3 ± 11.1**	**38.5 ± 2.4**	**553 ± 81.2**
			***V. uliginosum* (VAUG)**		
VAUG 016	163	<1.0	73.8	63.2	10.5
VAUG 017	122	<1.0	83.9	74.9	7.4
mean ± SEM	**142 ± 20.5**	<1.0	**78.9 ± 5.0**	**69.1 ± 5.8**	**8.9 ± 1.6**

phenolic acids that were investigated, caffeic, *p*-coumaric, and ferulic, were present at levels ranging from 150 to 5 μg/g of FW but did not exhibit the substantial quantities or differences when compared to the levels of chlorogenic and *p*-hydroxybenzoic acid just discussed.

Anthocyanidin Content. The HPLC analytical results for the presence and quantity of anthocyanidins in berries are illustrated in **Table 5**. The oval-leaf blueberry and the evergreen huckleberry exhibited by far the highest level of total anthocyanidins, whereas the cranberry and red huckleberry exhibited the least amount. These results are in very good agreement with the levels and trends already established with the spectrophotometric ACY results. The berry species that exhibited the highest levels of ACY (cascade, black, and evergreen huckleberries as well as the oval-leaf blueberry) typically contained cyanidin and/or delphinidin as the primary anthocyanidin(s). These berry species also contained, to a lesser extent, malvidin, peonidin, and petunidin. The half-high and highbush blueberries contained primarily delphinidin and malvidin as the primary anthocyanidin constituents followed by equal amounts of cyanidin and petunidin as well as very low (half-highbush) or undetectable (highbush) amounts of peonidin. The alpine bilberry was similar to the half-highbush blueberry species in that delphinidin was the primary anthocyanidin present, with decreasing amounts of cyanidin, malvidin, and petunidin and again very low amounts of peonidin. The remaining two species, wild cranberry and red huckleberry, both contained cyanidin as the primary anthocyanidin with either low amounts of delphinidin and peonidin (cranberry) or small amounts of malvidin and peonidin (red huckleberry). These two berry species also contained only three of the five anthocyanidins present in the other *Vaccinium* species.

DISCUSSION

There are marked inter- and intraspecies differences between berry groups in TPH, ACY, and antioxidant capacities (**Table 2**). The *Vaccinium* species differences in TPH, ACY, and antioxidant capacities are similar to what has been previously reported (*13*, *14*) in that the relative phytochemical contents are well correlated to the antiradical measures ($P < 0.005$: TPH versus ORAC $r^2 = 0.84$ or TPH versus FRAP $r^2 = 0.93$ and ACY versus ORAC $r^2 = 0.73$ or ACY versus FRAP $r^2 = 0.90$). Therefore, it is evident that the two *Vaccinium* species from wild populations, oval-leaf blueberry and evergreen huckleberry, that contained the highest TPH and ACY levels would also exhibit the greatest antiradical behavior. Conversely, the red huckleberry exhibited the lowest levels of TPH, ACY, and antiradical capacity.

Intraspecies differences in ACY and TPH contents among the wild populations of *Vaccinium* berries may be related to differences in the genetic diversity of the sampling areas and

Table 5. Anthocyanidin Content of Nine *Vaccinium* Species

cultivar or designation	μg/g of FW cyanidin	delphinidin	malvidin[a]	peonidin[a]	petunidin[a]
***V. angustifolium* × *corymbosum* (VAAC)**					
Northblue	134	341	232	24.4	145
Northcountry	112	256	129	26.7	91.1
Northsky	103	193	123	24.0	80.4
mean (%)[b]	**116 (17)**	**263 (39)**	**161 (24)**	**25.0 (4)**	**106 (16)**
***V. corymbosum* (VACM)**					
Bluecrop	54.1	174	131	<10.0	68.5
Bluejay	56.4	161	140	<10.0	84.3
Jersey	63.7	177	62.1	< 10.0	65.1
mean (%)[b]	**58.1 (14)**	**171 (41)**	**111 (27)**	**<10.0**	**72.6 (17)**
***V. deliciosum* (VADE)**					
VADE 019	267	414	143	56.5	123
VADE 021	329	432	76.5	38.1	113
mean (%)[b]	**298 (30)**	**423 (42)**	**110 (11)**	**47.3 (5)**	**118 (12)**
***V. membranaceum* (VAME)**					
VAME 098	503	413	84.3	57.7	109
VAME 103	595	416	140	122	123
VAME 106	622	432	83.9	71.7	113
mean (%)[b]	**573 (44)**	**420 (32)**	**103 (8)**	**83.8 (7)**	**115 (8)**
***V. ovalifolium* (VAOF)**					
VAOF 091	886	488	109	154	123
VAOF 093	1102	1052	195	151	231
VAOF 102	874	748	145	110	167
mean (%)[b]	**954 (44)**	**763 (35)**	**150 (7)**	**138 (6)**	**174 (8)**
***V. ovatum* (VAOV)**					
VAOV 006	1227	371	64.6	92.2	74.4
VAOV 007	1314	323	63.3	101	68.8
mean (%)[b]	**1271 (69)**	**347 (19)**	**64.0 (3)**	**96.6 (5)**	**71.6 (4)**
***V. oxycoccus* (VAOX)**					
VAOX 001	175	51.4	<10.0	67.2	<10.0
VAOX 002	175	54.0	< 10.0	63.9	< 10.0
mean (%)[b]	**175 (60)**	**52.7 (18)**	**<10.0**	**65.6 (22)**	**<10.0**
***V. parvifolium* (VAPA)**					
VAPA 004	82.8	<10.0	10.2	11.3	<10.0
VAPA 006	64.6	<10.0	10.4	11.6	<10.0
VAPA 010	67.0	< 10.0	10.2	11.3	< 10.0
mean (%)[b]	**71.5 (77%)**	**<10.0**	**10.3 (11)**	**11.4 (12)**	**<10.0**
***V. uliginosum* (VAUG)**					
VAUG 016	98.6	225	64.8	9.5	53.2
VAUG 017	207	505	160	41.9	114
mean (%)[b]	**153 (21)**	**365 (49)**	**112 (15)**	**25.7 (3)**	**83.6 (11)**

[a] Malvidin, peonidin, and petunidin are all expressed as μg of cyanidin equivalents/g of FW. [b] (%) = relative percentage with respect to the total anthocyanidins.

horticultural or environmental effects. It was noted that some berry samplings from different northwestern national forest sites showed considerable variations in the ACY contents. For example, the alpine bilberry samples were collected within a small area (several miles apart) within one national forest in Wyoming (**Table 1**). When these two samplings are compared, it becomes evident that one population had higher levels of ACY and antioxidant capacity (**Table 2**). This difference may be attributed to differences in the genetic makeup of the two populations (site VAUG 017 displayed marked genotypic diversity, whereas site VAUG 016 appeared to be qenetically homogeneous) and/or differences in the ripeness of the berries on the collection day, suggesting a horticultural effect (D. Barney, personal communication). Furthermore, the Kaniksu National Forest (VAOF 91) and Gifford Pinchot National Forest (VAOF 102) samplings of the oval-leaf blueberry (VAOF) exhibit reasonably similar results, whereas the Wenatchee National Forest (VAOF 093) collection contains higher levels of TPH, ACY, and antiradical capacity (**Table 2**). The basis for this intraspecies difference is not known, but may also stem from either genetic or environmental factors. In contrast to these two intraspecies differences, the other wild populations of *Vaccinium* species show relatively small degrees of variability in cases where there were significant distances between the sampling sites (VAME and VADE) or within smaller sampling areas (VAOV, VAOX, and VAPA). Future research and a more extensive multiyear sampling effort will be necessary to elucidate the significance of these inter- and intraspecies differences.

The primary focus of this investigation was to compare TPH, ACY, and antioxidant capacity within the nine species studied to determine the scope and magnitude of the differences. The species differences illustrated in the results (**Table 2**) are similar to those of Moyer et al. (*13*). They also evaluated the TPH, ACY, and antioxidant capacity of *V. membranaceum*, *V. ovalifolium*, *V. ovatum*, and *V. parvifolium* as well as 15 cultivars of *V. corymbosum*. The ACY results presented here and those within that study are quite similar. The TPH and antioxidant capacities (ORAC and FRAP) presented in the current study follow a similar pattern with respect to species differences reported by Moyer and associates (*13*), but the actual values reported herein are lower. This difference can be partially accounted for by the recovery efficiency of the extraction and analysis methods. Quality control samples were analyzed concurrently with berry samples, and matrix spike recoveries were documented for the TPH and antioxidant capacity assays. Typically, spike recoveries and thus method performance were on the order of 75%. It did not appear to be prudent to adjust the analytical results, considering the primary focus of this investigation was to characterize and compare the nine *Vaccinium* species. Although, if our results were adjusted for method performance, the antioxidant capacities would compare reasonably well with those of Moyer's (*13*), whereas our TPH values would still be lower. Overall, the data presented herein are reasonable when compared to the wide range of values for TPH, ACY, and antioxidant capacity established in the literature by various research groups (*13*, *14*, *17*, *20*, *32*, *33*).

An important question that arises from the preliminary screening of the *Vaccinium* berries relates directly to what are the species' polyphenolic profiles and which component(s) within these profiles may contribute the most to the differences in the bioactive potential. The second area of our investigation focused specifically on the polyphenolic profiles of the berry species and illustrates the differences in the contents of flavan-3-ols, flavonols, phenolic acids, and anthocyanins (**Tables 3**−**5**). Our compositional profiles and species differences are in good agreement with earlier reports for blueberry, cranberry, and alpine bilberry (*16*, *21*, *35*−*37*). The major differences in the total polyphenolic contents (**Tables 3**−**5**), and the species distributions of the components become quite evident when plotted together to illustrate the "polyphenolic profile" (**Figure 1**). The cascade, black, and evergreen huckleberries as well as the oval-leaf blueberry all appear to contain the majority of their antioxidant components as anthocyanins (65−86% of their polyphenolic profile). Conversely, the two domestic blueberry species contain a high percentage of chlorogenic acid (57−61%, respectively). The red huckleberry also contains a high percentage of phenolic acids (78%), but the predominant phenolic acid is *p*-hydroxybenzoic acid (48%). The two remaining *Vaccinium* species contain a more equal distribution of the flavanoid and phenolic acid components. The wild cranberry contains nearly equal amounts of the four polyphenolic groups and lacked a predominant component. The alpine bilberry exhibits an intermediate profile between the wild cranberry and other species in that it contained 50% anthocyanins, 20% phenolic acids, and 25% flavonols, respectively. These differences in the phy-

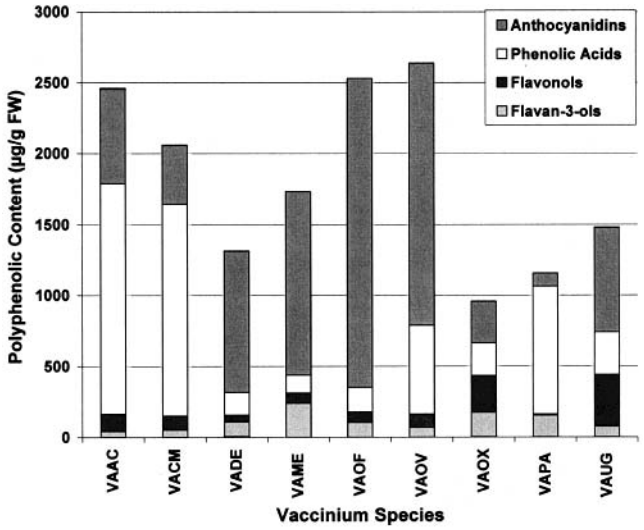

Figure 1. Contribution of anthocyanidins, phenolic acids, flavonols, and flavan-3-ols to the total polyphenolic profile in nine *Vaccinium* species.

tochemical distribution may prove to be important with respect to the nutritional health benefits of the berry species.

This concept of polyphenolic profiling is gaining in popularity for the analysis of berries that exhibit high antioxidant capacity because of the valuable information obtained (*16*, *21*, *34*−*36*). One specific investigation evaluated the antioxidant activity of berry phenolic extracts (blackberry, blueberry, red raspberry, strawberry, and sweet cherry) using two different copper-catalyzed in vitro assays—oxidation of human low-density lipoproteins (LDL), which is an early event in coronary disease, and liposome oxidation, which is relevant to oxidation in food systems (*34*). In the liposome model, the hydroxycinnamic acids that are present in higher concentrations in cherries and blueberries were shown to have the greatest activity. Furthermore, these cherry extracts, although they ranked the lowest in total polyphenolic content, exhibited the highest inhibition of oxidation. In the LDL model, blackberry extracts had the highest anthocyanin content and showed the greatest inhibition of oxidation followed by red raspberry extracts (third highest anthocyanins). The blueberry extracts that were second highest in anthocyanins were ranked second to last with this model. This result is confusing when it was assumed that the anthocyanin content is the most important component with respect to this model, although this order of activity becomes significant when the content of cyanidin glycosides that are the predominant anthocyanins in blackberries and red raspberries (*37*) is evaluated with respect to the LDL model (blueberries are high in delphinidin and malvidin; **Table 5**). From a review of this study, it becomes apparent that a berry's bioactive characteristics as measured using in vitro assays can be directly related to its specific polyphenolic profile.

The content and profile of anthocyanins present in fruits and berries, as alluded to above, have become important topics because of their potent antiradical behavior. The relevance of whether specific anthocyanins or anthocyanin profiles are predominant in health-promoting foods such as red wines (*30*), grapes (*38*), and berries (*16*, *39*) has become an important research question. Other areas that are being investigated surround whether the specific anthocyanin or other flavanoid components of foods are biologically available (*40*, *41*). What are the effects of jam and juice processing on the abundance and activity of the polyphenolic profiles (*42*, *43*)? What are the most potent forms of these bioactive compounds with respect to in vitro test systems (*5*, *6*, *44*) and in vivo models (*11*)? What foods are consumed in significant enough quantities to elevate the in vivo bioactive profile to produce a biological effect and provide a nutritional benefit? These important questions are being investigated by various research groups and will begin to provide the answers necessary to determine the relative significance of these polyphenolic profiles present in different fruits and vegetables.

In summary, flavanoid and phenolic acid component analysis produced a comprehensive illustration of the polyphenolic profiles present in the *Vaccinium* species studied. This phytochemical profiling of berries and other foods represents a semicomprehensive analysis of the antiradical components contained within and thus may lead to a better understanding of their nutritional health benefits. The diversity in the polyphenolic profiles between different berry species may relate to significantly different biological availability and activity relationships associated with reducing the risk of heart disease, specific forms of cancer, and other chronic diseases. These apparent health-promoting characteristics associated with the short- and long-term consumption of whole berries have not been extensively investigated and will be the necessary focus of future research.

ABBREVIATIONS USED

ACY, total anthocyanins; C3G, cyanidin 3-glucoside; DAD, diode array detector; DETC, diethyl dithiocarbamic acid; FRAP, ferric reducing ability of plasma; FW, fresh-frozen weight; GAE, gallic acid equivalents; HPLC, high-pressure liquid chromatograph; LDL, low-density lipoprotein; MSD, mass spectrometric detector; ORAC, oxygen radical absorbance capacity; SIM, selective ion monitoring; TE, Trolox equivalents; TFA, trifluoroacetic acid; TPH, total phenolics; VAAC, *Vaccinium angustifolium* × *corymbosum*; VACM, *Vaccinium corymbosum*; VADE, *Vaccinium deliciosum*; VAME, *Vaccinium membranaceum*; VAOF, *Vaccinium ovalifolium*; VAOV, *Vaccinium ovatum*; VAOX, *Vaccinium oxycoccus*; VAPA, *Vaccinium parvifolium*; VAUG, *Vaccinium uliginosum*.

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Received for review November 12, 2003. Revised manuscript received March 10, 2004. Accepted March 12, 2004. This work was supported by USDA-CSREES Initiative for Future Agriculture and Food Systems Program Bridge Grant 2001-04558.

JF0307595