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Comparison of Anthocyanin Pigment and Other Phenolic Compounds of *Vaccinium membranaceum* and *Vaccinium ovatum* Native to the Pacific Northwest of North America

JUNGMIN LEE,[†] CHAD E. FINN,[§] AND RONALD E. WROLSTAD*,[†]

Department of Food Science, Oregon State University, Corvallis, Oregon 97331, and Northwest Center for Small Fruit Research, U.S. Department of Agriculture—Agricultural Research Service, HCRL, 3420 NW Orchard Avenue, Corvallis, Oregon 97330

Two huckleberry species, *Vaccinium membranaceum* and *Vaccinium ovatum*, native to Pacific Northwestern North America, were evaluated for their total, and individual, anthocyanin and polyphenolic compositions. *Vaccinium ovatum* had greater total anthocyanin (ACY), total phenolics (TP), oxygen radical absorbing capacity (ORAC), and ferric reducing antioxidant potential (FRAP) than did *V. membranaceum*. The pH and °Brix were also higher in *V. ovatum*. Berry extracts from each species were separated into three different fractions—anthocyanin, polyphenolic, and sugar/acid—by solid-phase extraction. The anthocyanin fractions of each species had the highest amount of ACY, TP, and antioxidant activity. Each species contained 15 anthocyanins (galactoside, glucoside, and arabinoside of delphinidin, cyanidin, petunidin, peonidin, and malvidin) but in different proportions. Their anthocyanin profiles were similar by high-performance liquid chromatography with photodiode array detection (LC-DAD) and high-performance liquid chromatography with photodiode array and mass spectrometry detections (LC-DAD-MS). Each species had a different polyphenolic profile. The polyphenolics of both species were mainly composed of cinnamic acid derivatives and flavonol glycosides. The major polyphenolic compound in *V. membranaceum* was neochlorogenic acid, and in *V. ovatum*, chlorogenic acid.

KEYWORDS: ***Vaccinium*; huckleberry; anthocyanins; phenolics; antioxidant activity**

INTRODUCTION

Anthocyanins and polyphenolics are secondary metabolites of plants. They are a diverse group (>4000 flavonoids have been identified). These compounds contribute to food quality through appearance, taste, and health benefits (*1*). The health benefits of blueberries have been linked to their anthocyanin and phenolic contents (*2*).

Research in blueberries (*Vaccinium corymbosum* L.) has generated interest in other native *Vaccinium* sp., particularly the *Vaccinium* sp. called "huckleberries" and "bilberries", which have been shown to be high in anthocyanins and other phenolics compared to highbush blueberries (*3*). *Vaccinium myrtillus* L. (bilberry) fruit has one of the highest anthocyanin contents of berries examined (*4*). *Vaccinium ovatum* Pursh from section Pyxothamnus and *Vaccinium membranaceum* Douglas ex Torrey from section Myrtillus were examined in this study. *Vaccinium ovatum* (evergreen huckleberry) is the dominant huckleberry along the Pacific Coast of North America and is closely related to *Vaccinium consanguineum* Klotsch, native to Central America, and *Vaccinium floribundum* Kunth, native to Andean S. America. *Vaccinium floribundum* is wild-harvested in Andean countries and sold locally as fresh or processed fruit (*5*; Finn, personal communication). *Vaccinium membranaceum* (black huckleberry) grows in forested mountain areas of Washington, Oregon, and Idaho (*6*).

Vaccinium membranaceum has been an important part of Native Americans' diet, and limited amounts of their fruits are commercially available as canned or frozen berries and as preserves (*6*). The stems and leaves of *V. ovatum* have had more popularity in floral arrangements than its edible fruit (*6*, *7*). In Oregon, *V. ovatum* has recently been planted in commercial fields for their edible berries (Finn, personal communication). A small commercial huckleberry industry was based on established, managed fields in the early 1900s (*8*, *9*). Little is known about the chemical composition of *V. ovatum* fruit (*10*, *11*).

This study compared *V. ovatum* Pursh with native huckleberry, *V. membranaceum* Douglas ex Torrey. The objective of this study was to compare their chemical composition, their anthocyanin and polyphenolic profiles, and their antioxidant properties.

* To whom correspondence should be addressed: telephone 541-737-3591; fax 541-737-1877; e-mail ron.wrolstad@oregonstate.edu.
[†] Oregon State University.
[§] Northwest Center for Small Fruit Research.

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MATERIALS AND METHODS

Plant Material. Berries of both species were harvested from plants grown at the U.S. Department of Agriculture−Agricultural Research Service (USDA−ARS) in Corvallis, OR, from seed collected from wild stands. The huckleberries were established in 1995 by use of standard highbush blueberry practices. Berries of *V. membranaceum* ripen earlier than those of *V. ovatum*. *Vaccinium membranaceum* berries were collected in June 2003, while *V. ovatum* berries were harvested during August 2003. Samples were picked, placed in an icebox, and immediately frozen upon arrival at the laboratory (within 2 h of picking). All samples were stored at −70 °C until analysis. All visually ripe berries were picked and pooled by species ($n > 9$ genotypes for both species) for this study.

Reagents and Standards. Chlorogenic acid, caffeic acid, protocatechuic acid, catechin, epicatechin, vanillic acid, gallic acid, and β-phycoerythrin were obtained from Sigma Chemical Co. (St. Louis, MO). All solvents for this investigation were high-performance liquid chromatography- (HPLC-) grade. Chlorogenic acid isomers were obtained by the method described by Nagels et al. (*12*). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and TPTZ (tripyridyltriazine) were purchased from Fluka (Buchs, Switzerland). AAPH [2,2′-azobis(2-amidinopropane) dihydrochloride] was purchased from Wako (Richmond, VA).

Extraction. Anthocyanins and polyphenolics were extracted from frozen berries following the procedure described by Rodriguez-Saona and Wrolstad (*13*). Samples were liquid nitrogen-powdered in a mortar and pestle. Six grams of powdered sample was sonicated with 12 mL of 100% acetone, followed by re-extraction with 70% (v/v) aqueous acetone until the solution became colorless; it was then partitioned with chloroform (1:2 acetone:chloroform v/v) to obtain the aqueous fraction. The aqueous portion was collected, and residual acetone present was evaporated with a Büchi rotovapor (Westbury, NY) at 40 °C. The aqueous extract was dissolved to a final volume of 25 mL with distilled water. Samples were then stored at −70 °C until analysis. Samples for anthocyanin separation by HPLC were filtered through a 0.45 μm Millipore filter, type HA (Millipore Corp., Bedford, MA), before HPLC injection.

Berry Size, Titratable Acidity (TA), pH, and °Brix. Berry size was determined by counting the number of berries per 100 g of sample. Berry samples for determining TA, pH, and °Brix were initially homogenized by a mortar and pestle. TA was determined by titrating sample (5 g of homogenate + 45 mL of CO_2-free distilled water) with standardized 0.1 N NaOH to pH 8.1 by use of a pH meter. TA was expressed as citric acid equivalents (grams of citric acid per 100 grams of berries). The pH of the homogenate was determined by use of a Corning pH meter 340 (Corning Inc., Corning, NY) equipped with a Corning electrode. The homogenates were centrifuged and the supernatants were used in determining percent soluble solids. An auto Abbe refractometer model 10500 (Reighert-Jung, Lecia Inc., Buffalo, NY) was used to measure °Brix in percent soluble solids and temperature-compensated mode (21 °C).

Solid-Phase Extraction (SPE). Aqueous berry extracts were passed through a C-18 Sep-Pak minicolumn (Waters Associates, Milford, MA), rinsed with methanol, and activated with deionized water. SPE was performed as described in Rodriguez-Saona and Wrolstad (*13*).

Anthocyanins and polyphenolics were absorbed onto the column while sugars, acids, and other polar compounds that eluted with water (fraction 1) were removed and collected. The polyphenolic fraction (fraction 2) was obtained by eluting with 2 mL of ethyl acetate. Ethyl acetate was removed from the fraction with the Büchi rotovapor at 40 °C, and the residue was redissolved in 2 mL of deionized water. This fraction was filtered and injected into the LC for polyphenolics separation. Anthocyanins (fraction 3) were collected with acidified (0.01% HCl) methanol. The methanol was evaporated by use of a Büchi rotovapor at 40 °C. Pigments (fraction 3) were redissolved in 2 mL of acidified water (0.01% HCl). This fraction was also used for LC-MS analysis after filtering of samples. All fractions were kept at −70 °C until further analysis.

Determination of Total Anthocyanins and Total Phenolics. Total monomeric anthocyanins (ACY) were determined by the pH differential method (*14*). Absorbance was measured at 520 and 700 nm. ACY was expressed as cyanidin 3-glucoside (molar extinction coefficient of 26 900 L·cm^{-1}·mol^{-1} and molecular weight of 449.2). The unit for ACY was milligrams of anthocyanin per 100 grams of frozen berries. Total phenolics (TP) were measured by the Folin−Ciocalteu (FC) method (*15*). Absorbance was measured at 765 nm. TP was expressed as milligrams of gallic acid per 100 grams of frozen berries. A Shimadzu 300 UV−visible spectrophotometer (Shimadzu Inc., Kyoto, Japan) and 1-cm path length cells were used for both measurements. Measurements of ACY and TP on sample extracts were replicated two times.

Determination of Antioxidant Activity. Antioxidant activities of extracts were determined by ferric reducing antioxidant potential (FRAP) and oxygen radical absorbing capacity (ORAC) assays. FRAP assays were performed as described by Benzie and Strain (*16*), utilizing a 96-well ThermoMax microplate spectrophotometer (Molecular Devices, Foster City, CA) to measure the formation of ferrous−TPTZ complex ($\lambda_{max} = 595$ nm). FRAP measures the extract's ability to reduce ferric ion (Fe^{3+}) to ferrous ion (Fe^{2+}) in a solution of TPTZ prepared in sodium acetate at pH 3.6. Absorbance was measured at 595 nm. ORAC assays followed the method described by Cao et al. (*17*), with the alteration of utilizing a 96-well Cytofluor 4000 microplate fluorometer (PerSeptive Biosystems, Framingham, MA), which records rate and duration of fluorescence. β-Phycoerythrin acted as a target for the peroxyl radicals generated by AAPH (a peroxyl radical generator that destroys the fluorescence). Samples were monitored at 2-min intervals, for 2 h, at 485 nm (excitation wavelength) and 585 nm (emission wavelength). FRAP and ORAC values were expressed as micromoles of Trolox (a water-soluble tocopherol analogue) equivalents per gram of frozen fruit.

LC-DAD of Anthocyanins and Polyphenolics. Anthocyanins and polyphenolics were separated by reversed-phase HPLC on a Hewlett-Packard 1090 system (Agilent Technologies Inc, Wilmington, DE), equipped with a photodiode array detector (DAD). Absorbance spectra were recorded for all peaks. Flow rate was 1 mL/min. Hewlett-Packard HPLC-2D ChemStation software was used for data analysis.

Anthocyanin. A Prodigy 5-μm ODS (3) 100 Å (250 × 4.6 mm) column, fitted with 4.0 × 3.0 mm i.d. guard column, from Phenomenex (Torrance, CA) was used. Solvent A was 100% acetonitrile. Solvent B was 10% (v/v) acetic acid and 1% (v/v) phosphoric acid in water. The program used a linear gradient from 2% to 20% solvent A in 25 min; then a linear gradient of solvent A from 20% to 40% in 5 min, with simultaneous detection at 280, 320, and 520 nm (*18*). Injection volume was 20 μL. Column temperature was maintained at 40 °C.

Polyphenolics. A Synergi 4-μm Hydro-RP 80 Å (250 × 4.60 mm) column, fitted with 4.0 × 3.0 mm i.d. guard column, from Phenomenex (Torrance, CA) was used. Solvent A was 100% acetonitrile. Solvent B was 1% (v/v) formic acid in water. The program used a linear gradient from 5% to 25% solvent A in 50 min; then a linear gradient of solvent A from 25% to 50% in 5 min, then held for 5 min, with simultaneous detection at 260, 280, 320, and 520 nm. Injection volume was 100 μL. Column temperature was at room temperature.

LC-MS of Anthocyanins. A Hewlett-Packard 1090 system (Agilent Technologies Inc, Wilmington, DE) equipped with a photodiode array detector (DAD) and mass spectrometer (MS) was used to confirm the identification of the huckleberry anthocyanins. A Synergi 4-μm Hydro-RP 80 Å (250 × 2 mm, 4 μm) column, fitted with 4.0 × 3.0 mm i.d. guard column, from Phenomenex (Torrance, CA) was used. Absorbance spectra were collected for all peaks. Flow rate was 0.2 mL/min and injection volume was 20 μL. Solvent A was 5% formic acid and 80% acetonitrile (v/v), and solvent B was 5% formic acid. The initial solvent composition was 10% solvent A and 90% solvent B; then a linear gradient of 10% to 30% solvent A and 90% to 70% solvent B in 30 min was applied. Detection occurred simultaneously at 280, 320, and 520 nm. MS analysis was performed on a Perkin-Elmer Sciex API III (Toronto, Canada), equipped with an ion-spray source (ISV = 5500, orifice voltage = 50) in positive ion mode.

RESULTS AND DISCUSSION

The composition and characteristics of the two species are summarized in **Table 1**. *Vaccinium ovatum* (446 berries/

Table 1. Comparison of Fruit from *Vaccinium membranaceum* and *Vaccinium ovatum* Grown in Test Plots in Corvallis, OR[a]

	V. membranaceum	*V. ovatum*
pH	2.6 (±0.1)	2.8 (±0.1)
TA[b]	1.73 (±0.03)	1.63 (±0.01)
°Brix	12.7 (±0.01)	19.6 (±0.01)
°Brix/TA (sugar:acid ratio)	7.3	12.0
ACY[c]	167 (±1)	563 (±5)
TP[d]	617 (±4)	1169 (±10)
ACY/TP	0.271	0.482
berry size (no. of berries/100 g)	296 (±23)	446 (±31)
FRAP values[e]	42.1 (±4.8)	137.2 (±2.7)
ORAC values[e]	26.2 (±1.1)	103.4 (±4.4)
visual observation of berries	pigment present in flesh and skin; reddish-black berries	pigment mainly in the skin; black berries

[a] Values in parentheses are standard deviations. [b] Titratable acidity (TA) expressed as grams of citric acid per 100 grams of berries. [c] Total monomeric anthocyanin content (ACY) expressed as milligrams of cyanidin 3-glucoside (MW = 449.2 and extinction coefficient = 26 900) per 100 grams of berries. [d] Total phenolics (TP) expressed as milligrams of gallic acid per 100 grams of berries. [e] Expressed as micromoles of Trolox per gram of berries.

100 g)had smaller fruits than did *V. membranaceum* (296 berries/100 g). This might account for the high ACY and TP content of the *V. ovatum* fruit, since smaller berries mean a greater proportion of skin, where the anthocyanins are predominantly found, for equal masses of fruit. The total monomeric anthocyanin content of *V. ovatum* (563 mg of cyanidin 3-glucoside/100 g of frozen berries) was 3-fold greater than *V. membranaceum* (167 mg of cyanidin 3-glucoside/100 g of frozen berries). *Vaccinium ovatum* also had higher total phenolics content (1169 mg of gallic acid/100 g of frozen berries) than *V. membranaceum* (617 mg of gallic acid/100 g of frozen berries). Moyer et al. (*11*) reported two genotypes of *V. ovatum* to contain 336 and 357 mg of ACY/100 g, and 641 and 842 mg of TP/100 g. Also, Moyer et al. (*11*) reported that *V. membranaceum* contained 110−153 mg of ACY/100 g and 225−423 mg of TP/100 g by the same methodology. *Vaccinium ovatum* fruits were high in ACY and TP when compared to those of the bilberry (*V. myrtillus*), which was not available for this study, but Prior et al. (*4*) reported bilberry to contain 330 mg of ACY/100 g and 525 mg of TP/100 g. *Vaccinium ovatum* had a higher pH (2.8) and lower titratable acidity (1.63 g of citric acid/100 g of berries) values compared to *V. membranaceum* (pH = 2.6 and TA = 1.73 g of citric acid/100 g of berries). *Vaccinium ovatum* (19.6 °Brix) had higher percent soluble solids than *V. membranaceum* (12.7 °Brix). *Vaccinium membranaceum* had a lower sugar:acid ratio (7.3), which might contribute toward better storability when compared to *V. ovatum* (12.0), since low sugar:acid ratio has been shown to be a good indicator for prolonging fruit quality during storage (*19*). The FRAP and ORAC values were 3 and 4 times higher in *V. ovatum* samples, which was expected, as *V. ovatum* fruit had higher ACY and TP than *V. membranaceum* fruit.

Cultivated huckleberry samples were taken from a single growing location and growing season. Numerous research groups have reported differences in anthocyanin and phenolic contents in blueberry samples due to growing season and environmental factors (*5*, *20*, *21*).

Berry extracts from both species were further fractionated by solid-phase extraction with a C-18 Sep-Pak minicolumn. The corresponding ACY, TP, FRAP, and ORAC values are shown in **Table 2**. While the majority of the anthocyanins were present in the anthocyanin fraction, as expected, there were some anthocyanins present in the polyphenolics and sugar/acid fractions. The anthocyanin fraction had the highest TP value,

Table 2. Anthocyanin, Total Phenolics, and Antioxidant Activity of Fractions Obtained from Solid-Phase Extraction of Berries from Two *Vaccinium* Species

	V. membranaceum			*V. ovatum*		
	anthocyanin fraction	polyphenolic fraction	sugar/acid fraction	anthocyanin fraction	polyphenolic fraction	sugar/acid fraction
ACY[a]	104 (±1)	6 (±1)	14 (±7)	361 (±1)	43 (±1)	21 (±0)
TP[b]	241 (±2)	153 (±5)	8 (±0)	625 (±8)	354 (±2)	41 (±19)
FRAP[c]	23.8	11.8	4.1	73.6	32.4	6.0
ORAC[d]	15.7	9.9	3.1	44.9	24.8	6.3

[a] Total monomeric anthocyanin content (ACY) expressed as milligrams of cyanidin 3-glucoside (MW = 449.2 and extinction coefficient = 26 900) per 100 grams of berries. [b] Total phenolics content (TP) expressed as milligrams of gallic acid per 100 grams of berries. [c] Ferric reducing antioxidant potential, expressed as micromoles of Trolox per gram of berries. [d] Oxygen radical absorbing capacity, expressed as micromoles of Trolox per gram of berries.

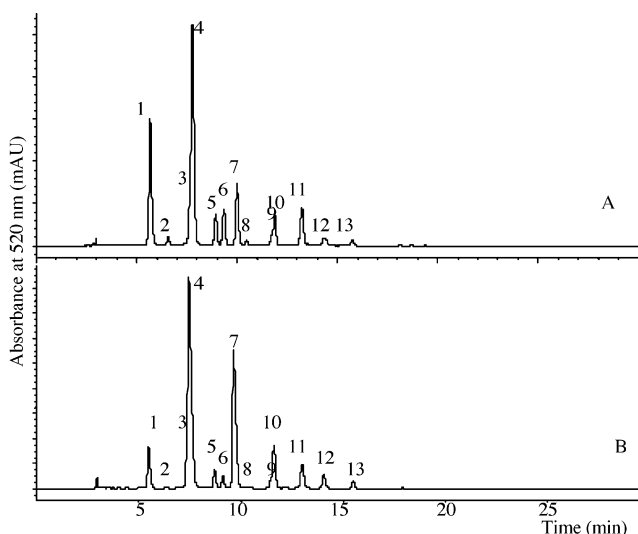

Figure 1. Anthocyanin HPLC profiles of *Vaccinium membranaceum* (**A**) and *Vaccinium ovatum* (**B**). Corresponding presumptive anthocyanin peak assignments and percent peak areas: 1, delphinidin 3-galactoside (A 20.3, B 6.9), 2, delphinidin 3-glucoside (A 1.3; B 0.4); 3, delphinidin 3-arabinoside (A 5.8, B 5.5); 4, cyanidin 3-galactoside (A 35.4, B 43.0); 5, cyanidin 3-glucoside (A 5.0, B 3.1); 6, petunidin 3-galactoside (A 5.7, B 2.2); 7, cyanidin 3-arabinoside (A 9.7, B 25.6); 8, petunidin 3-glucoside (A 0.8, B 0.3); 9, petunidin 3-arabinoside (A 1.4, B 1.4); 10, peonidin 3-galactoside (A 4.7, B 6.9); 11, malvidin 3-galactoside (A 7.1, B 2.6); 12, malvidin 3-glucoside (A 1.9, B 1.4); 13, malvidin 3-arabinoside (A 1.0, B 0.8).

compared to the polyphenolic fraction and sugar/acid fraction. The antioxidant activity of the sugar/acid fraction could be due to water-soluble compounds (such as sugars, acids, ascorbic acid, and glutathione) that possess antioxidant activities (*22*, *23*). The anthocyanin fractions appeared to be the major contributor to FRAP and ORAC values, while the sugar/acid fractions contributed the least. In most cases, FRAP analyses indicated higher antioxidant measurements than did ORAC analyses.

The anthocyanin profiles obtained from the LC-DAD of the two species appear to differ slightly (**Figure 1**). Peak assignments were made on the basis of their spectra and retention time. Both species had the same qualitative composition with different proportions of peak areas. From the LC-DAD results, *V. membranaceum* and *V. ovatum* had the same 13 different anthocyanins present. *Vaccinium membranaceum* had cyanidin glycosides (50.0%), delphinidin glycosides (27.4%), malvidin glycosides (10.0%), petunidin glycosides (7.9%), and peonidin glycosides (4.7%), in the order of most abundance (based on

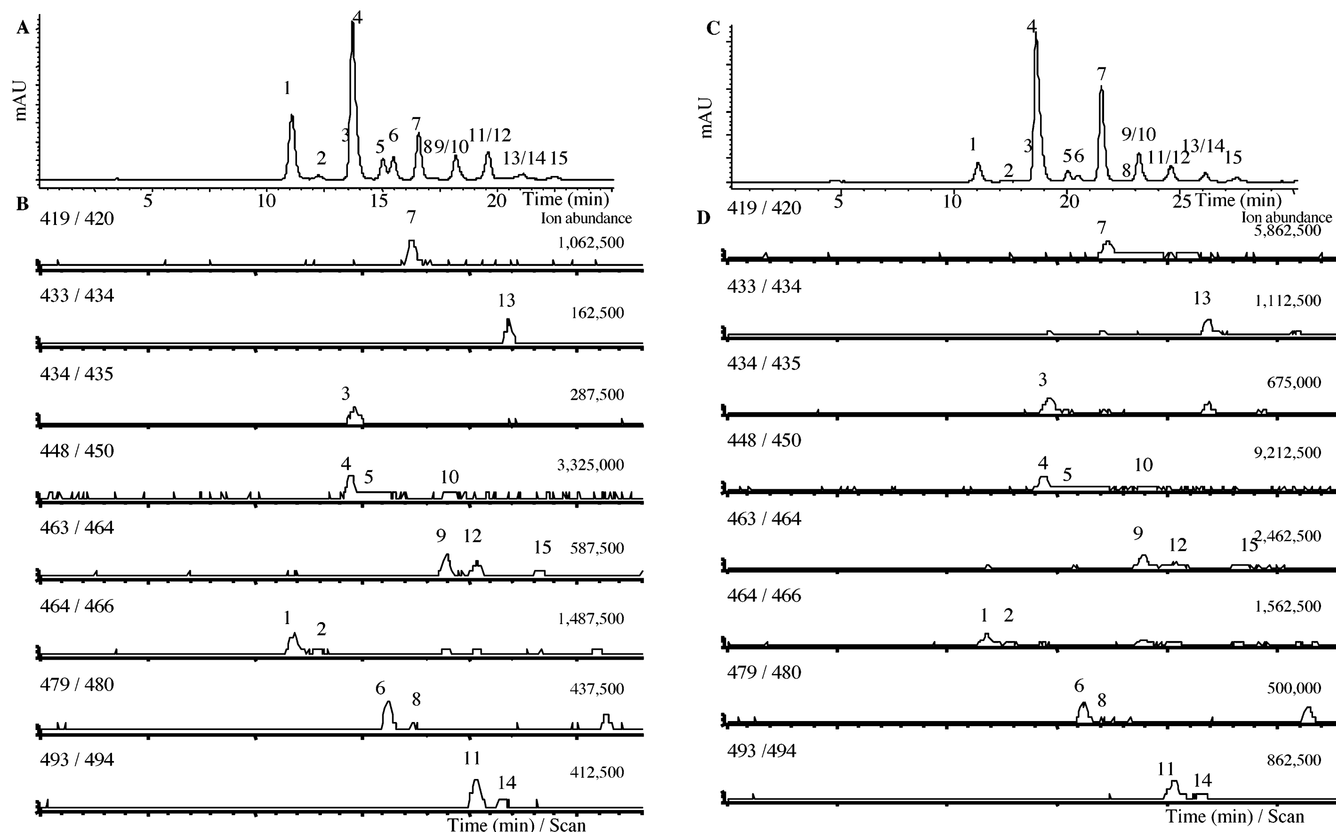

Figure 2. *Vaccinium membranaceum* and *Vaccinium ovatum* results from LC-DAD-MS: LC-DAD profile (**A**) and extracted ion chromatograms (**B**) of *V. membranaceum*, and LC-DAD profile (**C**) and extracted ion chromatograms (**D**) of *V. ovatum*. Corresponding anthocyanin peak assignments and masses: 1, delphinidin 3-galactoside (465.2); 2, delphinidin 3-glucoside (465.2); 3, delphinidin 3-arabinoside (435.0); 4, cyanidin 3-galactoside (449.2); 5, cyanidin 3-glucoside (449.2); 6, petunidin 3-galactoside (479.2); 7, cyanidin 3-arabinoside (419.0); 8, petunidin 3-glucoside (479.2); 9, peonidin 3-galactoside (463.2); 10, petunidin 3-arabinoside (449.0); 11, malvidin 3-galactoside (493.2); 12, peonidin 3-glucoside (463.2); 13, peonidin 3-arabinoside (433.0); 14, malvidin 3-glucoside (493.2); 15, malvidin 3-arabinoside (463.0).

percent peak area at 520 nm). *Vaccinium ovatum* had cyanidin glycosides (71.6%), delphinidin glycosides (12.9%), peonidin glycosides (6.9%), malvidin glycosides (4.8%), and petunidin glycosides (3.9%), in the order of most abundance (based on percent peak area). The major anthocyanin present in both species was cyanindin 3-galactoside (peak 4, **Figure 1**). This confirms the findings of Ballington et al. (*10*, *24*), who reported that 15 galactosides, glucosides, and arabinosides of delphinidin, cyanidin, petunidin, peonidin, and malvidin were present in both species. *Vaccinium membranaceum* had higher amounts of delphinidin galactoside than did *V. ovatum*. The second largest peak for *V. membranaceum* was delphinidin 3-galactoside, whereas the second largest peak for *V. ovatum* was cyanidin 3-arabinoside. Both huckleberries had a smaller amount of malvidin glycosides when compared to levels of the highbush blueberry *Vaccinium corymbosum* ‘Rubel’ (*25*). Cyanidin glycosides have higher antioxidant activity than other anthocyanins (*22*). In addition to the higher amounts of total anthocyanins and total phenolics in huckleberries, the presence of individual anthocyanins that are higher in antioxidant properties might also contribute to high antioxidant content of huckleberries in contrast with other blueberry relatives.

LC-DAD-MS was conducted on the huckleberry SPE extracts to firmly identify the individual anthocyanins. The LC-DAD-MS profiles, peak assignments, and masses of both species are provided (**Figure 2**). The individual peaks eluting at different times were analyzed by MS and the corresponding peaks’ mass-to-charge ratio was obtained from the MS. Based on LC-DAD-MS, there were 15 peaks present in both species. Peonidin 3-glucoside and peonidin 3-arabinoside were not detected by LC-DAD by use of phosphoric acid and acetic acid mobile phase but were found in low amounts with LC-DAD-MS (peaks 12 and 13). Separation was achieved by a different column and mobile phase (formic acid instead of a combination of acetic acid and phosphoric acid), with the LC-DAD-MS system, and the resolution was not as good as in the LC-DAD system. However, additional information was obtained with MS detection. Huckleberry anthocyanin peaks that coeluted in the LC-DAD system had different mass-to-charge ratios and therefore could be distinguished by MS detection. For example, malvidin 3-glucoside and peonidin 3-glucoside coeluted, and peonidin 3-glucoside could not be detected with LC-DAD but was detected by LC-DAD-MS. Since peonidin 3-glucoside and peonidin 3-arabinoside were present in low concentrations and coeluted with other anthocyanins, they were difficult to identify on the basis of LC-DAD alone. MS could also be helpful in identification of anthocyanins, due to the limited amount of authentic standards commercially available. Identification of anthocyanins was simplified by using a combination of retention time, peak spectra, and mass-to-charge ratio.

SPE was used to remove the anthocyanin fraction that interferes with the polyphenolic LC analysis. Further hydrolysis was performed. The polyphenolics in the two species were examined in their native forms. The LC chromatograms of the ethyl acetate fractions of the two berries are shown (**Figure 3**). Only the absorbances at 320 and 260 nm are shown. Tentative peak assignments were made on the basis of their retention times, UV−visible spectra, and standards (when available). The

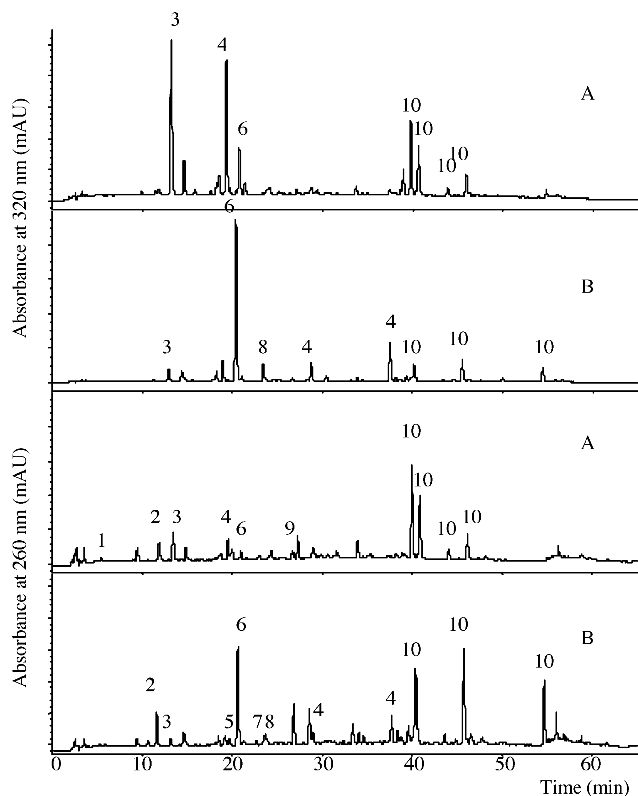

Figure 3. Polyphenolic HPLC separations of *Vaccinium membranaceum* (**A**) and *Vaccinium ovatum* (**B**) monitored at 320 and 260 nm. Corresponding tentative peak assignments: 1, gallic acid; 2, protocatechuic acid; 3, neochlorogenic acid; 4, cinnamic acid dervatives; 5, catechin; 6, chlorogenic acid; 7, vanillic acid; 8, caffeic acid; 9, epicatechin; 10, flavonol glycosides.

polyphenolic profiles of the two species were distinctly different. Neochlorogenic acid (3-*O*-caffeoylquinic acid, 26% of the total peak area measured at 320 nm) and a cinnamic acid derivative (peak 2, 21% of the total peak area measured at 320 nm) were the major polyphenolics present in *V. membranaceum,* as detected by LC under the conditions used in this study. This study is the first to report the presence of neochlorogenic acid as one of the major polyphenolics present in *V. membranaceum*. Neochlorogenic acid was reported in large quantities in Hawaiian *V. reticulatum* Smith and *V. calycinum* Smith leaves (*26*), both of which are also in the Myrtillus section. Neochlorogenic acid has been reported in the green berries and leaves of whortleberry (*V. arctostaphylos* L.; Sect. Hemimyrtillus) (*27*, *28*). Chlorogenic acid (5-*O*-caffeoylquinic acid, 43% of the total peak area measured at 320 nm) was the major polyphenolic present in *V. ovatum*. The *V. ovatum* polyphenolic profile was similar to that of ‘Rubel’ (data not shown). The major polyphenolic present in ‘Rubel’ is chlorogenic acid (*25*). Both species’ polyphenolic profiles were mainly composed of cinnamic acids (strong 320-nm absorbers) and flavonol glycosides (strong 260-nm absorbers). Minor peaks identified in the ethyl acetate fraction of *V. membranaceum* were gallic acid, protocatechuic acid, and epicatechin, while *V. ovatum* had protocatechuic acid, catechin, and vanillic acid minor peaks, which might have hydrolyzed during extraction and/or SPE.

Vander Kloet (*6*) provided three different phenograms to divide the 26 North American *Vaccinium* species into groups, which were dependent upon the mean character scores for quantitative and/or qualitative features of the species. None of these features included anthocyanin or polyphenolic data. Depending on the phenogram, *V. membranaceum* (Section Myrtillus), *V. ovatum* (Section Pyxothamnus), and *V. corymbosum* (Section Cyanoccocus) links to one another were strengthened or weakened. Data are insufficient and too highly variable to speculate on differences in the profiles of *Vaccinium* sections. The ranges of ACY, TP, and ACY/TP values of the various sections overlap.

In conclusion, the fruits of *V. ovatum* were higher in ACY, TP, antioxidant activities, and soluble solids than were those of *V. membranaceum*. The anthocyanin pigment profiles were qualitatively the same but differed in their proportions. The polyphenolic profiles were distinctive, with neochlorogenic acid being the major polyphenolic in *V. membranaceum* and chlorogenic acid predominating in *V. ovatum*. While the anthocyanin fraction was highest in antioxidant properties for both fruits, the polyphenolic fraction contained substantial antioxidant activity. Since *V. ovatum* ripens later than *V. membranaceum*, it could be an additional source to lengthen the huckleberry season for the fresh market and commercial processing.

ABBREVIATIONS USED

ACY, total monomeric anthocyanins; TP, total phenolics; ORAC, oxygen radical absorbing capacity; FRAP, ferric reducing antioxidant potential; LC-DAD, high-performance liquid chromatography with photodiode array detection; LC-DAD-MS, high-performance liquid chromatography with photodiode array and mass spectrometry detections; Trolox, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; TPTZ, tripyridyltriazine; AAPH, 2,2′-azobis(2-amidinopropane) dihydrochloride; TA, titratable acidity; SPE, solid-phase extraction; FC, Folin−Ciocalteu.

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