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Influence of Cultivar, Maturity, and Sampling on Blackberry (*Rubus* L. Hybrids) Anthocyanins, Polyphenolics, and Antioxidant Properties

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Total anthocyanin pigments increased from 74.7 to 317 mg/100 g fresh weight (FW) from underripe to overripe for Marion blackberries and from 69.9 to 164 mg/100 g FW for Evergreen blackberries. Total phenolics did not show a marked change with maturity with values slightly decreasing from underripe to ripe. Antioxidant activities, while increasing with ripening, also did not show the marked change that total anthocyanins exhibited. The impact of variation due to plots, subsampling, sample preparation, and measurement on Marion composition was examined in detail. Plot-to-plot and sample differences were the major contributors to variation, with sample preparation being an important contributor for some parameters. Measurement variation was a relatively small component of the total variation. Total anthocyanins for 11 blackberry cultivars ranged from 131 to 256 mg/100 g FW (mean = 198), total phenolics ranged from 682 to 1056 mg GAE/100 g FW (mean = 900), oxygen radical absorbance capacity ranged from 37.6 to 75.5 μmol TE/g FW (mean = 50.2), and ferric reducing antioxidant power ranged from 63.5 to 91.5 μmol TE/g FW (mean = 77.5).

KEYWORDS: Blackberries; *Rubus* L. hybrids; anthocyanins; polyphenolics; antioxidant properties; cultivar; sample variation; maturity

INTRODUCTION

Polyphenolics include several classes of phenolic compounds that are secondary plant metabolites and an integral part of both human and animal diets. While phenolics historically were considered in some instances to be antinutrients, interest in food phenolics has increased greatly because of their antioxidant capacity (free radical scavenging and metal chelating activities) and possible beneficial roles in human health, such as reducing the risk of cancer, cardiovascular disease, and other pathologies (*1*, *2*).

Blackberries are a rich source of anthocyanins and other polyphenolic antioxidants (*3*−*5*). Extensive studies have been done on blackberry anthocyanins, and their identities have been well-characterized as being solely cyanidin-based compounds (*5*−*7*). In general, five anthocyanins are detected in blackberries and they are identified as cyanidin 3-glucoside (major anthocyanin) (*5*, *6*, *8*, *9*), cyanidin 3-rutinoside (*6*, *8*), malonic acid acylated cyanidin 3-glucoside (*5*, *6*), xylose-cyanidin derivative (*6*, *9*), and cyanidin 3-dioxalyl-glucoside (*7*). Wada and Ou (*5*) also reported the presence of cyanidin 3-(6′-*p*-coumaryl)-glucoside in Marion berries and cyanidin 3-arabinoside in Evergreen blackberries, and Dugo and others (*10*) reported the presence of cyanidin 3-galactoside, cyanidin 3-arabinoside, pelargonidin 3-glucoside, and malvidin 3-glucoside in a commercial Italian blackberry. However, no other investigators have found these pigments in blackberries.

We (*11*) recently investigated the polyphenolics of blackberries and found that flavonols (primarily quercetin glycosides) were the major phenolics in the berries whereas procyanidins (catechin- and epicatechin-based) and ellagic acid derivatives predominated in seeds. The presence of ellagic acid derivatives, quercetin and kaempferol glycosides, catechin, and epicatechin confirmed identifications of previous workers (*12*−*18*).

Genetic and environmental factors, such as cultivar, maturity, UV light exposure, and harvesting method, play an important role in berry composition (*19*). It is well-known that levels of phenolics and the antioxidant capacity of blackberries are influenced by maturity (*3*) and that there is pronounced variation among cultivars (*20*). Small differences in ripeness can have an effect on sample-to-sample variation; however, the effects of plot-to-plot differences, sample preparation (milling, extraction, and isolation), and measurement have not been systematically investigated. Differences in methodology used for extraction and analysis and the way in which the quantitative results are expressed [on a dry weight or fresh weight (FW) basis] are reasons for the great variation in phenolic contents of fruits and vegetables as reported in the literature (*21*).

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The objectives of this study were to investigate the anthocyanins, polyphenolics, and antioxidant properties of blackberries, to evaluate the influence of cultivar and maturity, and to determine the variation caused by plot difference, subsampling, sample preparation, and analytical measurement.

MATERIALS AND METHODS

Reagents and Standards. Folin−Ciocalteau reagent was purchased from Sigma Chemical Co. (St. Louis, MO) as were citric acid and the following phenolic standards: gallic acid, (+)-catechin, ellagic acid, and quercetin 3-rutinoside (rutin). Acetone, chloroform, sodium carbonate, high-performance liquid chromatography (HPLC) grade methanol, HPLC grade ethyl acetate, formic acid, and phosphoric acid were purchased from EM SCIENCE (A division of EM Industries, Inc., Gibbstown, NJ). Acetic acid (glacial) and hydrochloric acid were obtained from Allied Chemical (General Chemical Division, Morristown, NJ) and J. T. Baker Inc. (Phillipsburg, NJ), respectively. Cranberry and blackberry juice concentrates were provided by Kerr Concentrates Inc. (Salem, OR).

Plant Materials. All blackberries were harvested at the Oregon State University (OSU) North Willamette Research and Extension Center (Aurora, OR) from late June to mid-August, 2002. Marion and Evergreen blackberries, the two major commercial varieties in the Pacific Northwest, were chosen for investigation at three different maturity stages based on visual appearance of the fruit: underripe (part red−part black), full ripe (shiny black), and overripe (dull black). Berries were handpicked by the senior author from three plots of Marion (kept separately) and from one plot of Evergreen; plots were in a completely randomized design with three blackberry plants per plot, about 1.5 m wide/plant. Marion blackberries ripen from mid-July through early August, whereas Evergreen blackberries ripen from mid-August through mid-September. Individual berries are typically about 5 g. Sampling was done on two different days from the same harvested pool of the same plot. Berries were transported (ca. 1 h) in insulated cooled containers to the OSU Food Science and Technology pilot plant where they were immediately frozen and stored at −23 °C.

Ripe berries from 11 blackberry cultivars (Marion, Waldo, Evergreen, Chester, Silvan, NZ 9128R-1, NZ 9351-4, ORUS 1843-3, ORUS 1380-1, ORUS 1489-1, and ORUS 1369-3) were selected for their genetic diversity and commercial importance and used as materials for the cultivar study. Berries were grown at the North Willamette Research and Extension Center and were provided by C. E. Finn, USDA-ARS (Corvallis, OR). Fruits were selected at optimum full ripeness, with the harvest date varying from mid-July to early September for the different selections. Evergreen and Chester berries were harvested from a single plot while berries of the other nine cultivars were harvested from three different plots and combined. Samples were stored at −23 °C.

Extraction of Anthocyanins and Polyphenolics. Berries (from eight to 10 fruits) were cryogenically milled with liquid nitrogen using a stainless steel Waring Blender as previously described (*22*). The powder (ca. 5 g) was mixed 1:1 (w/v) with acetone, sonicated with an ultrasonic cleaner (Branson Cleaning Equipment Co., Shelton, CT) for 3 min, and centrifuged. The remnant was re-extracted with 70% (v/v) aqueous acetone twice. The filtrates were combined and gently mixed with chloroform (1:2, v/v). After centrifugation at 3000 rpm for 30 min on an IEC International Centrifuge (model UV, International Equipment Co., Boston, MA), the aqueous phase (top portion) was collected and placed on a Büchi rotary evaporator (Brinkmann Instruments, Westbury, NY) at 40 °C under vacuum to remove residual acetone. The aqueous extract was then made up to a known volume with deionized water and stored at −70 °C until analyzed. For the maturity study, extractions were performed on two subsamples of each plot with two replicated extractions for each subsample. For the cultivar comparison trial, extractions were performed on three subsamples of each sample.

Total Soluble Solids (TSS) and Titratable Acidity (TA). TSS was measured using an Auto Abbe refractometer 10500 (Reichert-Jung, Leica Inc., Buffalo, NY). The instrument was set to measure % TSS with the temperature compensated mode. Measurements were made on fresh fruit. A Metrohm titration unit (Brinkmann, Metrohm Herisau, Switzerland) equipped with a TT A80 Titration Assembly (Radiometer A/S, Copenhagen, Denmark) was used for total TA determination. Measurements were made on the fruit extracts, and results were calculated as g citric acid/100 g FW. Measurements were replicated twice for the maturity study with no replication for the cultivar study.

Total Phenolics. The total phenolic content was determined with Folin−Ciocalteau reagent (Sigma Chemical Co.) by the method modified from Singleton and Rossi (*23*) using gallic acid as a standard. A series of seven test tubes each containing 7.5 mL of deionized water and 0.5 mL of reagent were prepared. To each test tube one of the following solutions was added, 0.5 mL of sample (diluted as necessary), 0.5 mL of 40, 120, or 200 ppm gallic acid dilution, or 0.5 mL of deionized water as a blank. All solutions were well-mixed using a VWR Vortexer (model G-560, Scientific Industries, Inc., Bohemia, NY) and held at room temperature for 10 min. Then, to each test tube, 3 mL of 20% Na_2CO_3 solution was added, mixed well, and placed in a heat block (VWR International, West Chester, PA) at 40 °C for 20 min. After the reaction, the test tubes were immediately cooled in an ice bath for 3 min. The absorbances of the samples and standards were measured at 755 nm, using a Shimadzu 300 UV spectrophotometer and 1 cm path length disposable cells. Results were calculated as mg gallic acid equivalent (GAE) per 100 g FW. Analyses were replicated twice for the maturity study with no replication for the cultivar study.

Total Monomeric Anthocyanins. The total anthocyanin content was determined using the pH differential method (*24*). Samples were diluted in pH 1.0 and pH 4.5 buffers, and absorbance measurements were made at 510 and 700 nm on a Shimadzu 300 UV spectrophotometer, using 1 cm path length disposable cells. The pigment content was calculated and expressed as cyanidin-3-glucoside (Cyd-3-glu)/100 g FW, using an extinction coefficient (ϵ) of 26900 L cm^{-1} mol^{-1} and a molecular weight of 449.2 g mol^{-1}. Analyses were replicated twice for the maturity study with no replication for the cultivar study.

Antioxidant Properties. Antioxidant properties were determined by the oxygen radical absorbance capacity (ORAC) and ferric reducing antioxidant power (FRAP) assays at the Linus Pauling Institute, Oregon State University. The ORAC assay was performed as described by Cao and others (*25*) and adapted for use in a 96 well microplate fluorometer (model Cytofluor 4000, PerSeptive Biosystems, Framingham, MA). β-Phycoerythrin was the target for the peroxyl radicals generated by 2,2′-azobis(2-amidinopropane)dihydrochloride. ORAC values, derived from triplicate analyses, were expressed as μmol Trolox equivalent (TE) per g FW. The FRAP assay (*26*) was adapted for use in a 96 well microplate spectrophotometer (ThermoMax, Molecular Devices, Foster City, CA). FRAP values, derived from duplicate analyses, were expressed as μmol TE per g FW. Both assays were performed on one randomly selected subsample.

Anthocyanin and Polyphenolic Purification. The method described by Skrede and others (*27*) for separation of blueberry anthocyanins from other phenolics was followed. The extract (1 mL) was applied to a C_{18} Sep-Pak cartridge containing 360 mg of sorbent (Waters Associates, Milford, MA), which had been previously activated with 5 mL of ethyl acetate, 5 mL of acidified (0.01% HCl) methanol, and 5 mL of acidified (0.01% HCl) water, respectively. The cartridge with the adsorbed extract was then washed with 10 mL of acidified (0.01% HCl) water, after which the cartridge was dried with a current of nitrogen for 3 min. Ethyl acetate (10 mL) eluted polyphenolics free from anthocyanins. Anthocyanins were eluted with 10 mL of acidified (0.01% HCl) methanol. Both eluents were evaporated to near dryness on a Büchi rotary evaporator (40 °C under vacuum) and taken up in deionized water. Samples were filtered through a 0.45 μM Millipore filter, type HA (Millipore Corp., Bedford, MA), before being injected onto the HPLC system.

HPLC. *Analytical HPLC for Anthocyanins. 1. Apparatus.* A Hewlett-Packard 1090 Liquid Chromatograph (Agilent Technologies, Palo Alto, CA), equipped with photodiode array detector and Gateway 2000 P5-90 computer with Hewlett-Packard $HPLC^{2D}$ Chemstation software, was used.

2. Column, Mobile Phases, and HPLC Conditions. Chromatographic analysis was done according to the method of Durst and Wrolstad (*28*) using a Prodigy ODS-3 column (5 μm), 250 mm × 4.60 mm i.d. (Phenomenex, Torrance, CA), fitted with an Allsphere 10 mm × 4.6

Table 1. TSS, TA, Maturity Index, Polyphenolic Composition, and Antioxidant Properties of Marion and Evergreen Blackberries at Three Maturity Stages[a]

cultivar	TSS (°Brix)	TA (citric acid, g/100 g)	maturity index (°Brix:acid)	total phenolics (mg GAE/100 g)	total anthocyanins (mg Cyd-3-glu/100 g)	ORAC[c] (μmol TE/g)	FRAP[c] (μmol TE/g)
				Marion			
underripe	9.46 ± 1.34a	2.32 ± 0.09b	4.10 ± 0.66a	975 ± 144a	74.7 ± 11.2a	43.0 ± 2.30	87.6 ± 1.86
ripe	13.5 ± 1.67b	1.28 ± 0.12a	10.8 ± 2.08b	903 ± 145a	221 ± 36.6b	60.9 ± 20.6	78.2 ± 1.15
overripe	16.0 ± 1.48c	1.29 ± 0.20a	12.6 ± 2.41b	1541 ± 376b	317 ± 35.3c	62.7 ± 12.3	102 ± 12.4
				Evergreen[b]			
underripe	11.0 ± 0.91	2.38 ± 0.15	4.62 ± 0.66	1090 ± 77.3	69.9 ± 10.6	46.1 ± 4.68	80.9 ± 8.21
ripe	15.7 ± 1.71	1.12 ± 0.20	14.0 ± 3.89	960 ± 101	131 ± 9.62	58.5 ± 2.23	94.8 ± 5.31
overripe	18.0 ± 0.75	0.47 ± 0.09	38.3 ± 9.07	1035 ± 46.5	164 ± 11.7	64.4 ± 2.06	91.1 ± 8.94

[a] Different letters in the same column for Marion indicate significant differences ($p \leq 0.05$); data expressed as means ± standard deviations ($n = 3$ plots) on a FW basis. [b] Data expressed as means ± standard deviations for two subsamples within one plot ($n = 1$ plot). [c] Data expressed as means ± standard deviations for two measurements, for one sample from one plot.

mm i.d. ODS-2 guard column (Alltech, Deerfield, IL). Mobile phase A consisted of 100% HPLC grade acetonitrile, and mobile phase B was a mixture of 1% phosphoric acid and 10% acetic acid (glacial) (v/v) in deionized water. Solvents and samples were filtered through a 0.45 μM Millipore filter type HA for aqueous or HV for organic solvent (Millipore Corp.).

The program was as follows: (a) 0 min, 2% A; (b) 0−25 min linear gradient from 2 to 20% A; (c) 25−30 min linear gradient from 20 to 40% A; (d) 30−34 min linear gradient from 40 to 2% A; 5 min posttime and gradient repeated. Simultaneous monitoring was performed at 280, 320, and 520 nm at a flow rate of 1 mL/min and an injection volume of 20 μL. Identification was made from matching UV−visible spectra and retention times with known anthocyanins from well-characterized cranberry and blackberry juices.

Analytical HPLC for Other Polyphenolics. 1. Apparatus. A Varian 5000 Liquid Chromatograph (Varian Instrument Group, Sunnyvale, CA) equipped with a Hewlett-Packard 1040A photodiode array detector and Gateway 2000 P5-90 computer with Hewlett-Packard HPLC^{2D} Chemstation software was used.

2. Column, Mobile Phases, and HPLC Conditions. Chromatographic analysis was done using a Synergi Hydro-RP column (4 μm), 250 mm × 4.60 mm i.d. (Phenomenex), fitted with an Allsphere 10 mm × 4.6 mm i.d. ODS-2 guard column (Alltech). Mobile phase A consisted of 100% HPLC grade methanol, and mobile phase B was 1% formic acid in deionized water. Solvents and samples were filtered through a 0.45 μM Millipore filter type HA for aqueous or HV for organic solvent.

The program was as follows: (a) 0 min, 10% A; (b) 0−50 min linear gradient from 10 to 35% A; (c) 50−55 min linear gradient from 35 to 70% A; (d) 55−60 min isocratic at 70% A; (e) 60−66 min linear gradient from 70 to 10% A; 5 min posttime and gradient repeated. Simultaneous monitoring was performed at 255, 280, 320, 360, and 520 nm at a flow rate of 1 mL/min and an injection volume of 20 μL. Identification was made from matching UV−visible spectra and retention times with authentic standards (when available). Quantitation of individual polyphenolic peaks was done by the external standard method. Catechin and procyanidins were determined as catechin at 280 nm; ellagitannins were determined as ellagic acid at 255 nm; flavonols were determined as rutin at 360 nm; ellagic acid and ellagic acid derivatives were determined as ellagic acid at 255 nm.

Electrospray Mass Spectrometry (ESMS). Low-resolution MS was obtained using ESMS. The instrument was a Perkin-Elmer SCIEX API III bimolecular mass analyzer (Ontario, Canada) equipped with an ion spray interface (ISV = 5500, orifice voltage = 50) and loop injection. The mass spectrometer was operated in the positive mode. The purified anthocyanin fraction was bled into the system by a 100 μL glass syringe connected with the infusion pump at a flow rate of 5 μL/min.

Tandem Mass Spectrometry (MS/MS). Collision-induced dissociation of the purified anthocyanin fraction was carried out using argon as the target gas. The mass of the parent ion was scanned in the first quadrupole (Q1), *m*/*z* selected and collisionally activated in Q2, and the daughter ions were analyzed in the third quadrupole (Q3). MS/MS was set in the multiple reaction monitoring mode and performed using a collision energy set of +30 eV.

Statistical Analyses. Variance components for different levels in the sampling design (plots, subsampling, sample preparation, and measurements) of Marion data were estimated by analysis of variance (ANOVA) method of components using the MIXED procedure in SAS version 8.1 (SAS Software, SAS Institute Inc., Cary, NC). Means and standard deviations of both cultivars were reported.

In the case of Marion data, maturity groups for each variate were compared at the plot level by (i) calculating the mean for each plot-by-maturity combination (3 × 3) and then (ii) analyzing the nine observations using an ANOVA model with plots (2 d.f.), maturity level (2 d.f.), and residual (4 d.f.). The residual measures the consistency of the maturity differences across plots. Tukey's-*b* procedure was used for the pairwise comparisons. Analyses were conducted in SPSS 12.0 Software (SPSS Inc., Chicago, IL), and the significance level was 0.05 unless otherwise indicated.

For the cultivar study, means and standard deviations of the samples were reported. The variability that was observed was a combination of measurement, sample preparation, and subsampling variability. Because of the lack of plot replication and the fact that the cultivar is confounded with plots, the 11 blackberry cultivars were not compared statistically. With regards to the ORAC and FRAP measurements, because there was only one replicate analyzed, measures of variability were not obtained except at the measurement level. No statistical analysis was done due to lack of replication.

RESULTS AND DISCUSSION

Influence of Maturity on Composition. The TSS increased considerably (9.46−16.0 °Brix for Marion, 11.0−18.0 °Brix for Evergreen) during the course of ripening (**Table 1**). The standard deviation for TSS was relatively high indicating considerable sample variation despite the careful attention given in sample collection. TA decreased (2.32 to 1.29 g/100 g FW for Marion, 2.38 to 0.47 g/100 g FW for Evergreen) during ripening as expected. The loss of acids was more rapid in Evergreen berries than in those of Marion. The °Brix:acid ratio of Evergreen blackberries also changed almost 10-fold during ripening. Perkins-Veazie and others (*29*) and Perkins-Veazie and Collins (*30*) observed similar trends in blackberries changing from mottled (50% black) to shiny black and dull black stages, respectively (5.85−7.92 °Brix, 2.45 to 0.98 g citric acid/100 g; $n = 4$).

The total anthocyanin content increased from 74.7 to 317 mg/100 g FW from underripe to overripe for Marion and from 69.9 to 164 mg/100 g FW for Evergreen (**Table 1**). The total anthocyanin content of Marion berries, which significantly increased with ripening, was about the same as that of Evergreen berries in the underripe stage but was almost twice as much in the ripe and overripe stages. Because the data were taken at a single point in time (rather than following groups of berries

Table 2. Variance Component Estimates for Blackberry Composition at Different Sampling Levels for Marion Blackberries

analysis	underripe	% of total variance	ripe	% of total variance	overripe	% of total variance
			TSSs			
plot	~0	~0	~0	~0	~0	~0
subsampling	~0	~0	2.4343	77.78	~0	~0
sample preparation	1.8753	99.96	0.6951	22.21	2.2766	99.98
measurement	0.0007	0.04	0.0003	0.01	0.0004	0.02
total variance	1.8760	100.00	3.1297	100.00	2.2770	100.00
CV (%)[a]	14.48		13.07		9.46	
			TA			
plot	~0	~0	~0	~0	0.0233	46.23
subsampling	0.0066	78.57	0.0137	91.95	0.0266	52.78
sample preparation	0.0017	20.24	0.0012	8.05	0.0004	0.79
measurement	0.0001	1.19	~0	~0	0.0001	0.20
total variance	0.0084	100.00	0.0149	100.00	0.0504	100.00
CV (%)	3.96		9.56		17.36	
			total phenolics			
plot	~0	~0	~0	~0	91751	52.81
subsampling	18678	80.05	18346	26.43	13034	7.50
sample preparation	4055.1	17.38	5076.9	73.14	67837	39.04
measurement	601.08	2.57	297.40	0.43	1119.6	0.65
total variance	23334	100.00	69412	100.00	173741	100.00
CV (%)	15.66		29.19		27.05	
			total anthocyanins			
plot	71.968	46.21	~0	~0	436.10	31.50
subsampling	55.718	35.78	742.57	51.60	~0	~0
sample preparation	1.0329	0.66	~0	~0	146.13	10.55
measurement	27.014	17.35	696.54	48.40	802.28	57.95
total variance	155.73	100.00	1439.1	100.00	1384.5	100.00
CV (%)	16.70		17.15		11.75	

[a] CV (%), percent coefficient of variance was 100 × (square root of total variance divided by overall mean response).

through time), we are comparing ripe earlier-ripening berries to unripe later-ripening berries. Therefore, we cannot conclude that the differences are solely due to ripening (since time of ripening is also different).

Total phenolics did not show as pronounced of a change with ripening. The change in total phenolics for Marion from underripe to ripe stages (975 to 903 mg/100 g FW) was not significant while the increase from ripe to overripe stages (903 to 1541 mg/100 g FW) was significant. Evergreen berries showed an apparent decrease from underripe to ripe (1090 to 960 mg/100 g) and an apparent increase from ripe to overripe (960 to 1035 mg/100 g). The total phenolic contents of Evergreen berries were slightly higher than those of Marion during underripe and ripe stages, but it was the reverse in the overripe stage. In general, total phenolics did not show the marked change with maturity that total anthocyanins did.

Wang and Lin (*3*) and Perkins-Veazie and others (*31*) observed the same trends, from green to ripe (0.93−153 mg/100 g FW) and from green to dull black stages, respectively, on total anthocyanin contents of several thornless blackberry cultivars. Bilyk and Sapers (*32*) also observed a positive correlation between total anthocyanin content and maturity in several thornless blackberry cultivars (79.3−112 mg/100 g; from red to black). Moreover, Wang and Lin (*3*) reported the reduction in total phenolic content from green to ripe stages (295 to 226 mg/100 g FW; $n = 3$).

In both Marion and Evergreen berries, the underripe stage had the lowest ORAC values (**Table 1**). The ORAC values increased from 43.0 to 62.7 μmol TE/g FW for Marions and from 46.1 to 64.4 μmol TE/g FW for Evergreens. Changes in the FRAP values with maturity for the two varieties were inconsistent with the lowest values in ripe berries for Marion (78.2 μmol TE/g FW) and in underripe berries for Evergreen (80.9 μmol TE/g FW). Our ORAC values (mean = 44.6−59.7 μmol TE/g FW; from underripe to ripe stages) were about 2−3 times higher than the range reported by Wang and Lin (*3*) (15.6−22.4 μmol TE/g FW; from pink to ripe stages, $n = 3$).

Variance Component Estimation. An advantage to having plot replication for Marion was accessibility to the estimates of the components of variance at different levels: plots, subsampling, sample preparation, and measurement (**Table 2**). In this and subsequent variance component tables, the entries are estimates of the variance contribution from each level after accounting for the variation at the lower (nested) levels. The estimation method was based on the expectations of the mean squares in nested ANOVA, except that negative estimates were set to zero when in the ANOVA higher level mean squares were slightly smaller than lower level mean squares (*33*). In each column, the plot variance component is based on just two plots, so the precision is low. The number of observations doubles moving down each level in the study design, so the precision for estimating variance components increases until it is greatest for measurement error.

One common trait that we found in the variance components of TSS and TA is that there was almost no variability at the measurement level (**Table 2**). This indicates the consistency of measurement devices, which in this case were the Auto Abbe refractometer and the Metrohm titration unit, respectively. Most of the TSS variation came from sample-to-sample differences (77.78% for ripe) and were caused by sample preparation (ranged from 22.21 to 99.98%).

On the other hand, most of the variation for total phenolics was from sample-to-sample differences (ranged from 7.50 to 80.05%) and sample preparation (ranged from 17.38 to 73.14%). The variation from measurement or the analysis was low (0.43−2.57%) when compared to the above.

For the total anthocyanin content, underripe and overripe berries were more susceptible to plot differences (31.50−

Table 3. Anthocyanins Distribution (% Total Peak Area at 520 nm) of Marion and Evergreen Blackberries at Different Maturity Stages[a]

cultivar	cyanidin 3-glucoside	cyanidin 3-rutinoside	cyanidin-xyloside	cyanidin 3-glucoside acylated with malonic acid	cyanidin 3-dioxalyl-glucoside	total anthocyanins (mg Cyd-3-glu/100 g)
			Marion			
underripe	57.0 ± 4.17a	37.0 ± 3.90b	0.01 ± 0.05a	1.61 ± 0.13b	4.39 ± 0.50b	74.7 ± 11.2a
ripe	72.6 ± 1.49b	24.2 ± 1.42a	0.18 ± 0.03b	1.12 ± 0.08a	1.91 ± 0.17a	221 ± 36.6b
overripe	71.0 ± 1.17b	25.8 ± 1.10a	0.23 ± 0.02c	1.06 ± 0.07a	1.92 ± 0.14a	317 ± 35.3c
			Evergreen[b]			
underripe	70.4 ± 2.29	6.89 ± 0.93	4.87 ± 0.40	7.22 ± 0.48	10.6 ± 1.27	69.9 ± 10.6
ripe	81.2 ± 0.37	2.71 ± 0.04	6.30 ± 0.15	4.72 ± 0.05	5.07 ± 0.42	131 ± 9.62
overripe	84.3 ± 0.16	1.91 ± 0.05	7.53 ± 0.18	3.36 ± 0.04	2.86 ± 0.12	164 ± 11.7

[a] Different letters in the same column for Marion indicate significant differences ($p \leq 0.05$); data expressed as means ± standard deviations ($n = 3$ plots) on a FW basis. [b] Data expressed as means ± standard deviations for two subsamples within one plot ($n = 1$ plot).

46.21%) than that of ripe berries (~0%). Considerable variations were found at sample preparation (35.78% for underripe, 51.60% for ripe) and analysis (ranged from 17.35 to 57.95%) levels.

Influence of Maturity on Anthocyanin Composition. HPLC chromatographic profiles of Marion and Evergreen at different maturity stages were qualitatively the same, but their proportions were different (**Table 3**). Five anthocyanins were identified, using LC-MS/MS, as cyanidin 3-glucoside (*m*/*z* 449.0), cyanidin 3-rutinoside (*m*/*z* 595.1), cyanidin-xyloside (*m*/*z* 419.0), cyanidin 3-glucoside acylated with malonic acid (*m*/*z* 535.1), and cyanidin 3-dioxalylglucoside (*m*/*z* 593.0). The primary anthocyanins in Marion berries were cyanidin 3-glucoside (57−73%) and cyanidin 3-rutinoside (24−37%). On the other hand, the anthocyanin in Evergreen blackberries was predominantly cyanidin 3-glucoside (70−85%).

The anthocyanin profiles for Marion differ from Evergreen in containing substantially more cyanidin-3-rutinoside (**Table 3**). Nevertheless, they show similar trends with ripening, with proportions of cyanidin 3-glucoside increasing (57.0−71.0% for Marion, 70.4−84.3% for Evergreen), increasing cyanidin-xyloside (0.01−0.23% for Marion, 4.87−7.53% for Evergreen), decreasing for cyanidin 3-rutinoside (37.0 to 25.8% for Marion, 6.89 to 1.91% for Evergreen), decreasing cyanidin 3-glucoside acylated with malonic acid (1.61 to 1.06% for Marion, 7.22 to 3.36% for Evergreen), and a marked decrease in cyanidin 3-dioxalylglucoside with ripening (4.39 to 1.92% for Marion, 10.6 to 2.86% for Evergreen). The standard deviation for cyanidin-xyloside was relatively high as compared to the other anthocyanins.

Sapers and others (*9*) reported similar trends in two thornless blackberry cultivars (from pink to black stages) with cyanidin 3-glucoside (37.5−73.8%) and cyanidin derivative containing xylose (1.3−10.2%) increasing and acid acylated derivative of cyanidin 3-glucoside (6.6 to 3.3%) and dicarboxylic derivative of cyanidin 3-glucoside (45.8 to 10.4%) decreasing with increasing ripeness. However, they (*9*) also reported the dicarboxylic derivative of cyanidin 3-glucoside (subsequently identified as cyanidin-3-dioxalyl-glucoside) to be about 4−6-fold higher than our results (mean = 7.50 to 2.39%; underripe to overripe). Their investigation was on Black Satin and Hull Thornless blackberries.

Variance Component Estimation. For the distribution of anthocyanins, the largest source of variation was generally the sample-to-sample (subsampling) component (**Table 4**). We observed that the major anthocyanins, such as cyanidin 3-glucoside and cyanidin 3-rutinoside, are most susceptible to sample-to-sample differences in underripe (55.07−64.67%) and ripe (98.19−98.33%) berries and to plot differences in overripe berries (55.72−61.08%). Overall, there were lesser variations at sample preparation and analysis levels. Cyanidin-xyloside was particularly low in underripe berries, which resulted in a very large coefficient of variation and relatively large variation between measurements on the same sample preparation.

Influence of Maturity on Polyphenolic Composition. HPLC chromatographic profiles of Marion and Evergreen polyphenolics at different maturity stages were qualitatively similar yet quantitatively very different (**Table 5**). Both varieties contained two major ellagitannin peaks (60−80%) and had flavonols and ellagic acid derivatives as minor compounds. Evergreen blackberries had more complex profiles of flavonols and ellagic acid derivatives than did Marion berries.

The changes in the amounts of the different polyphenolic classes with ripening were substantially different between Marion and Evergreen (**Table 5**). In general, Evergreen berries contained higher levels of procyanidins (5.9-fold), flavonols (1.8-fold), and ellagic acid derivatives (2-fold) but lower level of ellagitannins (1.6-fold) than those of Marion. In Marion berries, the trends for procyanidins and ellagitannins were similar, being lower at the ripe stage (not detected for procyanidins, 20.7 mg/100 g FW for ellagitannins) as compared to underripe (0.60 mg/100 g FW for procyanidins, 34.8 mg/100 g FW for ellagitannins) and overripe (13.8 mg/100 g FW for procyanidins, 32.3 mg/g for ellagitannins) stages. On the other hand, the concentrations of procyanidins and ellagitannins of Evergreen blackberries continuously decreased with maturity (38.9 to 19.5 mg/100 g FW for procyanidins, 34.6 to 17.6 mg/100 g FW for ellagitannins). The concentrations of flavonols increased with ripening (5.39−12.4 mg/100 g FW) for Marion berries, while those of Evergreen berries increased from underripe to ripe (14.1−16.0 mg/100 g FW), before slightly decreasing in overripe (15.0 mg/100 g FW). The only similarity between these two varieties was the changes in the concentration of ellagic acid derivatives, which the lowest were those of ripe berries (0.66 mg/100 g FW for Marion, 1.29 mg/100 g FW for Evergreen). The standard deviation for procyanidins, ellagitannins, flavonols, and ellagic acid derivatives was relatively high indicating considerable sample variation.

Bilyk and Sapers (*32*) reported a positive correlation between flavonol contents and blackberry maturity (9.01−15.8 mg/100 g FW for quercetin content, 0.7−1.74 mg/100 g for kaempferol content; from red to black).

Variance Component Estimation. In general, only overripe Marion berries had variations in the polyphenolic concentrations, except for ellagic acid derivatives, at the plot level (ranged from 37.88 to 88.79%) as compared with other maturity stages (**Table 6**). Variability of ellagitannins and flavonols mainly came from sample-to-sample differences in underripe (97.15% for ellagitannins, 94.43% for flavonols) and ripe berries (72.14% for

Table 4. Variance Component Estimates for Blackberry Anthocyanin Distribution at Different Sampling Levels for Marion Blackberries

anthocyanins	underripe	% of total variance	ripe	% of total variance	overripe	% of total variance
			cyanidin 3-glucoside			
plot	9.7381	44.39	~0	~0	1.0892	61.08
subsampling	12.081	55.07	2.4991	98.33	0.6609	37.06
sample preparation	0.1116	0.51	0.0217	0.86	0.0251	1.41
measurement	0.0061	0.03	0.0207	0.81	0.0080	0.45
total variance	21.936	100.00	2.5415	100.00	1.7832	100.00
CV (%)[a]	8.22		2.20		1.88	
			cyanidin 3-rutinoside			
plot	6.2735	33.50	~0	~0	0.8752	55.72
subsampling	12.110	64.67	2.2701	98.19	0.6687	42.57
sample preparation	0.3388	1.81	0.0297	1.29	0.0203	1.29
measurement	0.0034	0.02	0.0122	0.53	0.0066	0.42
total variance	18.726	100.00	2.3120	100.00	1.5708	100.00
CV (%)	11.70		6.29		4.86	
			cyanidin-xyloside			
plot	~0	~0	0.0001	8.32	0.0001	26.02
subsampling	~0	~0	0.0006	66.70	~0	12.57
sample preparation	~0	~0	~0	~0	0.0001	40.06
measurement	0.0024	100.00	0.0002	24.97	0.0001	21.35
total variance	0.0024	100.00	0.0009	100.00	0.0003	100.00
CV (%)	489.89		16.21		8.03	
			malonic acid acylated cyanidin 3-glucoside			
plot	0.0178	80.96	0.0058	75.20	0.0007	13.29
subsampling	~0	~0	0.0016	20.95	0.0036	72.89
sample preparation	0.0039	17.87	~0	0.26	0.0005	10.53
measurement	0.0002	1.17	0.0003	3.59	0.0002	3.30
total variance	0.0220	100.00	0.0077	100.00	0.0050	100.00
CV (%)	9.20		7.87		6.64	
			cyanidin 3-dioxalylglucoside			
plot	0.1936	59.96	0.0146	39.06	~0	~0
subsampling	0.0881	27.28	0.0220	58.81	0.0233	99.52
sample preparation	0.0398	12.31	0.0006	1.61	0.0001	0.28
measurement	0.0014	0.45	0.0002	0.52	~0	0.20
total variance	0.3229	100.00	0.0374	100.00	0.0234	100.00
CV (%)	12.95		10.11		7.98	

[a] CV (%), percent coefficient of variance was 100 × (square root of total variance divided by overall mean response).

Table 5. Concentrations of Polyphenolics (mg/100 g FW) in Marion and Evergreen Blackberries at Different Maturity Stages[a]

cultivar	procyanidins (as catechin)	ellagitannins (as ellagic acid)	flavonols (as rutin)	ellagic acid derivatives (as ellagic acid)
		Marion		
underripe	0.60 ± 2.95	34.8 ± 5.92b	5.39 ± 1.54a	0.96 ± 0.23a
ripe	ND[b]	20.7 ± 3.09a	6.84 ± 1.20b	0.66 ± 0.14a
overripe	13.8 ± 9.36	32.3 ± 5.66b	12.4 ± 1.38c	1.39 ± 0.42b
		Evergreen[c]		
underripe	38.9 ± 5.61	34.6 ± 1.98	14.1 ± 0.99	2.17 ± 0.34
ripe	27.1 ± 13.4	25.4 ± 3.32	16.0 ± 1.56	1.29 ± 0.27
overripe	19.5 ± 9.12	17.6 ± 2.12	15.0 ± 1.65	2.61 ± 0.52

[a] Different letters in the same column for Marion indicate significant differences ($p \leq 0.05$). [b] ND, not detected; data expressed as means ± standard deviations (n = 3 plots) on a FW basis. [c] Data expressed as means ± standard deviations for two subsamples within one plot (n = 1 plot).

ellagitannins, 91.99% for flavonols), while those of procyanidins were from the analysis level for underripe (100.00%) and overripe (42.36%) fruits. Nonetheless, concentrations of ellagic acid derivatives were most affected by sample preparation in ripe (54.53%) and overripe (53.76%) berries and by analysis in underripe berries (65.99%). Procyanidins were particularly low in underripe berries, which resulted in a very large coefficient of variation and relatively large variation between measurements on the same sample preparation. Procyanidins in ripe berries could not be detected.

Influence of Cultivar on Composition. Table 7 summarizes the compositions and antioxidant properties of 11 blackberry cultivars. The TSS of the blackberries ranged from 8.20 to 13.6 °Brix with a mean of 11.5. Waldo fruits (13.6 °Brix) had the highest TSS of the cultivars compared; NZ 9351-4 (12.4 °Brix) and ORUS 1369-3 (11.4 °Brix) were the highest of their selections. The TA ranged from 0.84 to 2.62 g/100 g FW (mean = 1.49). Chester fruits (0.84 g/100 g FW) were the least acidic of the cultivars compared; NZ 9128R-1 (1.13 g/100 g FW) and ORUS 1369-3 (1.30 g/100 g FW) were the least acidic selections. Our results of TSS and TA were similar to the ranges of Sapers and others (*34*) (7.7−13.9 °Brix, 0.4−1.3 g/100 g FW; n = 37) and the TSS of Fan-Chiang (*6*) (6.88−16.8 °Brix; n = 52). The TSS of Chester berries was very close to those reported by Himelrick and Nesbitt (*35*) (8.6 and 8.2 °Brix; years 2000 and 2001).

Table 6. Variance Component Estimates for Blackberry Polyphenolic Composition at Different Sampling Levels for Marion Blackberries

analysis	underripe	% of total variance	ripe	% of total variance	overripe	% of total variance
			procyanidins			
plot	~0	~0			38.423	37.88
subsampling	~0	~0			14.229	14.03
sample preparation	~0	~0			5.8096	5.73
measurement	8.6791	100.00			42.970	42.36
total variance	8.6791	100.00			101.43	100.00
CV (%)[a]	489.90				72.74	
			ellagitannins			
plot	~0	~0	~0	~0	39.563	88.79
subsampling	39.065	97.15	7.7003	72.14	3.6496	8.19
sample preparation	0.8910	2.22	2.6656	24.97	1.2009	2.70
measurement	0.2548	0.63	0.3075	2.88	0.1420	0.32
total variance	40.211	100.00	10.673	100.00	44.5551	100.00
CV (%)	18.21		15.75		20.64	
			flavonols			
plot	~0	~0	~0	~0	1.0622	45.19
subsampling	2.5493	94.43	1.5086	91.99	0.9213	39.19
sample preparation	0.0511	1.89	0.0400	2.44	0.2764	11.76
measurement	0.0994	3.68	0.0914	5.57	0.0909	3.87
total variance	2.6997	100.00	1.6400	100.00	2.3508	100.00
CV (%)	30.47		18.72		12.38	
			ellagic acid derivatives			
plot	~0	~0	~0	~0	~0	~0
subsampling	0.0195	34.01	0.0050	23.04	0.0295	16.29
sample preparation	~0	~0	0.0119	54.53	0.0973	53.76
measurement	0.0378	65.99	0.0049	22.43	0.0542	29.95
total variance	0.0573	100.00	0.0218	100.00	0.1810	100.00
CV (%)	24.95		22.51		30.65	

[a] CV (%), percent coefficient of variance was 100 × (square root of total variance divided by overall mean response).

Table 7. TSS, TA, Maturity Index, Polyphenolic Composition, and Antioxidant Properties of Selected Blackberry Cultivars[a]

cultivar	TSS (°Brix)	TA (citric acid, g/100 g)	maturity index (°Brix:acid)	total phenolics (mg GAE/100 g)	total anthocyanins (mg Cyd-3-glu/100 g)	ORAC[c] (μmol TE/g)	FRAP[c] (μmol TE/g)
Marion	12.9 ± 5.49	1.26 ± 0.12	10.2 ± 5.24	1005 ± 54.2	245 ± 33.9	56.2 ± 4.64	91.7 ± 0.47
Waldo	13.6 ± 0.67	1.83 ± 0.13	7.43 ± 0.85	940 ± 26.1	156 ± 30.1	54.1 ± 1.36	81.7 ± 7.15
Evergreen[b]	11.5 ± 2.32	0.91 ± 0.06	12.6 ± 3.28	959 ± 27.0	131 ± 6.25	75.5 ± 20.7	91.5 ± 3.61
Chester[b]	8.20 ± 1.05	0.84 ± 0.06	9.76 ± 1.88	697 ± 94.5	192 ± 30.6	48.9 ± 3.91	63.2 ± 0.14
Silvan	12.8 ± 1.44	1.13 ± 0.15	11.3 ± 2.90	779 ± 14.2	161 ± 8.22	37.6 ± 2.81	67.4 ± 1.19
NZ 9128R-1	12.1 ± 1.15	1.13 ± 0.04	10.7 ± 1.40	758 ± 46.6	176 ± 11.4	39.0 ± 1.58	63.5 ± 1.87
NZ 9351-4	12.4 ± 0.89	1.93 ± 0.13	6.42 ± 0.86	682 ± 14.6	186 ± 6.66	45.3 ± 1.13	57.8 ± 1.01
ORUS 1843-3	9.64 ± 0.76	2.09 ± 0.07	4.61 ± 0.49	954 ± 40.7	256 ± 35.2	46.4 ± 1.65	84.8 ± 0.26
ORUS 1380-1	11.1 ± 1.52	1.38 ± 0.41	8.04 ± 3.27	1026 ± 104	197 ± 63.2	42.8 ± 1.21	75.2 ± 0.40
ORUS 1489-1	11.1 ± 0.06	2.62 ± 0.28	4.24 ± 0.48	1056 ± 31.5	235 ± 47.2	54.4 ± 6.80	97.3 ± 1.59
ORUS 1369-3	11.4 ± 0.52	1.30 ± 0.16	8.77 ± 1.44	1040 ± 252	246 ± 31.1	51.8 ± 3.00	77.9 ± 7.02
mean ± SD	11.5 ± 2.24	1.49 ± 0.56	8.56 ± 3.37	900 ± 157	198 ± 48.8	50.2 ± 11.7	77.5 ± 13.1

[a] Single combined sample from three plots. [b] Sample from one plot; data expressed as means ± standard deviations (n = 3 subsamples) on a FW basis. [c] Data expressed as means ± standard deviations (n = 1 subsample).

Our data showed that the total phenolic and total anthocyanin contents varied greatly among cultivars. The total phenolic content of the samples ranged from 682 to 1056 mg/100 g FW (mean = 900). The ORUS selections (954−1056 mg/100 g FW) and Marion (1005 mg/100 g FW) had considerably higher total phenolics contents. Total anthocyanins for the 11 cultivars ranged from 131 to 256 mg/100 g FW (mean = 198). The ORUS selections (197−256 mg/100 g FW) and Marion (245 mg/100 g FW) were among the cultivars containing the highest total anthocyanin pigments. Some of the experimental ORUS selections were higher in total anthocyanins and total phenolics than the common commercial varieties indicating the potential for obtaining new cultivars with high pigment/phenolic content through classical plant breeding.

In this study, blackberries had a similar range in total phenolics to our earlier investigation (*11*) (822−844 mg/100 g, mean = 833; n = 2); however, it was approximately twice that reported by Wilska-Jeszka and others (*16*) (448 mg/100 g), Heinonen and others (*36*) (435 mg/100 g), Moyer and others (*4*) (275−678 mg/100 g, mean = 478; n = 32), Sellappan and others (*18*) (418−555 mg/100 g, mean = 486; n = 2), and Wada and Ou (*5*) (495−583 mg/100 g, mean = 539; n = 2), and it was four times higher than that of Wang and Lin (*3*) (204−248 mg/100 g, mean = 226; n = 3). Our total anthocyanins were similar to reports by Fan-Chiang (*6*) (70−201 mg/100 g, mean = 137; n = 52), Wang and Lin (*3*) (134−172 mg/110, mean = 153; n = 3), Moyer and others (*4*) (80−230 mg/100 g, mean = 145; n = 32), Sellappan and others (*18*) (110−123 mg/100 g, mean = 117; n = 2), and Siriwoharn and Wrolstad (*11*) (154−225 mg/100 g, mean = 190; n = 2) but were slightly higher than the results of Wilska-Jeszka and others (*16*) (115 mg/100 g).

Table 8. Anthocyanins Distribution (% Total Peak Area at 520 nm) of Selected Blackberry Cultivars[a]

cultivar	cyanidin 3-glucoside	cyanidin 3-rutinoside	cyanidin-xyloside	cyanidin 3-glucoside acylated with malonic acid	cyanidin 3-dioxalyl-glucoside	monomeric anthocyanins (mg of Cyd-3-glu/100 g)[d]
Marion	73.8 ± 1.07	22.8 ± 1.19	0.22 ± 0.01	1.12 ± 0.07	1.97 ± 0.07	245 ± 33.9
Waldo	87.4 ± 1.58	9.81 ± 1.18	ND	0.76 ± 0.08	2.02 ± 0.35	156 ± 30.1
Evergreen[b]	83.4 ± 0.67	2.59 ± 0.14	6.59 ± 0.43	3.92 ± 0.12	3.53 ± 0.26	131 ± 6.25
Chester[b]	89.2 ± 0.69	ND[c]	3.49 ± 0.61	2.43 ± 0.21	4.87 ± 0.30	192 ± 30.6
Silvan	69.8 ± 2.22	29.9 ± 2.32	0.13 ± 0.12	0.22 ± 0.02	ND	161 ± 8.22
NZ 9128R-1	80.7 ± 0.86	17.9 ± 0.14	0.56 ± 0.97	0.78 ± 0.11	ND	176 ± 11.4
NZ 9351-4	91.0 ± 0.68	7.95 ± 0.66	0.36 ± 0.03	0.72 ± 0.09	ND	186 ± 6.66
ORUS 1843-3	93.9 ± 0.46	5.77 ± 0.44	0.13 ± 0.01	0.15 ± 0.01	ND	256 ± 35.2
ORUS 1380-1	75.3 ± 4.36	21.4 ± 3.78	0.13 ± 0.11	0.69 ± 0.10	2.48 ± 0.59	197 ± 63.2
ORUS 1489-1	92.3 ± 0.91	ND	0.39 ± 0.03	1.40 ± 0.11	5.91 ± 0.81	235 ± 47.2
ORUS 1369-3	74.6 ± 1.85	24.5 ± 1.82	0.12 ± 0.01	0.79 ± 0.04	ND	246 ± 31.1
mean ± SD	82.9 ± 8.36	13.0 ± 10.4	1.10 ± 2.03	1.18 ± 1.07	1.89 ± 2.09	198 ± 48.8

[a] Single combined sample from three plots. [b] Sample from one plot. [c] ND, not detected. [d] On a FW basis; data expressed as means ± standard deviations ($n = 3$ subsamples).

The ORAC values of blackberries ranged from 37.6 to 75.5 μmol TE/g with a mean of 50.2. While Evergreen fruit (75.5 μmol TE/g) had the highest ORAC values of the cultivars compared, it was not highest in either total phenolics or total anthocyanins. FRAP values ranged from 57.8 to 97.3 μmol TE/g (mean = 77.5). ORUS 1489-1 (97.3 μmol TE/g), Marion (91.7 μmol TE/g), and Evergreen (91.5 μmol TE/g) were varieties containing the highest FRAP values.

Our ORAC results were higher than those reported by Jiao and Wang (*20*) (14.8−22.6 μmol TE/g; $n = 6$), Wang and Lin (*3*) (20.3−24.6 μmol TE/g; $n = 3$), Wada and Ou (*5*) (27.5−28 μmol TE/g; $n = 2$), and Siriwoharn and Wrolstad (*11*) (34.3−35.5 μmol TE/g; $n = 2$) but fell within in the range of Moyer and others (*4*) (26.7−78.8 μmol TE/g; $n = 32$). The FRAP values of blackberries were within the ranges of Moyer and others (*4*) (40.6−106 μmol TE/g; $n = 32$) and similar to those of Siriwoharn and Wrolstad (*11*) (74.2−79.1 μmol TE/g; $n = 2$).

Influence of Cultivar on Anthocyanin Composition. Table 8 summarizes the anthocyanin patterns for the 11 blackberry cultivars. We found that the proportions of anthocyanins varied greatly among cultivars. Nonetheless, three anthocyanin patterns were observed. The first pattern (I) was found in eight of the 11 studied cultivars. It consisted of cyanidin 3-glucoside as the major pigment, a second major pigment of cyanidin 3-rutinoside, traces of cyanidin-xyloside, cyanidin 3-glucoside acylated with malonic acid, and/or the presence of cyanidin 3-dioxalylglucoside. The following cultivars all exhibit the pattern: Marion, Waldo, Silvan, NZ 9128R-1, NZ 9351-4, ORUS 1843-3, ORUS 1380-1, and ORUS 1369-3. The second pattern (II) was similar to pattern I but with much smaller amounts of cyanidin 3-rutinoside, which was of the same order as cyanidin 3-glucoside acylated with malonic acid and cyanidin 3-dioxalylglucoside. It contained a larger proportion of cyanidin-xyloside than those obtained in other patterns. It was shown by Evergreen. The last pattern (III) resembles pattern I but with a larger proportion of cyanidin 3-dioxalylglucoside and an absence of cyanidin 3-rutinoside. Both Chester and ORUS 1489-1 exhibited that profile. These patterns are consistent with previous investigations in our laboratory (*6*, *7*). Sapers and others (*9*) observed similar patterns in several thornless blackberry varieties.

There are considerable differences with respect to the proportions of individual anthocyanins among the different cultivars (**Table 8**). Blackberries contained cyanidin 3-glucoside ranging from 69.8 to 93.9% (mean = 82.9), cyanidin 3-glucoside acylated with malonic acid ranging from 0.15 to 3.92% (mean = 1.18), and cyanidin 3-rutinoside, cyanidin-xyloside, and cyanidin 3-dioxalylglucoside all ranging from not detected to 29.9 (mean = 13.0), 6.59 (mean = 1.10), and 5.91% (mean = 1.89), respectively. Our data fell within the range reported by Fan-Chiang (*6*) and matched that of Stintzing and others (*7*) for Evergreen blackberries. However, Sapers and others (*9*) reported almost 6- and 3-fold higher values in cyanidin-containing xylose (mean = 6.4%; $n = 8$) and cyanidin 3-dioxalylglucoside (mean = 5.6; $n = 8$), respectively, and a 9-fold lower value in cyanidin 3-rutinoside (mean = 1.5; $n = 8$).

We discovered a negative correlation between total anthocyanins and cyanidin-xyloside ($r = -0.42$), cyanidin 3-glucoside acylated with malonic acid ($r = -0.35$), and cyanidin 3-dioxalylglucoside ($r = -0.06$) and a slight positive correlation with cyanidin 3-glucoside ($r = 0.10$) and cyanidin 3-rutinoside ($r = 0.05$).

Influence of Cultivar on Polyphenolic Composition. The qualitative composition of blackberry polyphenolics was similar for all cultivars yet quantitatively very different (**Table 9**). The procyanidin concentration ranged from 3.29 to 27.2 mg/100 g FW (mean = 14.1), ellagitannins ranged from 7.77 to 27.2 mg/100 g FW (mean = 18.8), flavonols ranged from 4.06 to 11.9 mg/100 g FW (mean = 7.02), and ellagic acid derivatives ranged from 0.46 to 1.63 mg/100 g FW (mean = 0.94). Evergreen and Waldo berries had the highest flavonol (11.9 mg/100 g FW) and procyanidin (27.2 mg/100 g FW) concentrations, respectively, whereas the ORUS selections were highest in both ellagitannins (27.2 mg/100 g for ORUS-1489-1) and ellagic acid derivatives (1.63 mg/100 g for ORUS-1843-3).

The procyanidin and flavonol concentrations were quite similar to those reported by Heinonen and others (*36*) (10.8 mg/100 g for procyanidins, 8.3 mg/100 g for flavonols). The flavonol concentration was four times higher than those reported by Bilyk and Sapers (*32*) (mean = 1.55 mg/100 g; $n = 9$), approximately half that of Siriwoharn and Wrolstad (*11*) (11.6−17.8 mg/100 g, mean = 14.7; $n = 2$), and one-third that reported by Fukumoto and Mazza (*37*) (mean = 24 mg/100 g; $n = 3$).

Moreover, the flavonol concentrations of Marion and Evergreen were considerably lower than those reported by Wada and Ou (*5*) (11 mg/100 g, Marion; 24 mg/100 g, Evergreen). There may well be flavonols that were not separated with this analytical procedure since this particular gradient was mainly modified for the separation of ellagitannins. These values are considerably lower than our previous investigation (*11*) where we reported a three times higher concentration of ellagic acid

Table 9. Concentrations of Polyphenolics (mg/100 g FW) in Selected Blackberry Cultivars[a]

cultivar	procyanidins (as catechin)	ellagitannins (as ellagic acid)	flavonols (as rutin)	ellagic acid derivatives (as ellagic acid)
Marion	3.29 ± 5.70	21.8 ± 2.19	8.63 ± 2.83	0.84 ± 0.33
Waldo	27.2 ± 6.17	23.6 ± 2.34	8.73 ± 2.46	0.81 ± 0.32
Evergreen[b]	23.8 ± 5.32	21.9 ± 1.67	11.9 ± 1.84	1.38 ± 0.47
Chester[b]	8.49 ± 8.10	7.77 ± 0.38	4.21 ± 0.69	0.46 ± 0.30
Silvan	22.1 ± 19.1	14.9 ± 2.18	5.46 ± 0.85	0.61 ± 0.28
NZ 9128R-1	7.33 ± 6.49	16.1 ± 1.43	6.19 ± 0.56	0.88 ± 0.17
NZ 9351-4	10.7 ± 9.32	8.78 ± 1.10	4.06 ± 1.50	1.04 ± 0.25
ORUS 1843-3	15.2 ± 6.04	21.3 ± 0.51	8.76 ± 1.47	1.63 ± 0.58
ORUS 1380-1	13.5 ± 11.7	25.1 ± 0.48	7.14 ± 0.99	1.10 ± 0.42
ORUS 1489-1	8.42 ± 7.34	27.2 ± 4.18	6.96 ± 0.83	0.91 ± 0.54
ORUS 1369-3	15.5 ± 6.81	18.7 ± 3.28	5.18 ± 0.48	0.71 ± 0.15
mean ± SD	14.1 ± 10.6	18.8 ± 6.40	7.02 ± 2.61	0.94 ± 0.45

[a] Single combined sample from three plots. [b] Sample from one plot; data expressed as means ± standard deviations ($n = 3$ subsamples) on a FW basis.

derivatives (1.64−3.62 mg/100 g, mean = 2.63; $n = 2$). Because we used the same analytical methods as our previous investigations, we believe these to be due to seasonal or plot-to-plot variations.

In conclusion, in Marion and Evergreen blackberries, the total anthocyanin content increased considerably during the course of ripening, while total phenolic content and antioxidant properties did not show such pronounced changes. Identities of five anthocyanins were confirmed in both berries. The anthocyanin profiles of Marion and Evergreen were qualitatively the same, but their proportions were different at different maturity stages with proportions of cyanidin 3-glucoside and cyanidin-xyloside increasing and decreasing for cyanidin 3-rutinoside, cyanidin 3-glucoside acylated with malonic acid, and cyanidin 3- dioxalylglucoside. The changes in the amounts of the different polyphenolic classes with ripening were also substantially different between Marion and Evergreen berries.

The effect of subsampling, sample preparation, and analytical measurement on compositional variation in Marion berries was determined. Sample-to-sample differences and sample preparations were major contributors to variation in total phenolic and total anthocyanins contents. For total anthocyanin content, ripe and overripe berries were more susceptible to sample-to-sample difference, sample preparation, and analytical measurement than those of underripe berries. Most variation in the distribution of anthocyanins was from sample-to-sample differences, especially in the less ripe fruit, with cyanidin 3-glucoside and cyanidin 3-rutinoside being more susceptible than other anthocyanins. Variability of ellagitannins and flavonols mainly came from sample-to-sample differences, while that of procyanidins was from analytical measurements. The ellagic acid derivative content was almost unaffected by sample-to-sample differences, sample preparation, or analytical measurement. It should be noted that samples used in this study were collected from one location with the same growing conditions and fertilization. Still, substantial variations were found. Thus, even more variation would be expected in materials obtained from different farms or fields, such as that in the commercial sector. Plot-to-plot differences also greatly influenced anthocyanin composition and overripe blackberry polyphenolic composition. As a result, this factor should be taken into account especially for one sample studies.

Total phenolic and total anthocyanin contents varied greatly among cultivars. The ORUS selections and Marion contained the highest total anthocyanin and total phenolic contents among the 11 cultivars studied. ORUS 1489-1, Marion, and Evergreen were varieties containing the highest ORAC and FRAP values. The proportion of anthocyanins also varied greatly among cultivars. Nonetheless, three anthocyanin patterns were observed. Evergreen and Waldo berries had the highest flavonol and procyanidin concentrations, respectively, whereas the ORUS selections were highest in both ellagitannins and ellagic acid derivatives. Although Marion and Evergreen blackberries were still an excellent source for dietary antioxidants and anthocyanin pigments, the results showed the potential for obtaining new cultivars with higher pigment and phenolic contents through classical plant breeding.

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