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Underutilized Chokeberry (*Aronia melanocarpa*, *Aronia arbutifolia*, *Aronia prunifolia*) Accessions Are Rich Sources of Anthocyanins, Flavonoids, Hydroxycinnamic Acids, and Proanthocyanidins

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Supporting Information

ABSTRACT: Polyphenols from underutilized black, purple, and red aronia (*Aronia melanocarpa*, *Aronia prunifolia*, and *Aronia arbutifolia*) and 'Viking' (*Aronia mitschurinii*) berries were characterized. Anthocyanin and nonanthocyanin flavonoids were quantitated by UHPLC-DAD-MS and proanthocyanidins by normal-phase HPLC. On a dry weight basis, anthocyanins were mainly cyanidin-3-galactoside, highest in black aronia (3.4−14.8 mg/g) and lowest in red aronia (0.5−0.8 mg/g) as cyandin-3-galactoside equivalents. Berries from 'Viking' and the red accession UC021 had substantially more proanthocyanidins than the other accessions, with 3.3 and 3.8 mg catechin equiv/g, respectively. Chlorogenic acids and quercetin glycosides were most abundant in purple UC047 berries, at 17.3 and 1.3 mg/g, respectively. In contrast to anthocyanin content, total phenol values were highest in berries from red and purple accessions and attributed to phenolic acid and proanthocyanin content. Thus, red, purple, and black aronia berries are rich sources of polyphenols with various levels of polyphenol classes.

KEYWORDS: *chokeberry, aronia, polyphenol, flavonoid, proanthocyanidin, anthocyanin, quercetin, chlorogenic acid*

INTRODUCTION

Berry-derived polyphenols have garnered interest for their antioxidant and anti-inflammatory capacities.[1−3] Increased consumption of anthocyanin-rich foods is associated with reduced hypertension and cardiovascular disease risk in adults.[4,5] *Aronia melanocarpa* (Michx.) Elliott (black chokeberry) berries are one of the richest known sources of dietary polyphenols and anthocyanins.[6,7] Accordingly, clinical and preclinical studies indicate that *A. melanocarpa* juice and polyphenol-rich extracts possess a wide range of bioactivities, including modulation of endothelial function, blood cholesterol levels, inflammation, oxidative stress, and blood pressure.[8−11]

Aronia berries are primarily cultivated in Europe, although acreage in the United States has recently increased. The predominant commercial aronia cultivar is the black-berry-producing 'Viking' (*Aronia mitschurinii* A.K. Skvortsov & Maitul), which was likely hybridized by backcrossing a *Sorbus aucuparia* L. (mountain ash) × *A. melanocarpa* with *A. melanocarpa*.[12,13] Native aronia plants include *A. melanocarpa*, *Aronia prunifolia* (Marshall) Rehder, and *Aronia arbutifolia* (L.) Pers., which are primarily differentiated by berry color and secondarily by foliage pubescence.[12−14] 'Viking' aronia berry polyphenols have been found to consist of cyanidin anthocyanins, proanthocyanidins, flavonols, chlorogenic acid, and neochlorogenic acid.[15,16] Despite this prior work, little is known about the polyphenol composition of other aronia species and genotypes. *A. arbutifolia* produces red and *A. prunifolia* produces purple berries, but their berry polyphenol and anthocyanin profiles are unknown. Likewise, there is limited information about berry polyphenols from wild *A. melanocarpa* accessions.

Defining the polyphenol profiles of *A. melanocarpa*, *A. prunifolia*, and *A. arbutifolia* is necessary to further develop the nutritional properties of this crop and may complement ongoing efforts to characterize their origins and phylogenetic relationships. Underutilized aronia accessions could be selectively used to improve the nutritional, sensory, or other functional properties of commercial varieties of aronia berries. Therefore, the primary objective of this study was to characterize the polyphenol profiles of black, purple, and red aronia berries with defined accession histories. The secondary objective was to develop a single UHPLC-MS method suitable for quantitating aronia anthocyanins, phenolic acids, and flavonols to facilitate broader efforts to characterize polyphenols from commercial and native aronia accessions.

MATERIALS AND METHODS

Chemicals and Reagents. Sephadex LH-20 was purchased from GE Healthcare (Waukesha, WI, USA). Ethyl alcohol was from Pharmco-AAPER (Brookfield, CT, USA). HPLC grade dichloromethane, LC-MS grade methanol, acetone, reagent alcohol, acetic acid, and LC-MS grade formic acid were from Fisher Scientific (Fair Lawn, NJ, USA). Resveratrol, resveratrol-3-glucoside, (+)-catechin, (−)-epicatechin, neochlorogenic acid, chlorogenic acid, caffeic acid, rutin, quercetin-3-glucoside, quercetin-3-galactoside, cyanidin-3-galactoside,

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Table 1. Color, Origin, Harvest Date, and Moisture Content of *Aronia* Accessions Grown in a Test Plot in Storrs, CT, USA

accession	berry color	origin	species	harvest date	plants harvested	moisture (%)
UC021	red	cv. 'Erecta'	*A. arbutifolia* (L.) Pers.	Sept 15, 2011	2	72.2
UC057	red	Manahawkin, NJ	*A. arbutifolia* (L.) Pers.	Sept 15, 2011	1	73.4
UC053	red	Millville, NJ	*A. arbutifolia* (L.) Pers.	Sept 15, 2011	3	70.6
UC047	purple	Plainfield, MA	*A. prunifolia* (Marshall) Rehder	Aug 23, 2011	2	65.1
UC011	purple	Nobleboro, ME	*A. prunifolia* (Marshall) Rehder	Aug 23, 2011	1	64.7
UC033	purple	Groton, CT	*A. prunifolia* (Marshall) Rehder	Aug 23, 2011	1	69.2
PI578096	purple	USDA collection; VA	*A. prunifolia* (Marshall) Rehder	Sept 15, 2011	1	74.5
UC007	black	Chaplin, CT	*A. melanocarpa* (Michx.) Elliot	Aug 23, 2011	1	63.6
UC009	black	Nobleboro, ME	*A. melanocarpa* (Michx.) Elliot	Aug 13, 2011	3	66.8
PI636375	black	USDA collection; Russian Federation	*A. melanocarpa* (Michx.) Elliot	Aug 13, 2011	3	67.5
AMES27010	black	USDA collection; MI	*A. melanocarpa* (Michx.) Elliot	Aug 13, 2011	3	67.0
'Viking'	black	commercial cultivar	*A. mitschurinii* A.K. Skvortsov & Maitul	Aug 13, 2011	3	76.0

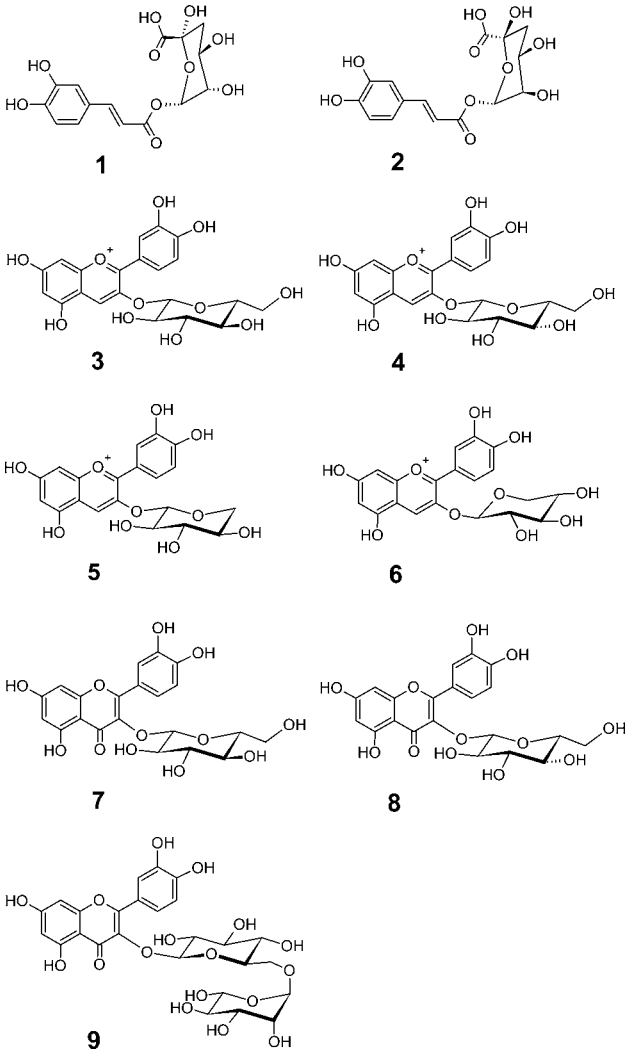

Figure 1. Structures of polyphenols quantitated in aronia berries: (**1**) neochlorogenic acid; (**2**) chlorogenic acid; (**3**) cyanidin-3-galactoside; (**4**) cyanidin-3-glucoside; (**5**) cyanidin-3-arabinoside; (**6**) cyanidin-3-xyloside; (**7**) quercetin-3-galactoside; (**8**) quercetin-3-glucoside; (**9**) quercetin-3-rutinoside.

and all other chemicals were from Sigma-Aldrich (St. Louis, MO, USA).

Aronia Cultivation and Harvest. *A. mitschurinii*, *A. melanocarpa*, *A. arbutifolia*, and *A. prunifolia* accessions originating from locations in the United States and the Russian Federation (n = 1−3 plants/accession) were grown in a test plot in Storrs, CT, USA (Table 1). Plants were identified by Dr. Mark Brand, and voucher specimens were deposited at the University of Connecticut George Safford Torrey Herbarium (CONN) in Storrs, CT, USA. Plant accessions labeled "PI" are identified by their entry in the USDA National Plant Germplasm System, whereas accessions with "UC" or "Ames" labels were unique to Dr. Brand's collection.[13] Plants were started by germination from seeds or through propagation by cuttings or rhizome division, maintained outdoors, in rows, without the use of pesticides or irrigation, for at least 3 years prior to harvest. Fertilizer and herbicides were used to maintain plant beds. Berries were harvested at apparent ripeness, which was determined by pigmentation, tenderness, and the onset of berry shriveling in similar aronia genotypes. For each accession, 100−250 g of berries from all available plants (n = 1−3) was harvested by hand to achieve a representative sample of the entire plant canopy. At harvest, fruit clusters were immediately stored on ice for 2−3 h and then held at 13 °C for a maximum of 48 h. Berries were then cleaned of stems, leaves, and debris and frozen at −80 °C. Only intact, apparently edible berries were used for subsequent analysis. Frozen berries were lyophilized for 24−48 h in a Labconco Freezone 4.5 lyophilizer (Kansas City, MO, USA). For each accession, equivalent portions of lyophilized berries from each plant were pooled to create a ∼25 g composite sample of plants and then ground into a powder using an IKA A11 Basic Grinder (St. Louis, MO, USA). Aronia powders were stored at −80 °C, and composite samples were analyzed by UHPLC-MS within 6 months. Total phenol and DMAC analyses were completed within 9 months of collection.

Polyphenol Extraction. Polyphenols were extracted from lyophilized composite berry powder according to a previously described method, with minor modifications.[17] Berry powder (2 g) was diluted in 40 mL of 70% acetone, 29.5% ultrapure water, and 0.5% acetic acid, sonicated for 5 min, and centrifuged at 950*g* for 10 min. The pellet was re-extracted twice, and supernatants were stored at −80 °C. The pellet was diluted in acidified acetone−water as above, agitated on a test tube rocker for 12 h at 23 °C in darkness to limit any degradation associated with light exposure, and centrifuged as above. The supernatants were combined and dried at 40 °C in a rotary evaporator. Dried extract was reconstituted in 12 mL of 20% methanol in water (v/v), and aliquots were dried under a stream of nitrogen gas at 23 °C.

UHPLC-MS Analysis of Polyphenols. Dried extracts were reconstituted with 1.3 mL of 5% formic acid, 0.5 mL of 100% methanol, and 0.2 mL of 1 mM daidzein in 30% methanol as an internal standard. UHPLC-MS analysis was conducted with a Shimadzu Nexera system equipped with a DGU-20A5 solvent degasser, an SIL-30AC autosampler, a CTO-30A column oven, an SPD-M20A diode array detector, an LCMS-2020 quadrupole mass spectrometer with a DUal Ion Source (DUIS) (Columbia, MD, USA), and a Nitroflowlab nitrogen gas generator (Parker-Balston, Haverhill, MA, USA). Diluted extracts or standards (1 μL) were injected onto a 2.1 mm × 50 mm i.d., 1.7 μm, Kinetex PFP 100A column (Phenomenex Inc., Torrance, CA, USA) and eluted with mobile

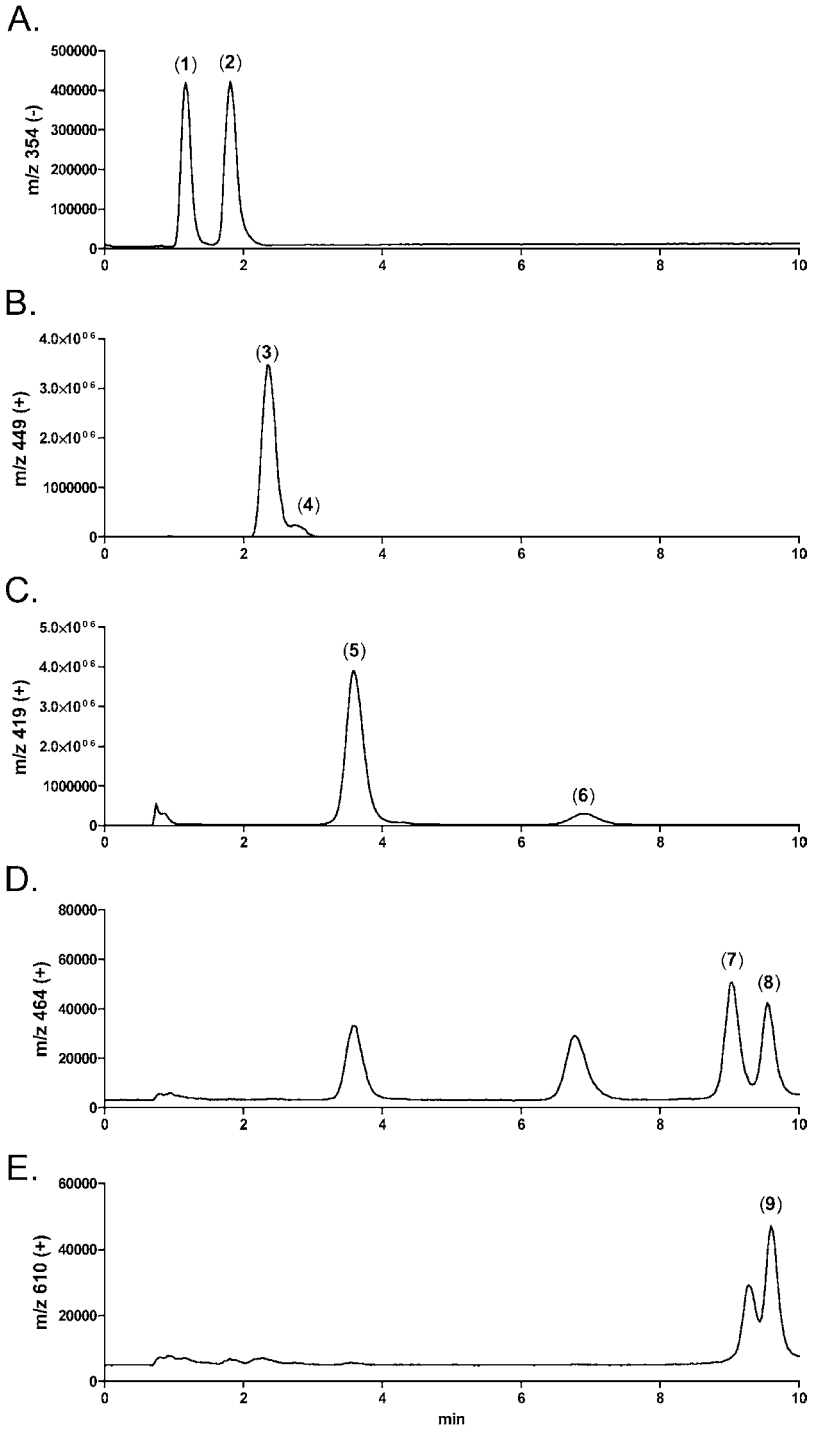

Figure 2. UHPLC-MS chromatograms of polyphenols determined in a 1 μL injection of 'Viking' aronia berry extract. Peaks correspond to (A) m/z 354 (−) (**1**) neochlorogenic acid, (**2**) chlorogenic acid; (B) m/z 449 (+) (**3**) cyandin-3-galactoside, (**4**) cyanidin-3-glucoside; (C) m/z 419 (+) (**5**) cyanidin-3-arabinoside, (**6**) cyanidin-3-xyloside; (D) m/z 464 (−) (**7**) quercetin-3-galactoside, (**8**) quercetin-3-glucoside; and (E) m/z 610 (−) (**9**) quercetin-3-rutinoside.

phases of (A) 5% formic acid in water and (B) 30% methanol in water at 0.2 mL/min. Gradient conditions were 20% B from 0 to 6 min, a linear gradient to 50% B at 12 min, and descending to 20% B over 2 min, and 4 min of column equilibration at 20% B. MS instrument settings were 15 L/min drying gas, 1.5 L/min nebulizing gas, a 250 °C desolvation line, a 500 °C heat block, a detector at 1.1 kV, operating in selected ion monitoring mode cycling between positive and negative ionization modes, and an event time of 0.2 s in DUIS mode.

Compounds identified and quantitated in aronia berries included neochlorogenic acid (nCga), chlorogenic acid (Cga), cyanidin-3-galactoside (Cy3Gal), cyanidin-3-glucoside (Cy3Glu), cyanidin-3-arabinoside (Cy3A), cyanidin-3-xyloside (Cy3X), quercetin-3-galactoside (Q3Gal), quercetin-3-glucoside (Q3Glu), and quercetin-3-rutinoside (Q3R) (Figure 1). Polyphenols except for Cy3A and Cy3X were identified on the basis of comparison with authentic standards, mass spectra, and UV spectral characteristics. Cy3A and Cy3X were identified on the basis of mass spectra, UV absorbance, and expected retention time. Anthocyanins were quantified at 520 nm as Cy3Gal equivalents (CyE), whereas all other compounds were quantified by MS using authentic external standards. Anthocyanins and quercetin

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Table 2. Analytical Parameters, Reproducibility, and Limits of Quantification (LOQ) and Detection (LOD) for UHPLC-MS Analysis of Polyphenols from Aronia Berries

				% RSD[c]		LOQ		LOD	
compound[a]	retention time (min)	m/z (mode)	quantification[b]	interday	intraday	ng OC[d]	μg/g dw[e]	ng OC	μg/g dw
nCga (**1**)	1.2	354 (−)	M − H	16.13	2.97	0.6	14.4	0.5	12.0
Cga (**2**)	1.9	354 (−)	M − H	11.09	2.49	0.7	16.8	0.5	12.0
Cy3Gal (**3**)	2.2	449 (+)	UV	0.34	0.52	1.4	33.6	1.2	28.8
Cy3Glu (**4**)	2.6	449 (+)	UV	8.89	1.65	1.4	33.6	1.2	28.8
Cy3A (**5**)	3.4	419 (+)	UV	0.75	0.45	1.4	33.6	1.2	28.8
Cy3X (**6**)	6.6	419 (+)	UV	5.22	0.94	1.4	33.6	1.2	28.8
Q3Gal (**7**)	9.3	464 (−)	M − H	16.62	5.36	0.5	12.0	0.4	9.6
Q3Glu (**8**)	9.8	464 (−)	M − H	14.18	5.55	0.5	12.0	0.4	9.6
Q3R (**9**)	9.9	610 (−)	M + H	34.00	2.06	0.4	9.6	0.4	9.6
quercetin	14.4	303 (+)	M + H	19.90	3.07	0.5	12.0	0.4	9.6

[a](**1**) neochlorogenic acid; (**2**) chlorogenic acid; (**3**) cyanidin-3-galactoside; (**4**) cyanidin-3-glucoside; (**5**) cyanidin-3-arabinoside; (**6**) cyanidin-3-xyloside; (**7**) quercetin-3-galactoside; (**8**) quercetin-3-glucoside; (**9**) quercetin-3-rutinoside. [b]Quantitation by UV absorbance (UV) as cyanidin-3-galactoside equivalents, positive ionization (M + H) or negative ionization (M − H) as noted. [c]RSD, relative standard deviation. [d]OC, on column. [e]dw, dry weight.

were detected in positive ion mode, whereas all other polyphenols were detected in negative ion mode. The internal standard daidzein was quantified at m/z (+) 254 with a retention time of 12.0 min, and MS areas under the curve were normalized to the internal standard response. Standard curves were run daily, were linear, and had correlation coefficients of >0.999 for cyanidin-3-galactoside from 5 to 500 ng OC and of >0.996 for hydroxycinnamic acids from 5 to 50 ng OC and for flavonols from 1 to 50 ng OC. Limits of detection (LOD) and limits of quantification (LOQ) were determined by injecting standards at progressively higher concentrations until the signal-to-noise ratio approached 3 and 10, respectively.[18] Additional compounds screened for, but not detected in, berry extracts included eriodictyol-3-glucuronide, quercetin-3-vicianoside, quercetin-3-robobinoside, pelargonidin-3-galactoside, pelargonidin-3-arabinoside, caffeic acid, protocatechuic acid, resveratrol, and resveratrol-3-glucoside.[16,19,20]

Proanthocyanidin Quantitation. Proanthocyanidins from aronia berry extracts were isolated by Sephadex LH-20 chromatography and quantitated as (+)-catechin equivalents (CE) by normal phase HPLC.[21] Dried aronia extract was reconstituted in 30% methanol in water and applied to a 2.5 cm diameter column with ∼6 g of Sephadex LH-20 equilibrated in 30% methanol. Columns were first eluted with 50 mL each of 30% methanol in water and 50% aqueous ethanol. Proanthocyanidins were then eluted with 100 mL of 90% acetone in water. Proanthocyanidin fractions were dried by rotary evaporation, reconstituted with 10 mL of acetone, dried with nitrogen gas, and stored in sealed tubes at −20 °C.

Proanthocyanidin fractions were resolved using a Dionex Ultimate 3000 UHPLC (Thermo Scientific, Sunnyvale, CA, USA) equipped with a temperature-controlled autosampler, a column oven, a diode array detector, a fluorescence detector, and a 250 mm ×4.6 mm i.d., 5 μm, Hypersil silica column (ThermoFisher, Bellefonte, PA, USA). The mobile phase consisted of methanol (A), dichloromethane (B), and 50% aqueous acetic acid (v/v) (C). Following 1 or 5 μL injections, the initial conditions were 82% B with 4% C held for the duration of the run. B decreased to 67.6% over the course of 30 min and then to 56.4% at 45 min and was 1% at 55 min. Then, 1% B was held for 5 min, followed by a 5 min increase to 82%, and a 5 min equilibration at 82% B for a total of 75 min. The autosampler temperature was set to 18 °C, and the column temperature was set to 25 °C. Proanthocyanidin monomers to oligomers were quantitated as CE, with polymerization <10 by fluorescence detection with 276 nm excitation and 316 nm emission. Polymers >10 were quantified by absorption at 280 nm because of the large differential of the fluorescence response between oligomers and polymers. Catechin standard curves were linear from 6.25 to 125 ng OC (r^2 > 0.9992).

Proanthocyanidins were also determined by the 4-(dimethylamino)-cinnamaldehyde (DMAC) assay as previously described.[20,22,23] Aronia extracts were suspended in 250 μL of an acetone/water/acetic acid solution, 70:29.5:0.5 (v/v/v) and diluted 100-fold with 91% ethanol in water. (+)-Catechin solutions from 1.56 to 100 μg/mL in 91% ethanol in water were used for standard curves. Standards and aronia samples (70 μL) were incubated with 280 μL of a 1 mg/mL DMAC solution in microtiter wells and read at 640 nm at 23 °C every 60 s for 15 min using a Biotek Synergy HT (Winooski, VT, USA) spectrophotometer. Maximum absorbance values were used to calculate (+)-catechin equivalents, typically at 15 min.

Statistical Analysis. Results are expressed as means ± standard deviation of three analytical replicates, unless indicated otherwise. Data were analyzed using two-way or one-way ANOVA as described, and significance was defined as $P \leq 0.05$. Tukey's multiple-comparison test was performed when data were significant by ANOVA, where significance was $P \leq 0.05$. Principal component analysis was performed using Minitab 16 statistical software (Minitab Inc., State College, PA) using polyphenol content as variables.

RESULTS AND DISCUSSION

Analysis of Polyphenols by UHPLC-UV-MS. Resolution of the predominant aronia berry polyphenols was achieved by a UHPLC-UV-MS method within 10 min (Figure 2). Resolution of Q3Gal from Q3Glu and Cy3Gal from Cy3Glu limited the ability to reduce the run time further. LODs were from 0.4 to 0.5 μg on column (OC) for flavonols by MS and 1.2 μg OC for anthocyanins by UV (Table 2). LOQs were from 0.4 to 0.7 μg OC for flavonoids and were 1.4 μg OC for anthocyanins. Intraday variation was <5.6% for all polyphenols, with an average of 2.45%, whereas interday variation was below 17% with the exception of Q3R, which was 34%. Thus, the described method is a rapid and reproducible means to quantitate aronia berry polyphenols.

Several polyphenols were previously quantified in aronia berries, but not detected by using the present method. Pelargonidin-3-arabinoside has been observed in *A. melanocarpa* at 0.02 mg/g fresh weight (FW), and pelargonidin-3-galactoside has been reported in trace amounts.[19] Quercetin-3-vicianoside and eriodictyol-7-glucuronide have been reported in aronia fruit at 0.05 and 0.24 mg/g fw, respectively.[24] Caffeic acid, a precursor to chlorogenic acid, was previously observed in wild aronia berries at a concentration of 1.4 mg/g fw.[25] Protocatechuic acid was present in a commercial ethanolic aronia extract, which may have resulted from cyanidin degradation during the extraction process.[20,26] It is unclear if the presence of caffeic acid and protocatechuic acid in aronia

Table 3. Polyphenol Contents of Aronia Berries Determined by UHPLC-MS[a]

	hydroxycinnamic acids (mg/g dry weight)		anthocyanins[b] (mg/g dry weight)				flavonols (mg/g dry weight)		
accession	nCga (1)[c]	Cga (2)	Cy3Gal (3)	Cy3Glu (4)	Cy3A (5)	Cy3X (6)	Q3Gal (7)	Q3Glu (8)	Q3R (9)
UC021	nd[d]	8.45 ± 0.17	0.58 ± 0.00	nd	0.02 ± 0.00	nd	0.184 ± 0.005	0.126 ± 0.005	0.159 ± 0.004
UC057	nd	11.2 ± 0.3	0.73 ± 0.00	0.013 ± 0.001	0.08 ± 0.00	nd	0.494 ± 0.006	0.211 ± 0.000	0.482 ± 0.009
UC053	1.09 ± 0.02	4.10 ± 0.07	0.41 ± 0.00	0.008 ± 0.001	0.06 ± 0.00	nd	0.257 ± 0.003	0.121 ± 0.002	0.350 ± 0.004
UC047	3.82 ± 0.02	13.5 ± 0.0	3.88 ± 0.01	0.050 ± 0.001	0.06 ± 0.00	nd	0.411 ± 0.003	0.285 ± 0.006	0.640 ± 0.005
UC011	3.38 ± 0.00	6.68 ± 0.05	2.08 ± 0.04	0.045 ± 0.001	1.01 ± 0.02	0.055 ± 0.002	0.347 ± 0.002	0.233 ± 0.005	0.180 ± 0.001
UC033	3.76 ± 0.02	7.16 ± 0.05	1.53 ± 0.01	0.038 ± 0.002	0.82 ± 0.01	0.015 ± 0.000	0.403 ± 0.008	0.240 ± 0.003	0.460 ± 0.008
PI578096	2.37 ± 0.01	3.11 ± 0.02	2.49 ± 0.01	0.041 ± 0.002	0.71 ± 0.00	nd	0.150 ± 0.003	0.170 ± 0.003	0.176 ± 0.002
UC007	3.22 ± 0.05	6.42 ± 0.10	2.21 ± 0.01	0.049 ± 0.001	1.05 ± 0.01	0.057 ± 0.002	0.320 ± 0.011	0.239 ± 0.005	0.181 ± 0.005
UC009	3.14 ± 0.06	3.87 ± 0.06	14.50 ± 0.10	0.191 ± 0.003	0.18 ± 0.00	nd	0.468 ± 0.005	0.371 ± 0.003	0.189 ± 0.002
PI636375	6.54 ± 0.04	3.32 ± 0.02	4.50 ± 0.05	0.109 ± 0.012	2.16 ± 0.02	0.126 ± 0.004	0.558 ± 0.003	0.424 ± 0.002	0.177 ± 0.003
AMES27010	2.16 ± 0.04	5.06 ± 0.15	3.95 ± 0.01	0.098 ± 0.003	2.04 ± 0.01	0.150 ± 0.005	0.526 ± 0.012	0.302 ± 0.008	0.158 ± 0.003
'Viking'	2.50 ± 0.02	3.88 ± 0.01	9.00 ± 0.05	0.469 ± 0.008	4.06 ± 0.02	0.391 ± 0.004	0.461 ± 0.003	0.389 ± 0.001	0.159 ± 0.001

[a]Data are expressed as mean ± standard deviation of triplicate determinations of a composite sample; statistical significance levels by two-way ANOVA were $P < 0.0001$ for polyphenol and genotype and $P = 0.0001$ for their interaction. [b]Anthocyanins are expressed as cyandin-3-galactoside equivalents. [c](1) neochlorogenic acid; (2) chlorogenic acid; (3) cyanidin-3-galactoside; (4) cyanidin-3-glucoside; (5) cyanidin-3-arabinoside; (6) cyanidin-3-xyloside; (7) quercetin-3-galactoside; (8) quercetin-3-glucoside; (9) quercetin-3-rutinoside. [d]nd, below limit of detection.

extracts may be an artifact of the extraction process or dependent on extraction solvent. *trans*-Resveratrol was reported in aronia wine at a concentration of 8.67 μg/mL; however, stilbenes, such as resveratrol and resveratrol-3-glucoside were not detected in our aronia extracts.[27] The present analysis used lyophilized whole berries. It is unclear if fermentation increases stilbene content or if it is necessary to use a more selective method of ethanol or ethyl acetate extraction and SPE cleanup to quantitate aronia stilbenes from dried berries.[28,29] Nonetheless, the present UHPLC-MS method is an efficient and reproducible means to characterize the majority of aronia nonproanthocyanidin polyphenols.

Polyphenol Content by UHPLC-UV-MS. Cy3Gal, Cy3Glu, and Cy3A were detected in 12 aronia accessions, whereas Cy3X was observed in only some black and some purple berries (Table 3). Red aronia berries had the least total anthocyanin content with 0.63 mg CyE/g dw, whereas black had the greatest, with 9.06 mg CyE/g dw ($P = 0.0171$) (Table 4). UC009 and 'Viking' had the highest concentrations of total anthocyanins; however, UC009 had relatively high Cy3Gal (14.5 mg/g dw) and little of the other anthocyanins, whereas 'Viking' had relatively high Cy3Gal and Cy3A with 9 and 4.06 mg CyE/g dw, respectively. The order of anthocyanin abundance among aronia berries was generally Cy3Gal > Cy3A > Cy3Glu > Cy3X (if present). Wu et al. observed a similar order of anthocyanin content in aronia berries; however, Cy3X was in higher quantities than Cy3Glu.[19]

In contrast to anthocyanin content, flavonol and hydroxycinnamic acid contents were not related to berry color. UC047 had the highest flavonol content by a considerable margin (about 64% greater than the second highest accession), with a sum of 1.33 mg/g dw, and UC053 had the least flavonols (Table 3). Notably, two red accessions (UC057 and UC053) and two purple accessions (UC033 and UC047) had 0.85−3-fold more Q3R than other accessions. Aronia color did not correlate with quercetin glycoside content. All but one aronia accession had more Q3Gal than Q3Glu in berries. Eriodictyol-7-glucuronide, quercetin-3-vicianoside, and quercetin-3-robinobioside have previously been observed in black aronia berries.[16,24]

Hydroxycinnamic acid content was greatest in purple accessions and the red accession UC057, but otherwise similar across aronia berries, ranging from 5.2 to 17.3 mg/g dw (Table 3). UC047 had the most hydroxycinnamic acids among aronia berries, of which 78% was Cga. Cga was present in all aronia accessions at 3.1−13.5 μg/g dw. nCga was present in purple and black accessions, but detected in only one red accession, at a level at least 50% less than that of the black and purple berries. Thus, further work is warranted to determine if nCga can differentiate red from purple and black aronia varieties. nCga contents of purple and black berries were similar; however, PI636375 had at least 71% more nCga than other accessions. Similarly, Slimestad et al. reported that Norwegian black aronia berries had more nCga than Cga, with 1.23 and 0.61 mg/g fw, respectively.[21]

Aronia Proanthocyanidin Content. Aronia berry proanthocyanidins were mainly polymers >10, with only 0.02−0.38% monomer to oligomers based on normal phase HPLC analysis (Figure 3; Table 5). This disparity required separate injections to facilitate quantification without detector saturation. Proanthocyanidin content of aronia berries was not significantly different by berry color. UC057 and UC021 represented the lowest and highest concentrations of proanthocyanidins at 1.1−

Table 4. Polyphenol Contents of Aronia by Berry Color as Sum of Polyphenol Content on a Dry Weight Basis[a]

	aronia berry color			
polyphenol (units)	red	purple	black	*P* value of means
anthocyanins (μg CyE[b]/g)	0.63 ± 0.17 (0.48−0.82)	3.21 ± 0.65 (2.4−3.99)	9.06 ± 5.06 (3.37−14.87)	0.0171
hydroxycinnamic acids (μg/g)	8.27 ± 3.01 (5.19−11.16)	10.95 ± 4.87 (5.48−17.28)	8.02 ± 1.61 (6.38−9.85)	0.4166
nonanthocyanin flavonoids (μg/g)	0.79 ± 0.36 (0.47−1.18)	0.92 ± 0.37 (0.50−1.34)	0.98 ± 0.15 (0.74−1.16)	0.6837
proanthocyanidins, HPLC (mg CE/g)	2.17 ± 1.34 (1.10−3.67)	1.93 ± 0.14 (1.72−2.29)	2.30 ± 0.62 (1.56−3.26)	0.7662
proanthocyanidins, DMAC (mg CE[c]/g)	12.15 ± 7.28 (4.25 −18.6)	9.28 ± 4.49 (5.24−12.9)	10.34 ± 1.52 (9.25−13.5)	0.7040
total phenols (mg GAE[d]/g)	185 ± 56 (151−250)	196 ± 41 (148−246)	164 ± 32 (127−197)	0.5168

[a]Data are expressed as mean ± standard deviation (range) for $n = 3$, red; $n = 4$, purple; and $n = 5$, black aronia accessions. Standard deviation reflects biological replicates within berry color; statistical analysis was by one-way ANOVA by the pooled mean values of the sum of the polyphenol class for each color. [b]CyE, cyanidin-3-galactoside equivalents. [c]CE, catechin equivalents. [d]GAE, gallic acid equivalents.

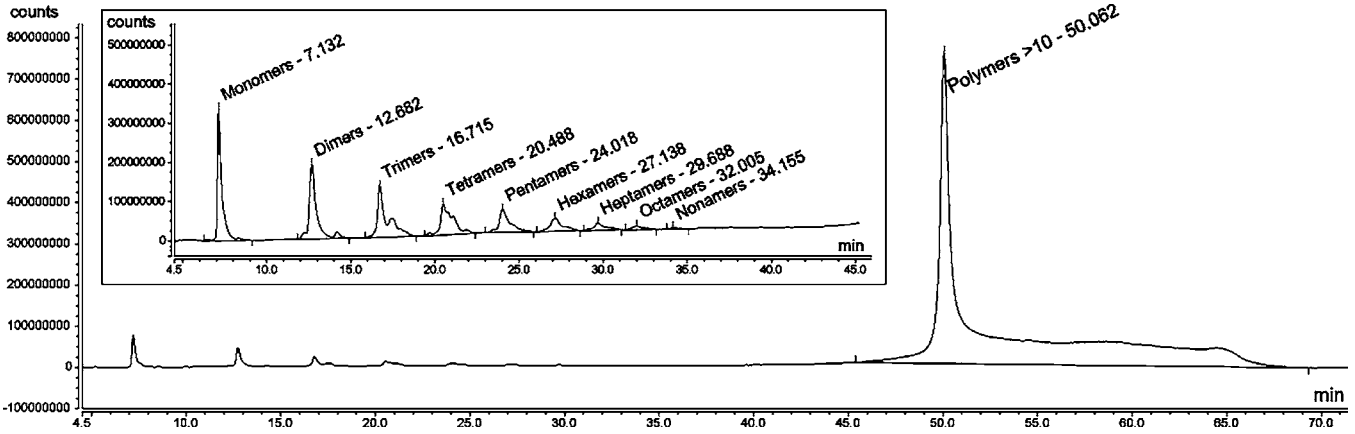

Figure 3. Normal-phase HPLC resolution of *Aronia arbutifolia* accession UC021 (red chokeberry) proanthocyanidins following purification by Sephadex LH-20. Injection volume was 1 or 5 μL (inset) with fluorescence detection by excitation at 276 nm and emission at 316 nm.

Table 5. Proanthocyanidin Content of Aronia Berries Determined by Normal Phase HPLC (Micrograms per Gram Dry Weight)[a]

accession	monomers	dimers	trimers	4−6-mers	7−10-mers	polymers	sum
UC021	0.04 ± 0.00	1.32 ± 0.15	3.16 ± 0.26	6.69 ± 0.52	1.41 ± 0.15	3658 ± 277	3671 ± 278
UC057	0.05 ± 0.00	0.48 ± 0.04	0.78 ± 0.06	1.65 ± 0.14	0.27 ± 0.03	1096 ± 36	1099 ± 36
UC053	0.01 ± 0.00	0.14 ± 0.02	0.75 ± 0.13	2.24 ± 0.54	0.50 ± 0.15	1727 ± 25	1731 ± 25
UC047	0.09 ± 0.00	1.24 ± 0.09	1.89 ± 0.09	3.75 ± 0.19	0.71 ± 0.03	2009 ± 42	2017 ± 42
UC011	0.19 ± 0.01	0.69 ± 0.09	0.87 ± 0.09	1.99 ± 0.21	0.45 ± 0.06	1968 ± 66	1972 ± 67
UC033	0.02 ± 0.00	0.55 ± 0.07	1.77 ± 0.12	3.63 ± 0.36	0.75 ± 0.11	1987 ± 136	1994 ± 137
PI578096	0.01 ± 0.00	0.14 ± 0.04	0.45 ± 0.06	1.58 ± 0.25	0.47 ± 0.09	1721 ± 42	1724 ± 43
UC007	0.01 ± 0.00	0.25 ± 0.04	0.74 ± 0.07	1.70 ± 0.21	0.36 ± 0.08	2331 ± 1	2334 ± 2
UC009	0.01 ± 0.00	0.39 ± 0.04	0.86 ± 0.07	1.75 ± 0.19	0.36 ± 0.05	2290 ± 77	2293 ± 77
PI636375	0.01 ± 0.00	0.22 ± 0.04	0.49 ± 0.03	1.02 ± 0.05	0.21 ± 0.01	1562 ± 41	1564 ± 41
AMES27010	0.02 ± 0.00	0.28 ± 0.07	0.63 ± 0.09	1.32 ± 0.17	0.24 ± 0.03	2057 ± 112	2060 ± 112
'Viking'	0.01 ± 0.00	0.10 ± 0.00	0.22 ± 0.02	0.48 ± 0.04	0.10 ± 0.01	3258 ± 210	3259 ± 210

[a]Proanthocyanidin content was determined following aqueous acidic acetone extraction of *A. mitschurinii*, *A. melanocarpa*, *A. arbutifolia*, and *A. prunifolia* berries (Table 1) and Sephadex LH-20 purification; data are expressed as mean ± standard deviation of duplicate determinations of a composite sample representing equal masses from available plants (Table 1); proanthocyanidins are expressed as (+)-catechin equivalents; statistical significance levels among values by two-way ANOVA were $P < 0.0001$ for degree of polymerization, genotype, and their interaction.

3.67 mg CE/g dw, respectively. A study by Wu et al. found the proanthocyanidin concentration of aronia berries to be 6.64 mg/g fw, which is >6-fold higher than the aronia samples in the present study.[19] They also reported that 81.7% of aronia proanthocyanidins were polymers, which is less than the present study. These differences may be due to the use of purified cocoa and blueberry proanthocyanidin standards for quantitation compared to catechin equivalents used in the present study. Aronia proanthocyanidins have been identified as procyanidin B-type, containing (epi)catechin subunits.[19,30]

Aronia berry proanthocyanidin content determined by the DMAC assay ranged from 4.3 to 18.6 mg CE/g dw (Table 4). DMAC and HPLC proanthocyanidin values of berries were not highly correlated ($r = 0.449$, $P = 0.143$). The lower HPLC proanthocyanidin content of berries relative to DMAC could reflect incomplete recovery of tannins after Sephadex LH-20 fractionation. The DMAC assay may overestimate proanthocyanidin by reactions with interfering compounds, but could also underestimate polymeric proanthocyanidins.[21]

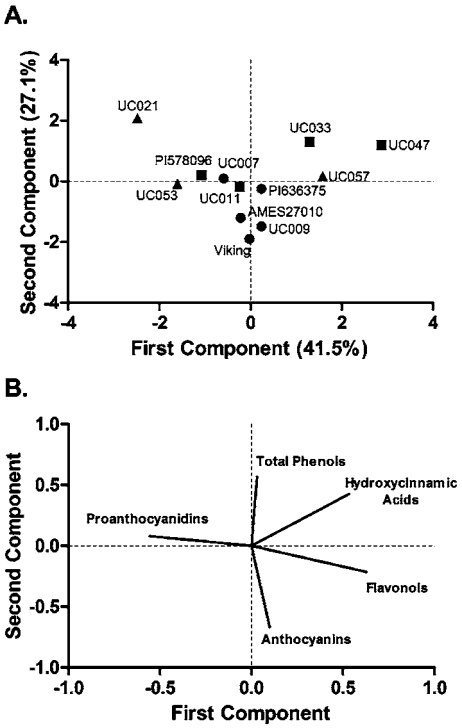

Figure 4. Principal component analysis of the polyphenol contents of *Aronia mitschurinii*, *A. melanocarpa*, *A. prunifolia*, and *A. arbutifolia* berries: (A) biplot of principal components 1 and 2, where ▲ are red, ● are black, and ■ are purple berries; (B) loading plot of the first two principal components of aronia polyphenol content.

Principal Component Analysis. Principal component analysis was used to distinguish aronia accessions by berry color and polyphenol distribution (Figure 4). UC021, UC009, 'Viking', and UC047 were particularly distinct in their distribution of phenol classes. The red accession UC021 had relatively lower levels of anthocyanins and flavonoids, higher proanthocyanidins, and the highest total phenol value among aronia berries in the present study. The purple accession UC033 did not show increased levels of any particular polyphenol, but had a comparatively high total phenol value. The only polyphenol classes with consistent grouping to polyphenol content were black accessions and anthocyanin content.

Polyphenol Content on Fresh Weight Basis. Comparing aronia berry polyphenol content on a fresh weight basis is necessary to estimate the potential nutrient intake from defined serving sizes. The moisture content of aronia berries varied from 63.6% (UC007) to 76% ('Viking'). On a fresh weight basis, Cy3Gal content was greatest in UC009 and lowest in the red aronia accessions. Because moisture content varied <20%, rank order of polyphenol content between accessions on a fresh weight basis did not change drastically relative to dry weight basis. UC007 berry Q3Gal was 5% higher compared to 'Viking' by fresh weight, but 'Viking' had 30.6% more Q3Gal than UC007 by dry weight. On the basis of the present study, consuming 100 g of fresh berries would supply 16−490 mg anthocyanin, 140−320 mg hydroxycinnamic acids, and 13−37 mg quercetin glycosides. Black aronia berry, black currant, and black elderberry have comparatively higher anthocyanin content than other berries.[31]

In conclusion, the color of black, purple, and red aronia berries was apparently due to the abundance of cyanidin anthocyanins rather than the presence of peonidin or petunidin anthocyanins or the variation of copigmenting polyphenols. Black and purple aronia berries contained 1.9−17-fold more cyanidins than red accessions. In contrast, aronia proanthocyanidins and flavonoids were unrelated to berry color. nCga was at low levels or absent in red berries, so this compound could potentially be used to distinguish *A. arbutifolia* from *A. prunifolia*. The anthocyanin profiles of aronia berries remained nearly uniform despite their color differences, with Cy3Gal being the primary anthocyanin, followed by Cy3A, Cy3Glu, and Cy3X if present. Relative to the underutilized varieties analyzed, the commercial 'Viking' cultivar has a comparable polyphenol content. Underutilized aronia berries, such as *A. arbutifolia* and *A. prunifolia*, have unique polyphenol profiles warranting further investigation of their comparative nutraceutical and commercial values. Future analytical efforts should establish the extent that interplant differences, cultivation, environment, and yearly differences contribute to the variability in aronia polyphenols. Also, further effort is needed to distinguish aronia genotypes to more accurately compare polyphenol or other nutritionally relevant contents.

■ ASSOCIATED CONTENT

Supporting Information

Table S1: Sources and Propagation of Aronia Accessions Utilized in the Present Study. Table S2: Anthocyanin Content of Aronia Berries Determined by UHPLC-MS as mg/g Fresh Weight. Table S3: Proanthocyanidin Content of Aronia Berries Determined by Normal phase HPLC (mg/g fresh weight). Table S4: Polyphenol Composition of Aronia Berries as Sum of Polyphenol Classes on a Fresh Weight Basis. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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