Anthocyanins and other polyphenolics in American elderberry (*Sambucus canadensis*) and European elderberry (*S. nigra*) cultivars

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Abstract

BACKGROUND: Ten genotypes representing two elderberry species, *Sambucus canadensis* L. (eight genotypes) and *S. nigra* L. (two genotypes), were examined for their anthocyanins (ACY), total phenolics (TP),°Brix, titratable acidity (TA), and pH over two growing seasons.

RESULTS: Overall, fruit generally had higher ACY, TP, ACY/TP,°Brix, and pH in 2005 than 2004. All samples of *S. canadensis* had similar anthocyanin profiles to one another, but were distinctly different from *S. nigra*. Both species had cyanidin-based anthocyanins as major pigments. Previously unreported anthocyanins were identified in some samples in this study. Trace levels of delphinidin 3-rutinoside were present in all elderberry samples except cv. 'Korsør'. Also, petunidin 3-rutinoside was detected in cvs 'Adams 2', 'Johns', 'Scotia', 'York', and 'Netzer' (*S. canadensis*). The identified polyphenolics of both species were mainly composed of cinnamic acids and flavonol glycosides. The major polyphenolic compounds present in *S. canadensis* were neochlorogenic acid, chlorogenic acid, rutin, and isorhamnetin 3-rutinoside, while chlorogenic acid and rutin were found to be major polyphenolic compounds in *S. nigra*.

CONCLUSION: Sufficient variability was seen among these genotypes to suggest that a successful breeding program could be carried out to improve levels of the various compounds evaluated in this study.

Keywords: *Sambucus canadensis*; *S. nigra*; elderberry; elder; anthocyanin; polyphenolic; minor crop

INTRODUCTION

Elderberries (family Caprifoliaceae) are large deciduous shrubs or small trees native mostly to the northern hemisphere, although they have become naturalized throughout much of the temperate and subtropical regions where humans live.[1] Because their fruit are highly desirable to birds, elderberry rapidly colonizes moist areas along railways, roadways, forest edges, and fence lines. The large, pinnately compound leaves are typically dark green, although ornamental selections have been identified that are variegated, lime green, and dark purple and are popular plants for landscaping. Hundreds of small white hermaphroditic flowers are borne in flat umbels and likewise the fruit are individually small (0.3–0.6 cm) but collectively the hundreds of fruits produce very large clusters. Fruit of the cultivated elderberry are very dark purple, nearly black, but the various species range from bright red to blue and dark purple. The mild-flavored fruits ripen in mid to late summer. For commercial harvest, the entire cluster is picked and the entire crop is processed into juice or purée.[2] Elderberry bark, roots, stems, flowers, and fruit have been used by Native American cultures as medicine, foods and to produce toys and tools.[3]

Commercial elderberry production is concentrated within Oregon in the USA, Denmark, Italy, and Austria. In addition, wild harvested fruit is sold commercially in a number of areas, particularly the midwestern USA. In Kansas, commercial processors, particularly wineries that have relied on wild harvested fruit, are now driving the establishment of commercial plantings. While European and US production practices are similar, Europe relies on cultivars derived from *S. nigra* (commonly known as European elder) and the USA on cultivars derived from *S. canadensis*

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(commonly known as American elder) and *S. nigra*.[2] Most of the botany, plant and fruit development are similar for these two species. However, *S. nigra* tends to be a single- or few-trunked large shrub whereas *S. canadensis* can have many canes and can spread aggressively by underground rhizomes.[1,2]

A number of *S. canadensis* cultivars have been developed in the USA, primarily from the New York and Nova Scotia Agricultural Experiment Station breeding programs (e.g. 'Adams 1', 'Adams 2', 'Johns', 'York', 'Nova'). The origin of many of the European *S. nigra* plantings is less clear, although the Danish developed cultivars 'Allesø', 'Korsør,' 'Sambu', and 'Haschberg'.

Small fruits containing anthocyanins and other polyphenolics have received much attention, due to their potential health benefits.[4–7] Anthocyanins and polyphenolics are important fruit quality indicators, and strongly influence the appearance and flavor of berries and berry products. *Sambucus nigra* has been examined for its potential as a natural colorant and as a botanical supplement for human nutrition by numerous researchers.[8–12] Fruit products from *S. nigra* and *S. canadensis* have been studied for their stability in response to heat and light.[10,13] *Sambucus nigra* has no acylated anthocyanins whereas *S. canadensis* contains the more stable acylated anthocyanins.[11,12,14] Food scientists have examined different cultivars and processing methods for improving the yield and stability of anthocyanins, polyphenolics, and antioxidant activities of small fruit products.[7–9,11,15–21] Ultimately, the final processed product is directly influenced by the starting material, so berry quality is crucial. Horticulturists have been interested in developing plants with the superior plant growth and yield habits of *S. nigra* combined with the superior fruit quality of *S. canadensis* (Finn CE, personal communication).

Only three studies have examined the anthocyanin composition of *S. canadensis*,[13,22,23] and in many studies cultivars have rarely been identified.[11–13,19–26] Brønnum-Hansen and Hansen[12] examined 26 cultivars of *S. nigra*, but did not show individual cultivar data. Kaack and Austed[19,20] reported the total and individual anthocyanin content of 13 cultivars of *S. nigra*. Research papers describing the anthocyanin composition of different *S. canadensis* cultivars appear to be nonexistent. Numerous factors influence the phenolic content of any fruit or fruit product, including: species, cultivar, ripeness, growing season, yield, field management practices, environmental factors, post-harvest storage, and processing factors.[7,12,27–30] The objective of this study was to compare overall chemical composition, and the anthocyanin and other polyphenolic profiles of eight different genotypes of *S. canadensis* ('Adams 1', Adams 2', 'Johns, 'Scotia', 'York', 'Gordon B', 'Netzer', and 'Harris 2') with two different genotypes of *S. nigra* ('Korsør' and 'Haschberg'). This is the first paper to report the anthocyanin and phenolic composition of ten elderberries established at the USDA-ARS breeding program at Corvallis, OR, USA.

MATERIALS AND METHODS

Plant material

Two species and ten different genotypes were examined in this study (Table 1). The two species included the European elder (*S. nigra*) and the American elder (*S. canadensis*). The genotypes represented commonly grown commercial cultivars in addition to selections from the wild in the midwestern USA by Patrick Byers with Missouri State University (Mountain Grove, MO, USA). 'Harris 2' was included because to the human eye it looked much less purple and more red than the other genotypes. Berries of all genotypes representing both species were harvested from plants grown at the US Department of Agriculture Agricultural Research Service (USDA-ARS) National Clonal Germplasm Repository (Corvallis, OR, USA). The cultivars were planted in single plant plots in a randomized complete block with four blocks. Samples were harvested from early July to mid August 2004 and in August 2005 (two-season replication). When an entire umbel of fruit was ripe (based on color), the umbels were cut, kept cool in an ice chest containing ice, and

Table 1. Elderberry (*Sambucus* sp.) genotypes evaluated in this study, their origin, and the number of samples evaluated. All genotypes were grown at the U.S. Department of Agriculture-Agricultural Research Service (USDA-ARS) Corvallis, OR, USA

			Number of genotypes sampled for the two harvest years	
Genotype ($n = 10$)	Species	Origin	2004	2005
Adams 1	*S. canadensis*	Wild selection from New York, 1926	3	3
Adams 2	*S. canadensis*	Wild selection from New York, 1926	4	3
Johns	*S. canadensis*	Wild selection from Ontario released in Nova Scotia, 1954	3	3
Scotia	*S. canadensis*	Adams 2 open pollinated, Nova Scotia, 1959	3	3
York	*S. canadensis*	Adams 2 × Ezyoff, New York, 1964	3	3
Gordon B	*S. canadensis*	Wild selection, Missouri, ~1999	3	3
Netzer	*S. canadensis*	Wild selection, Missouri, ~1999	2	2
Harris 2	*S. canadensis*	Wild selection, Missouri, ~1999	1	1
Korsør	*S. nigra*	Denmark	3	3
Haschberg	*S. nigra*	Wild selection, Austria	3	3

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immediately frozen upon arrival at the laboratory (within 2 h of collecting). Fruit were collected from all elderberry plants that produced ripe berries. Berry samples were then stored at −20 °C until analysis. After freezing, the bags containing the samples were shaken and gently dropped to remove the berries easily from the vegetative umbel tissue, before extracting the berries. Details of the original collection locations are provided in Table 1. Each genotype was analyzed separately.

Reagents and standards

All chemicals used in this study were obtained from Sigma Chemical Co. (St Louis, MO, USA). All solvents and chemicals for this investigation were of analytical and high-performance liquid chromatography (HPLC) grade. Chlorogenic acid isomers were obtained by the method described by Nagels *et al*.[31]

Extraction

Frozen elderberries were extracted as described by Rodriguez-Saona and Wrolstad.[32] Briefly, samples were liquid nitrogen powdered using a mortar and pestle. Five grams of powdered sample were sonicated for 10 min with 10 mL of acidified methanol (0.1% v/v formic acid), followed by two additional re-extractions with acidified methanol. The supernatant was collected, and methanol was evaporated with a Labconco rotovapor (Westbury, NY, USA) at 40 °C. The aqueous extract was redissolved to a final volume of 25 mL with distilled water. Extracts were then kept at −80 °C until analysis. Extractions were replicated twice.

°Brix, titratable acidity (TA), pH, and berry size

The powdered samples were centrifuged and the supernatants were utilized to determine percent soluble solids (°Brix). A Leica AR200 refractometer (Reighert Inc., Depew, NY, USA) was used to measure °Brix. TA was determined by titrating each sample (5 g of homogenate + 45 mL of CO_2-free distilled water) with standardized 0.1 mol L^{-1} NaOH to pH 8.1 using a pH meter. TA was expressed as citric acid equivalents (g citric acid 100 g^{-1} berries). The pH of the purées was determined with a Mettler-Toledo SevenMulti pH meter (Mettler-Toledo Inc., Columbus, OH, USA) equipped with a Mettler-Toledo InLab 410 electrode. Berry size was determined by counting the number of berries per 100 g sample. All measurements were conducted in duplicates.

Total monomeric anthocyanins (ACY) and total phenolics determination

ACY were determined using the pH differential method.[33] Absorbance was measured at 520 and 700 nm. ACY were expressed as cyanidin 3-glucoside (molar extinction coefficient of 26 900 L cm^{-1} mol^{-1} and molecular weight of 449.2 g mol^{-1} was used). The unit for ACY was milligrams of cyanidin 3-glucoside equivalent 100 g^{-1} berries. Total phenolics (TP) were measured by the Folin–Ciocalteu (FC) method.[34] Absorbance was measured at 765 nm. TP was expressed as milligrams of gallic acid equivalent 100 g^{-1} berries. A SpectraMax M2 microplate reader (Molecular Devices Corp., Sunnyvale, CA, USA) was used for both measurements. Measurements of ACY and TP on sample extracts were run in duplicate.

High-performance liquid chromatography/diode array detection/electrospray ionization tandem mass spectrometry (HPLC/DAD/ESI-MS/MS) anthocyanin analysis

An HP1100 system equipped with a DAD and XCT ion trap mass spectrometer (Agilent Technologies Inc., Palo Alto, CA, USA) was used to quantify and confirm the identification of the elderberry anthocyanins. Quantification was conducted with the results obtained from the LC/DAD. A Synergi Hydro-RP 80Å (150 × 2 mm, 4 μm) column fitted with 4.0 × 3.0 mm i.d. guard column (Phenomenex, Torrance, CA, USA) was used. Absorbance spectra were collected for all peaks. The solvent flow rate was 0.2 mL min^{-1}. Solvent A was acetic acid–TFA–acetonitrile–water (10.0%:0.2%:5.0%:84.8%, v/v/v/v). Solvent B was acetonitrile. The initial solvent composition was 99% solvent A; then a linear gradient of 99% to 90% solvent A in 30 min; 90% to 70% solvent A in 10 min; 70% to 60% solvent A in 5 min; while detection occurred simultaneously at 520 and 280 nm. Column temperature was maintained at 25 °C. The ESI parameters were as follows: positive mode; nebulizer pressure, 30 psi; N_2 drying gas, 10 mL min^{-1}; drying gas temperature, 350 °C; trap drive, 74.5; skimmer, 40 V; octopole RF amplitude, 200 Vpp; capillary exit, 205.2 V. Scan range was 150–1000 *m/z*. Trap ICC was 20 000 units and accumulation time was 200 ms. Fragmentation amplitude was 1.50 V and threshold ABS was 10 000 units for the MS/MS condition. Extracts were filtered (0.45 μm Millipore Millex-FH syringe filter, Bedford, MA, USA) before injection. Injection volume was 5 μL. Peak assignments were made based on retention time, UV-visible spectra, and mass-to-charge ratio of the molecular ion and fragmented ion obtained by HPLC-DAD and MS analyses. Anthocyanins were quantified using cyanidin 3-glucoside as external standard.

HPLC/DAD/ESI-MS/MS polyphenolic analysis

Polyphenolics were isolated by solid-phase extraction (SPE) using a C-18 Sep-Pak mini column (Waters Associates, Milford, MA, USA) as described by Kim and Lee.[35] Briefly, ethyl acetate fraction was collected and evaporated using a Labconco RapidVap vacuum evaporation system (Labconco Corporation, Kansas City, MO, USA) and the polyphenolics were redissolved in 1 mL water. Individual polyphenolic

analyses were conducted on the same analytical instrument and column used for individual anthocyanin analyses. Absorbance spectra were collected for all peaks. The solvent flow rate was 0.2 mL min^{-1} and injection volume was 20 μL. Solvent A consisted of 2% acetic acid in water (v/v). Solvent B was 0.5% acetic acid in water and acetonitrile (50%:50%, v/v). The initial solvent composition was 90% solvent A; then a linear gradient of 90% to 60% solvent A in 60 min; 60% to 1% solvent A in 10 min; then held for 6 min with simultaneous detection at 280, 320, and 370 nm. Column temperature was 25 °C. The ESI parameters were as follows: negative mode; nebulizer pressure, 30 psi; N_2 drying gas, 12 mL min^{-1}; drying gas temperature, 350 °C; trap drive, 52.5; skimmer, −40 V; octopole RF amplitude, 187.1 Vpp; capillary exit, −128.5 V. Scan range was 50–1000 m/z. Trap ICC was 100 000 units and accumulation time was 200 ms. Fragmentation amplitude was 1.50 V and threshold ABS was 10 000 units for the MS/MS condition. Cinnamic acids were quantified as chlorogenic acid, and flavonol glycosides were expressed as quercetin 3-rutinoside (rutin) by the external standard method. Quantification was conducted with the results obtained from LC/DAD. Peak assignments were made according to UV-visible spectra, retention time, co-chromatography with authentic standards (when available), and mass spectra information (mass-to-charge ratio of the molecular ion and fragmented ion).

Statistical analysis

Analysis of variance (ANOVA) was conducted and the significant difference among the different cultivars was determined at the 95% confidence level using Bonferroni test. Correlation was determined on the two total anthocyanin values obtained by pH differential method and HPLC. Cluster analysis was performed on the individual anthocyanins and polyphenolics of each genotype using squared Euclidean distance and the Ward's method. Statistica for Windows version 7.0 was used (StatSoft, Inc., Tulsa, OK, USA).

RESULTS AND DISCUSSION

Two species and ten different genotypes were examined in this study (Table 1). There was significant interaction between genotypes and growing seasons for ACY (from the pH differential method), TP, ACY/TP, °Brix, TA, pH, and berry size ($P \leq 0.05$, Table 2). ACY content of the elderberry samples ($n = 10$) ranged from 140 mg ('Netzer') to 280 mg ('Adams 1') cyanidin 3-glucoside 100 g^{-1}

Table 2. Comparison of fruit from *Sambucus canadensis* and *S. nigra* grown in test plots at Corvallis, OR. Results are from two growing seasons. There were interaction effects between cultivars and growing seasons for all measurements ($P \leq 0.05$, ANOVA table not shown). The values are separated by growing season

Cultivar	ACY[a]	TP[b]	ACY/TP	°Brix	TA[c]	pH	Berry size[d]
2004 growing season							
Adams 1	280 (17)cd	442 (17)d–f	0.63 (0.02)a	12.1 (0.1)a–d	1.01(0.04)b	3.9 (0.04)bc	840 (21)a–c
Adams 2	251 (23)cd	404 (17)b–f	0.61 (0.04)a	12.2 (0.2)a–d	1.03(0.03)b	3.9 (0.02)bc	986 (28)bc
Johns	178 (14)a–c	340 (16)a–e	0.52 (0.02)a	12.6 (0.2)a–e	0.71 (0.04)a	4.2 (0.04)de	702 (21)ab
Scotia	246 (6)b–d	374 (6)a–f	0.66 (0.02)a	13.8 (0.1)ef	0.96 (0.01)b	3.9 (0.01)bc	965 (21)bc
York	178 (8)a–c	336 (12)a–d	0.53 (0.02)a	12.8 (0.1)b–e	0.66 (0.02)a	4.2 (0.02)d–f	680 (19)ab
Gordon B	263 (8)cd	413 (16)c–f	0.63 (0.02)a	11.7 (0.3)a–c	0.73 (0.02)a	4.3 (0.01)d–f	1619 (126)d
Netzer	140 (5)ab	351 (13)a–f	0.40 (0.00)a	13.4 (0.2)c–f	0.67 (0.04)a	4.4 (0.02)ef	1392(40)d
Harris 2	165 (4)*	327 (7)*	0.50 (0.00)*	13.2 (0.1)*	0.53 (0.04)*	4.3 (0.01)*	1414 (90)*
Korsør	176 (4)a–c	387 (11)a–f	0.46 (0.02)a	13.9 (0.1)ef	1.18 (0.03)c	3.8 (0.01)a–c	723 (28)ab
Haschberg	170 (12)ab	364 (17)a–f	0.46 (0.01)a	11.8 (0.3)a–c	1.43 (0.03)d	3.8 (0.06)ab	867 (43)a–c
2005 growing season							
Adams 1	430 (14)ef	536 (16)b–d	0.80 (0.02)cef	13.6 (0.1)b–e	0.75 (0.01)c–f	4.1 (0.02)b	788 (22)a–c
Adams 2	444 (14)ef	562 (11)b–d	0.79 (0.01)bef	13.8 (0.1)b–f	0.75 (0.02)c–f	4.1 (0.03)b	882 (27)bc
Johns	290 (16)b–d	418 (12)a	0.69 (0.02)bf	13.7 (0.2)b–f	0.48 (0.02)a–c	4.5 (0.00)c	635 (16)ab
Scotia	410 (6)ef	499 (10)bc	0.82 (0.01)ce	15.4 (0.2)fg	0.65 (0.02)b–e	4.2 (0.02)b	935 (43)bc
York	268 (6)bc	397 (7)a	0.67 (0.01)bf	14.1 (0.2)c–g	0.50 (0.01)a–c	4.4 (0.03)c	628 (21)ab
Gordon B	389 (17)d–f	532 (21)b–d	0.73 (0.01)cf	13.2 (0.2)b–e	0.59 (0.02)a–d	4.5 (0.03)c	1545 (52)d
Netzer	197 (7)a	391 (10)a	0.51 (0.03)a	14.8 (0.2)d–g	0.62 (0.02)a–e	4.4 (0.01)c	1632 (129)d
Harris 2	106 (2)*	277 (5)*	0.38 (0.01)*	11.2 (0.0)*	0.57 (0.00)*	4.2 (0.00)*	1499 (128)*
Korsør	343 (11)c–f	582 (10)cd	0.59 (0.01)a	14.6 (0.3)d–g	0.85 (0.03)ef	4.1 (0.04)b	815 (13)a–c
Haschberg	268 (16)bc	510 (20)bc	0.52 (0.01)a	12.0 (0.3)a	1.23 (0.05)g	3.8 (0.04)a	966 (26)bc

* 'Harris 2' was not included in the statistical analysis.

[a] Total anthocyanins (ACY) determined by pH differential method were expressed as mg cyanidin-3-glucoside (MW = 449.2 and extinction coefficient = 26 900) 100 g^{-1} berries.

[b] Total phenolics (TP) were expressed as mg gallic acid 100 g^{-1} berries.

[c] TA was expressed as g citric acid 100 g^{-1}.

[d] Number of berries 100 g^{-1}. Totals with different lower-case letters (within a column for the different growing seasons) were significantly different (Bonferroni test, $P \leq 0.05$). Values in parenthesis are standard errors.

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berries in the 2004 season (mean = 243 mg 100 g^{-1} for *S. canadensis*; mean = 173 mg 100 g^{-1} for *S. nigra*) and 106 mg ('Harris 2') to 444 mg ('Adams 2') cyanidin 3-glucoside 100 g^{-1} berries in 2005 (mean = 362 mg 100 g^{-1} for *S. canadensis*; mean = 306 mg 100 g^{-1} for *S. nigra*). 'Netzer' and 'Harris 2' had the lowest ACY content among the genotypes for both seasons. 'Adams 1', 'Adams 2', 'Scotia', and 'Gordon B' had higher levels of ACY than both *S. nigra* samples for the two growing seasons. Despite its small berry size, 'Netzer' had low ACY level, which is most likely due to the uneven ripening observed. Brønnum-Hansen and Hansen[12] reported *S. nigra* ACY content ranging from 200 to 1000 mg 100 g^{-1} berries (values obtained from 26 cultivars examined) determined by pH differential method (expressed as cyanidin 3-glucoside). 'Korsør' examined by Kaack and Austed[20] had higher ACY (1095 mg 100 g^{-1}, determined by HPLC) levels then the 'Korsør' sample collected in this study (176 mg and 343 mg 100 g^{-1} by the pH differential method, and 400.2 and 806.1 mg 100 g^{-1} by HPLC). In another study by Kaack,[19] the ACY values ranged from 518 mg ('Finn Sam') to 1028 mg ('Samocco') 100 g^{-1} of fruit (determined by absorbance at 530 nm and expressed as cyanidin 3-glucoside) for 11 cultivars of *S. nigra*. 'Harris 2' had the least amount of TP present in 2004 (327 mg/100 g) and 2005 (277 mg 100 g^{-1}) samples. 'Adams 1', 'Adams 2', and 'Korsør' had the highest levels of TP in both growing seasons. ACY and TP values were consistently higher for all fruits, except 'Harris 2', grown in 2005 when compared to 2004. Fruit from 'Harris 2' was included in the chemical analysis because of the unique color observed in the field compared to the other elderberry genotypes (visually bright red compared to the other dark purple colored elderberries), but was not included in the statistical analysis due to the lack of replication (samples were obtained from a single plot). Comparing the two *S. nigra* samples, 'Haschberg' had lower ACY, TP,°Brix, pH, and larger fruit than 'Korsør'. The average ACY/TP of *S. canadensis* (0.64 in 2004 and 0.77 in 2005) was higher than the average ACY/TP of *S. nigra* (0.46 in 2004 and 0.56 in 2005).

Elderberries had a pH from 3.8 to 4.5 and a TA from 0.48 to 1.43 g of citric acid of berries. Overall, the sample of *S. canadensis* had higher °Brix, pH, and berry size than *S. nigra* for both growing seasons. TA values of the two species decreased in 2005 when compared to 2004 values. 'Gordon B' and 'Netzer' had the smallest berries. Kaack[16,19] reported a TA ranging from 0.80 to 1.26 g citric acid 100 g^{-1} fruit present in 12 *S. nigra* cultivars.

Figure 1 shows chromatograms obtained from the anthocyanin HPLC analysis. Representative chromatograms, 'Adams 1' for *S. canadensis* and 'Korsør' for *S. nigra* samples, are shown in Figs 1(A) and 1(B), respectively. *Sambucus canadensis* 'Adams 2', 'Johns', 'Scotia', 'York', and 'Netzer' had the same 11 anthocyanins present (Table 3): cyanidin 3-sambubioside-5-glucoside (second major pigment present), cyanidin 3,5-diglucoside, cyanidin 3-sambubioside, cyanidin 3-glucoside, cyanidin 3-rutinoside, delphinidin 3-rutinoside (trace levels present), cyanidin 3-(*Z*)-*p*-coumaroyl-sambubioside-5-glucoside, cyanidin 3-*p*-coumaroyl-glucoside, petunidin 3-rutinoside (trace levels present), cyanidin 3-(*E*)-*p*-coumaroyl-sambubioside-5-glucoside (major pigment present), and cyanidin 3-*p*-coumaroyl-sambubioside. The *S. canadensis* genotypes 'Adams 1', 'Gordon B' and 'Harris 2' had the same anthocyanin profile as 'Adams 2', 'Johns', 'Scotia', 'York', and 'Netzer' with the exception of petunidin 3-rutinoside, which was not detected in the former and only at trace levels in the latter. This is the first time delphinidin 3-rutinoside and petunidin 3-rutinoside have been reported to be present in *S. canadensis*. Cyanidin-based anthocyanins were the major anthocyanins present in *S. canadensis*. All *S. canadensis* samples had more acylated anthocyanins (>60% of the total pigment present) than non-acylated anthocyanins.

Sambucus nigra 'Korsør' and 'Haschberg' had five and seven individual anthocyanins, respectively (Table 3). Both *S. nigra* samples contained cyanidin 3-sambubioside-5-glucoside, cyanidin 3,5-diglucoside, cyanidin 3-sambubioside, cyanidin 3-glucoside, and pelargonidin 3-glucoside (present in trace levels). 'Haschberg' had two additional peaks (trace levels of cyanidin 3-rutinoside and delphinidin 3-rutinoside). This is the first report to identify delphinidin 3-rutinoside present in *S. nigra* sample (only detected in 'Haschberg'). 'Korsør' examined by Kaack and Austed[20] also had cyanidin 3-glucoside (703 mg 100 g^{-1} fresh berries determined by HPLC = 64.2% based on total peak area) as the major pigment. The *S. nigra* samples examined by Watanabe *et al*.[21] and Inami *et al*.[13] were found to have slightly more cyanidin 3-sambubioside than cyanidin 3-glucoside. Bridle and García-Viguera[25] reported cyanidin 3-sambubioside-5-glucoside as the major anthocyanin in the *S. nigra* sample they tested, but Brønnum-Hansen and Hansen[12] reported cyanidin 3-glucoside as the major pigment of *S. nigra.* There were no acylated pigments found in 'Korsør' and 'Haschberg'. Both species contained 3-sambubioside-5-glucoside, 3,5-diglucoside, 3-sambubioside (second major pigment present), and 3-glucoside (major pigment present) of cyanidin. *Sambucus nigra* also had cyanidin-based anthocyanins as the major anthocyanins. According to Wu *et al*.,[26] they have identified three additional minor anthocyanins present in *S. nigra* (cyanidin 3-rutinoside, pelargonidin 3-glucoside, and pelargonidin 3-sambubioside) – the first time a non-cyanidin-based anthocyanin was reported in elderberries. 'Korsør' and 'Haschberg' contained trace levels of pelargonidin 3-glucoside, but pelargonidin 3-sambubioside was not detected. Wu *et al*.[26] also reported the two major anthocyanins in their *S. nigra* as cyanidin

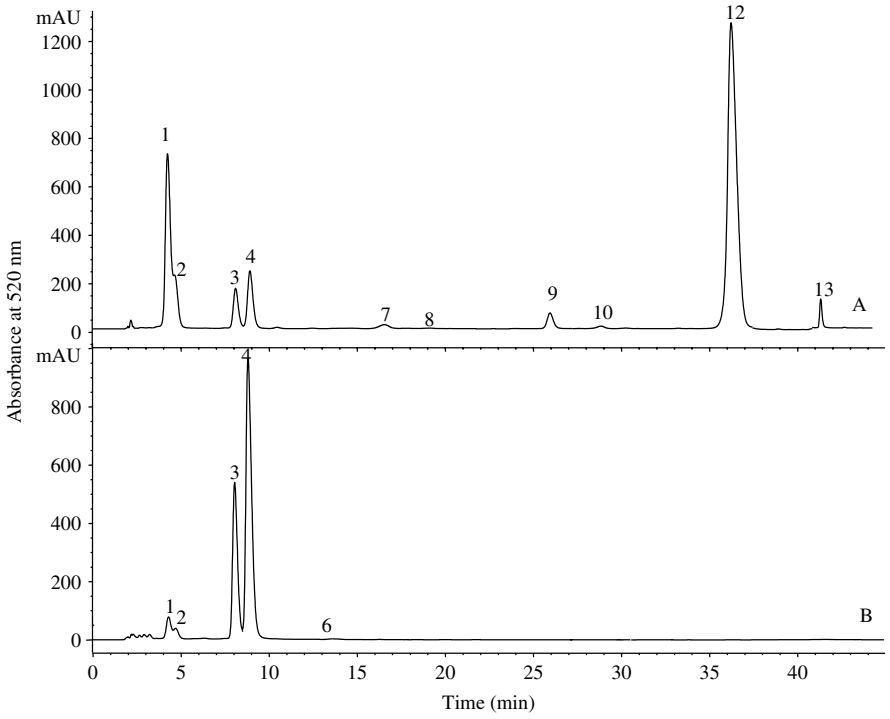

Figure 1. Anthocyanin separation of *Sambucus canadensis* 'Adams 1' (A) and *S. nigra* 'Korsør' (B) monitored at 520 nm. Corresponding peak assignments are in Table 3.

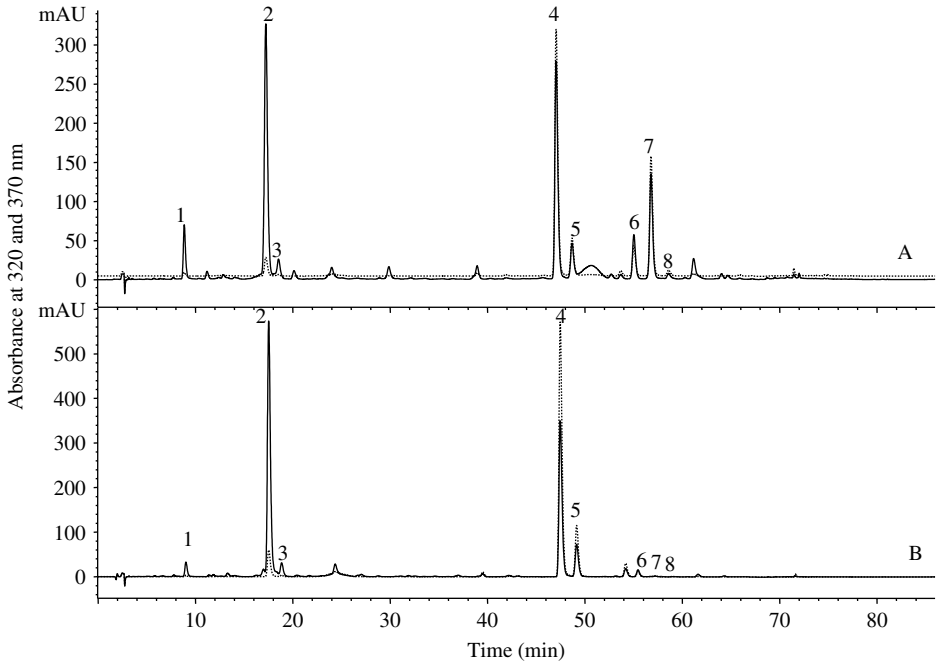

Figure 2. Polyphenolics profile of *Sambucus canadensis* 'Adams 1' (A) and *S. nigra* 'Korsør' (B). Cinnamic acids were monitored at 320 nm (solid line) and flavonol glycosides were monitored at 370 nm (dotted line). Corresponding peak assignments are listed in Table 4.

3-glucoside (739.8 mg $100\,g^{-1}$ fresh weight) and cyanidin 3-sambubioside (545.9 mg $100\,g^{-1}$ fresh weight), determined by HPLC. Various researchers have examined both species of elderberries (without describing cultivars), and reported the same four peaks as samples used in this study.[13,22,23]

Despite the unique visual appearance of 'Harris 2' observed during harvest in the field (visually bright red berries compared to other cultivars), from HPLC analysis the anthocyanin profile was not unique (Table 3). From these results, the visual difference was likely due to the amount of pigment present ('Harris 2' contained low amounts of total anthocyanin based on both analytical methods and both seasons). This visual difference might also be due to other factors not covered in this study.

The total anthocyanin values determined by the two methods, pH differential method and HPLC, were

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Table 3. Distribution of individual anthocyanins present in *Sambucus* samples obtained by HPLC. 'Korsør' and 'Haschberg' had unique anthocyanin profiles (cyanidin 3-glucoside and cyanidin 3-sambubioside were the major anthocyanin present) compared to the other elderberry samples (cyanidin 3-(E)-*p*-coumaryl-sambubioside-5-glucoside was the major pigment). Anthocyanins are listed in the order of elution. Units for all values are mg cyanidin 3-glucoside 100 g^{-1} berries. Masses of the molecular ions and their fragments of the identified anthocyanins are listed

Identified compound ($[M+H]^+$ ion, fragments)	Cyanidin 3-sambubioside-5-glucoside (743, 581, 449, 287)	Cyanidin 3, 5-diglucoside (611, 449, 287)	Cyanidin 3-sambubioside (581, 287)	Cyanidin 3-glucoside (449, 287)	Cyanidin 3-rutinoside (595, 449, 287)	Pelargonidin 3-glucoside (433, 271)	Cyanidin-based anthocyanin (785, 623, 449, 287)	Delphinidin 3-rutinoside (611, 465, 303)	Cyanidin 3-(*Z*)-*p*-coumaroyl-sambubioside-5-glucoside (889, 727, 449, 287)	Cyanidin 3-*p*-coumaroyl-glucoside (595, 449, 287)	Petunidin 3-rutinoside (625, 479, 317)	Cyanidin 3-(*E*)-*p*-coumaroyl-sambubioside-5-glucoside (889, 727, 449, 287)	Cyanidin 3-*p*-coumaroyl-sambubioside (727, 287)	
Peak	1	2	3	4	5	6	7	8	9	10	11	12	13	Sum
2004 growing season														
Adams 1	134.6 (13.6)	37.3 (2.8)	27.5 (2.3)	42.9 (3.2)	ND	ND	5.2 (0.3)	tr	26.4 (2.0)	2.4 (0.5)	ND	341.4 (20.4)	9.0 (0.6)	626.6 (36.4)d–f
Adams 2	108.1 (11.5)	32.2 (3.5)	26.2 (2.4)	37.3 (4.0)	ND	ND	4.6 (0.5)	tr	17.7 (1.7)	2.7 (0.2)	tr	324.2 (29.4)	8.7 (0.8)	561.7 (52.4)c–f
Johns	102.2 (10.2)	26.2 (2.0)	10.7 (0.9)	7.7 (0.4)	ND	ND	3.2 (0.3)	tr	18.5 (2.5)	3.3 (0.2)	tr	216.4 (19.4)	2.7 (0.2)	390.9 (32.1)a–ce
Scotia	107.0 (2.9)	55.2 (0.8)	10.5 (0.7)	27.8 (1.5)	ND	ND	3.8 (0.2)	tr	21.1 (2.5)	2.2 (0.1)	tr	296.9 (9.7)	3.8 (0.2)	528.3 (12.1)b–f
York	102.9 (4.0)	27.4 (1.3)	9.6 (1.7)	8.0 (0.3)	ND	ND	3.3 (0.2)	tr	19.6 (4.2)	3.5 (0)	tr	219.6 (9.7)	2.8 (0.1)	396.6 (19.4)a–ce
Gordon B	100.6 (6.6)	50.7 (3.2)	5.3 (0.6)	2.8 (0.2)	ND	ND	13.1 (3.0)	tr	23.0 (3.0)	4.5 (0.4)	ND	359.6 (33.3)	2.5 (0.2)	562.6 (48.7)b–f
Netzer	54.7 (3.4)	6.3 (0.4)	1.7 (0.1)	0.3 (0)	ND	ND	7.9 (0.3)	tr	10.1 (2.9)	1.7 (0)	tr	195.8 (4.2)	1.4 (0.1)	280.0 (5.5)a–c
Harris 2	61.3 (1.2)	28.1 (0.7)	3.3 (0)	3.3 (0.2)	ND	ND	5.6 (0)	tr	15.0 (0.3)	2.1 (0)	ND	211.2 (0)	2.1 (0.1)	332.1 (2.0)*
Korsør	16.0 (1.0)	8.2 (0.4)	122.2 (2.7)	253.7 (4.1)	ND	tr	ND	ND	ND	ND	ND	ND	ND	400.2 (6.6)a–e
Haschberg	32.2 (2.2)	11.2 (1.0)	143.0 (12.3)	204.6 (17.3)	tr	tr	ND	tr	ND	ND	ND	ND	ND	391.0 (31.9) a–ce
2005 growing season														
Adams 1	181.2 (5.0)	56.5 (1.4)	39.4 (1.5)	56.4 (1.6)	ND	ND	8.2 (0.5)	tr	22.8 (2.0)	3.6 (0.7)	ND	552.6 (23.7)	14.1 (1.0)	934.6 (31.5)d–f
Adams 2	194.7 (12.4)	61.1 (2.4)	33.6 (7.0)	62.7 (3.5)	ND	ND	10.9 (2.7)	tr	27.1 (2.0)	4.6 (0.2)	tr	594.2 (31.1)	16.3 (1.2)	1005.2 (47.8) d–f
Johns	167.8 (10.9)	41.2 (3.0)	14.6 (1.0)	10.1 (0.8)	ND	ND	5.7 (0.5)	tr	16.3 (1.7)	4.9 (0.4)	tr	367.3 (33.2)	4.5 (0.4)	632.1 (50.1) b–d
Scotia	176.9 (6.8)	94.6 (2.6)	15.0 (0.4)	40.3 (2.2)	ND	ND	6.7 (0.2)	tr	23.9 (1.2)	3.7 (0.1)	tr	520.2 (13.4)	6.8 (0.3)	888.1 (22.2) d–f
York	146.8 (3.4)	36.8 (1.0)	12.6 (0.4)	7.8 (0.5)	ND	ND	5.6 (0.3)	tr	19.2 (1.4)	4.4 (0.2)	tr	351.5 (11.0)	4.3 (0.2)	589.0 (11.6) a–c

Gordon B	149.6 (6.6)	74.6 (6.4)	6.4 (0.5)	3.8 (0.7)	ND	ND	15.3 (0.7)	tr	27.5 (1.6)	6.5 (0.3)	ND	555.0 (28.5)	3.9 (0.5)	842.6 (40.0)d–f
Netzer	79.6 (2.6)	8.6 (0.4)	3.2 (0.6)	1.9 (0.3)	ND	ND	10.2 (1.0)	tr	18.9 (0.5)	2.4 (0.1)	tr	267.4 (16.8)	1.8 (0.3)	394.0 (22.5)ab
Harris 2	40.8 (0.3)	14.7 (0.1)	1.7 (0.2)	1.3 (0.1)	ND	ND	3.3 (0.1)	tr	13.2 (0.6)	1.1 (0.1)	ND	130.6 (8.5)	1.1 (0.2)	207.7 (9.0)*
Korsør	37.3 (2.5)	18.3 (1.0)	269.1 (16.4)	481.4 (24.0)	ND	tr	ND	ND	ND	ND	ND	ND	ND	806.1 (39.8)cd
Haschberg	59.2 (1.6)	19.5 (0.6)	268.1 (20.6)	309.7 (18.3)	tr	tr	ND	tr	ND	ND	ND	ND	ND	656.5 (40.7)b–d

* 'Harris 2' values were not included in the statistical analysis.
Totals with different lower-case letters (within a column for the different growing seasons) were significantly different (Bonferroni test, $P \leq 0.05$). Values in parentheses are standard errors. ND, not detected in sample; 'tr' represents trace levels detected and was not included in the quantification.

different. The total anthocyanin content obtained by HPLC was 2.0–2.3 times higher than the values from the pH differential method. The external standard used was 89.8% pure. Purity (by obtaining the extinction coefficient) of the purchased cyanidin 3-glucoside standard was determined as described by Lee *et al*.[33] Although the HPLC-obtained anthocyanin values were expressed taking into consideration the purity of the external standard used, HPLC-obtained values were higher than ACY. Further studies should be conducted on the comparison of total anthocyanin content of a given sample based on pH differential method (Table 2) and values obtained by HPLC (Table 3). The trends were similar despite the method of analysis, and total anthocyanin values obtained by both methods were significantly correlated with each other ($R = 0.98$, $P \leq 0.05$).

SPE was used to remove the anthocyanin fraction that interferes with the polyphenolic HPLC analysis. The polyphenolics in the two species were examined in their native forms and only the ethyl acetate fraction was analyzed. The HPLC chromatograms of the ethyl acetate fractions are shown (Fig. 2). Both species contained eight polyphenolics; three cinnamic acids (neochlorogenic acid, chlorogenic acid, and cryptochlorogenic acid) and five flavonol glycosides (quercetin 3-rutinoside, quercetin 3-glucoside, kaempferol 3-rutinoside, isorhamnetin 3-rutinoside, and isorhamnetin 3-glucoside) (Table 4 and Fig. 2). The proportion of the individual polyphenolics differed between species. There was a slight difference in polyphenolic pattern among the different cultivars within *S. canadensis*. Neochlorogenic acid (3-caffeoylqunic acid), chlorogenic acid (5-caffeoylqunic acid), quercetin 3-rutinoside, and isorhamnetin 3-rutinoside were the major polyphenolics present in *S. canadensis* (Table 4 and Fig. 2A show 'Adams 1'). Chlorogenic acid and quercetin 3-rutinoside were the major polyphenolics in *S. nigra* (Table 4 and Fig. 2B show 'Korsør'). Isorhamnetin 3-glucoside was present at low levels in *S. nigra*. Neochlorogenic acid, cryptochlorogenic acid, kaempferol 3-rutinoside, isorhamnetin 3-rutinoside, and isorhamnetin 3-glucoside were identified for the first time in *S. canadensis* and *S. nigra* berries. Quercetin-based glycosides were the major flavonol glycosides in all cultivars. Flavonols were conjugated mainly with rutinose (>75% of total flavonol glycosides), while the remainders were conjugated with glucose (<25% of total flavonol glycosides). In 2004, 'Adams 1', 'Netzer', 'Korsør', and 'Haschberg' had the highest level of total cinnamic acids and flavonol glycosides (combined values of 78.3, 111.8, 85.7, and 108.5 mg $100\,g^{-1}$ of berries, respectively), while 'Adams 1', 'Adams 2', 'York', 'Netzer', 'Korsør', and 'Haschberg' had the highest total values of cinnamic acids and flavonol glycosides in 2005 (72.5, 81.8, 55.6, 89.2, 103.8, and 140.3 mg $100\,g^{-1}$ berries, respectively). There were greater levels of flavonol glycosides than cinnamic acids in all cultivars except 'Harris 2'. In 2004, 'Harris 2' had more cinnamic acids (59%)

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Table 4. Polyphenolic profiles (listed in elution order) of *Sambucus canadensis* and *S. nigra* ethyl acetate fractions. Detection at 320 nm (for cinnamic acids) and 370 nm (for flavonol glycosides). Cinnamic acids are expressed as mg chlorogenic acid 100 g^{-1} of berries, and flavonol glycosides are expressed as mg rutin 100 g^{-1} berries. Masses of the molecular ions and their fragments of the identified polyphenolics are listed

Identified compound ([M – H]$^-$ ion, fragments)	3-caffeoylquinic acid (neo-chlorogenic acid) (353, 191, 179)	5-caffeoylquinic acid (chloro-genic acid) (353, 191)	4-caffeoylquinic acid (crypto-chlorogenic acid) (353, 191, 173)	Quercetin 3-rutinoside (rutin) (609, 301, 255)	Quercetin 3-glucoside (isoquercitrin) (463, 301)	Kaempferol 3-rutinoside (593, 285, 257)	Isorhamnetin 3-rutinoside (624, 315, 300, 271)	Isorhamnetin 3-glucoside (477, 314)	Total cinnamic acids	Total flavonol glycosides	Sum of the polyphenolics identified
Peak	1	2	3	4	5	6	7	8			
2004 growing season											
Adams 1	4.1 (1.0)	17.5 (1.9)	1.7 (0.2)	33.0 (2.1)	4.5 (0.4)	2.2 (0.2)	14.7 (1.1)	0.7 (0.0)	23.2 (2.7)ab	55.1 (3.5)b–g	78.3 (4.2)a–e
Adams 2	3.3 (0.6)	15.2 (17.2)	1.2 (0.2)	32.7 (2.5)	3.9 (0.3)	1.9 (0.1)	16.7 (1.3)	0.8 (0.1)	19.8 (2.8)ab	56.0 (3.9)c–f	75.8 (5.1)a–d
Johns	6.0 (0.9)	17.2 (1.9)	1.3 (0.2)	25.6 (0.9)	3.9 (0.3)	0.9 (0.0)	16.5 (1.1)	0.9 (0.0)	24.5 (2.7)ab	47.9 (2.0)a–f	72.4 (3.3)a–d
Scotia	4.4 (0.5)	15.3 (1.3)	1.3 (0.2)	20.3 (3.3)	3.3 (0.2)	0.6 (0.1)	14.5 (2.4)	0.5 (0.1)	20.9 (1.8)ab	39.3 (6.0)a–f	60.2 (6.1)a–d
York	3.7 (1.0)	8.1 (3.2)	0.9 (0.4)	21.6 (2.2)	2.6 (0.5)	0.6 (0.1)	11.0 (2.5)	0.7 (0.2)	12.6 (4.3)ab	36.6 (3.8)a–e	49.2 (6.6)a–c
Gordon B	9.5 (1.7)	13.4 (2.9)	1.8 (0.7)	18.0 (0.9)	2.7 (0.1)	1.2 (0.2)	5.5 (0.3)	0.1 (0.0)	24.6 (4.9)ab	27.5 (1.3)a–c	52.1 (5.3)a–c
Netzer	15.8 (3.4)	25.5 (4.5)	5.4 (1.1)	41.9 (2.7)	4.8 (0.5)	2.7 (0.2)	14.9 (1.0)	0.9 (0.1)	46.6 (8.9)bc	65.1 (4.2)c–g	111.8 (0.3)ce
Harris 2	11.4 (1.9)	15.5 (2.0)	3.0 (0.4)	15.0 (0.6)	2.1 (0.2)	0.8 (0.2)	3.0 (0.2)	0.3 (0.0)	29.9 (4.2)*	21.1 (1.2)*	51.0 (5.4)*
Korsør	1.1 (0.2)	26.4 (3.7)	1.2 (0.2)	46.5 (3.9)	9.5 (0.7)	0.7 (0.1)	0.3 (0.2)	tr	28.7 (4.1)a–c	57.0 (4.5)b–g	85.7 (6.7)b–e
Haschberg	0.7 (0.1)	28.1 (2.5)	1.6 (0.2)	72.7 (8.9)	3.9 (0.4)	0.7 (0.1)	0.7 (0.1)	0.1 (0.1)	30.4 (2.7)a–c	78.1 (9.3)dfg	108.5 (9.6)ce
2005 growing season											
Adams 1	2.9 (0.6)	13.4 (2.8)	1.0 (0.2)	29.4 (2.9)	6.5 (0.6)	1.9 (0.1)	16.0 (1.4)	1.2 (0.1)	17.4 (3.6)a–c	55.1 (5.0)a–e	72.5 (4.7)a–e
Adams 2	‘2.8 (0.7)	12.4 (2.6)	0.9 (0.2)	36.9 (2.1)	6.7 (0.2)	2.2 (0.1)	18.6 (0.8)	1.2 (0.1)	16.2 (3.4)ab	65.7 (2.9)c–e	81.8 (2.0)c–e
Johns	5.6 (0.8)	10.4 (1.0)	0.9 (0.2)	15.4 (1.8)	4.5 (0.8)	0.6 (0.1)	9.5 (1.1)	0.7 (0.1)	17.0 (1.6)ab	30.7 (3.8)a–c	47.6 (4.9)a–c
Scotia	3.6 (0.8)	12.2 (0.9)	1.0 (0.1)	17.2 (1.4)	5.3 (0.3)	0.5 (0.0)	11.8 (0.7)	0.8 (0.0)	16.9 (1.5)ab	35.6 (2.3)a–d	52.5 (1.6)a–d
York	4.3 (0.8)	9.7 (1.6)	0.9 (0.1)	20.3 (1.9)	5.3 (0.3)	0.7 (0.0)	13.5 (0.6)	1.0 (0.0)	14.9 (2.5)ab	40.7 (2.5)a–e	55.6 (1.0)a–e
Gordon B	7.9 (1.8)	12.4 (1.4)	2.0 (0.2)	20.7 (2.2)	3.4 (0.2)	1.5 (0.2)	5.4 (0.6)	tr	22.3 (2.9)a–d	31.0 (3.1)a–c	53.2 (2.3)a–c
Netzer	11.2 (3.8)	22.3 (4.0)	3.8 (0.8)	34.0 (7.2)	7.7 (3.4)	1.8 (0.3)	8.0 (2.6)	0.5 (0.2)	37.2 (3.9)b–e	52.0 (7.6)a–e	89.2 (8.4)a–e
Harris 2	11.8 (2.7)	15.4 (1.9)	2.4 (0.1)	19.5 (3.6)	2.5 (0.9)	1.2 (0.4)	5.6 (3.0)	0.4 (0.2)	29.7 (0.9)*	29.2 (8.0)*	58.9 (7.1)*
Korsør	4.4 (3.0)	35.9 (6.2)	2.5 (0.5)	42.6 (7.8)	14.9 (2.6)	1.1 (0.2)	2.2 (1.8)	0.3 (0.2)	42.8 (5.8)c–e	61.1 (8.7)b–e	103.8 (7.3)b–e
Haschberg	0.9 (0.2)	34.7 (5.4)	1.9 (0.3)	95.6 (9.5)	5.2 (0.3)	1.2 (0.1)	0.7 (0.1)	tr	37.6 (5.9)b–e	102.7 (9.7)f	140.3 (10.4)f

* ‘Harris 2’ values were not included in the statistical analysis.

Totals with different lower-case letters (within a column for the different growing seasons) were significantly different (Bonferroni test, $P \leq 0.05$). Values in parentheses are standard errors; ‘tr’ represents trace levels detected and was not included in the quantification.

present than flavonol glycosides (41%), and in 2005 had equal amounts of cinnamic acids and flavonol glycosides.

Määttä-Riihinen *et al*.[36] reported their elderberry sample (*S. nigra* cv. unknown) contained chlorogenic acid, quercetin 3-rutinoside, quercetin 3-glucoside, and quercetin 3-arabinoside (tentative identification). Quercetin 3-arabinoside was not detected in any elderberry samples analyzed for this study. Isorhamnetin 3-glucoside and 3-rutinoside have been reported in the flowers of *S. nigra*.[37] Määttä-Riihinen *et al*.[36] also found approximately 1–5 mg of high molecular weight procyanidins 100 g^{-1} fresh weight. There was a clear distinction between the two elderberry species based on the distribution of individual anthocyanins and polyphenolics (dendogram from cluster analysis not shown). However, a distinction between cultivars within a species was more difficult to define.

All processed elderberry samples that have been tested as a juice, concentrate, natural colorant, and as dietary supplements in the literature were produced from *S. nigra*.[8,10–12,17,18,20] *Sambucus canadensis* should be a better choice since its major anthocyanins are acylated. Stintzing *et al*.[15] reported an increase in antioxidant activity when a cyanidin-based anthocyanin was acylated with a cinnamic acid. The naturally acylated pigment of *S. canadensis* might provide additional benefits besides improved pigment stability. Turker *et al*.[14] demonstrated the storage stability of acylated cyanidin-based anthocyanins in black carrot to exceed that of non-acylated cyanidin-based anthocyanins, at three storage temperatures (4, 25, and 40 °C) over a 90-day period.

The production of a number of minor crops (*Vaccinium* L., *Ribes* L., and *Lonicera* L.) is expanding rapidly in the USA and around the world and numerous papers have reported the berries of these to contain 14–593 mg ACY 100 g-1 berries and 191–1790 mg TP 100 g^{-1} berries.[27,38–40] Elderberry samples analyzed in this study, using identical methods, fall within those reported ranges for ACY and TP.[27,38–40]

In conclusion, the ten genotypes of *S. canadensis* and *S. nigra* had varying levels of ACY, TP, pH, TA: total anthocyanin by HPLC, and total polyphenolics by HPLC. While the species had different anthocyanin and polyphenolic patterns, the cultivars within each species had similar anthocyanin and polyphenolic profiles. *Sambucus canadensis* may be a better choice for processing due to its acylated anthocyanins, which would give the final product more color stability when compared to products made from *S. nigra*. From this study, *S. canadensis* 'Adams 1', 'Adams 2', 'Scotia' and 'Gordon B' had the highest ACY, TP, and acylated pigments (in both growing seasons), and should be recommended as a source of natural colorants or other processed products. In order to consider improving a crop through traditional plant breeding, the first criteria to consider is whether there is variability present for the traits of interest. This study identifies genotypes that have either high or low levels of various compounds. The nature of this study does not allow for any strong statements to be made about the genetic behavior of any of the traits evaluated. However, 'Adams 2' is the maternal parent for 'Scotia' and 'York'. These three genotypes grouped together for some characteristics, such as having trace levels of petunidin 3-rutinoside; however, for most traits they did not. Based on the overall evaluation 'Adams 2' and 'Scotia' would be recommended but 'York' would not. Crosses among the better-performing (e.g. food value, chemistry, nutraceutical, and horticultural traits) genotypes within each species or crosses between these sexually compatible species should allow the production of populations within which superior genotypes could be further selected that are better than any of the parents evaluated here. This is the first paper to report the anthocyanin and other polyphenolic composition of these elderberries at the USDA-ARS breeding program at Corvallis, OR, USA. This paper will also fill in some gaps in the literature concerning anthocyanin and polyphenolic composition of elderberry fruit.

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