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Characterization of Anthocyanins and Proanthocyanidins in Some Cultivars of *Ribes*, *Aronia*, and *Sambucus* and Their Antioxidant Capacity

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Anthocyanins and proanthocyanidins were characterized by HPLC-ESI-MS/MS coupled with a diode array and/or fluorescent detector in seven cultivars of *Ribes nigrum* (black currant) and *Ribes rubrum* (red currant, Red Lake), six cultivars of *Ribes grossularia* (gooseberries), *Aronia melanocarpa* (chokeberry), and *Sambucus nigra* (elderberry). Thirty-one different anthocyanins were detected in these berries, but not every anthocyanin was observed in each berry. A number of minor anthocyanins were identified from these berries for the first time. The concentrations of individual anthocyanins in all of the berries were quantified using relevant anthocyanidin 3-glucoside standards. Among the berries studied in this paper and in berries in general, chokeberry has the highest total anthocyanin concentrations [1480 mg/100 g of fresh weight (FW)], whereas the lowest total anthocyanin concentration in the berries studied was found in the gooseberry cv. Careless, which contained only 0.07 mg/100 g of FW. Two cultivars of gooseberries (Marigold and Leveller) did not contain any anthocyanins. Total proanthocyanidin concentrations in the berries studied ranged from 23 to 664 mg/100 g of FW in elderberry and chokeberry, respectively. Procyanidin or prodelphinidin polymers were the predominant components (>65% w/w) in most of the berries. The lipophilic and hydrophilic antioxidant capacities were measured by the oxygen radical absorbance capacity ($ORAC_{FL}$) procedure. The total antioxidant capacity varied from 21 μmol of TE/g of FW in Careless gooseberry to 161 μmol of TE/g of FW in chokeberry. Total phenolics in the berries in general paralleled hydrophilic antioxidant capacity.

KEYWORDS: Anthocyanin; proanthocyanidin; antioxidant; ORAC; phenolics; black currant; gooseberry; chokeberry; elderberry; red currant

INTRODUCTION

Considerable interest has developed over the years in fruits and vegetables containing high concentrations of flavonoids, due to their potential biological and health-promoting effects (*1*−*3*). These biological effects are in part due to their antioxidant capacity (*4*, *5*). Among all common fruits and vegetables in the diet, berries, especially those with dark blue or red colors, have the highest antioxidant capacities (*6*).

The targeted berries in this project are sometimes referred to as the "purple berries" (except for gooseberries and red currants), which are known for their high antioxidant capacity and potential health benefits. These berries are under consideration for commercialization in the northeastern United States. As in other berries, anthocyanins and proanthocyanidins are important polyphenolic components in *Ribes*, *Aronia*, and *Sambucus*. Anthocyanins are water-soluble glycosides and acylglycosides of anthocyanidins, which are polyhydroxy and polymethoxy derivatives of the 2-phenylbenzopyrylium (flavylium) cation (**Figure 1**) (*7*). Proanthocyanidins (PAs) are oligomeric and polymeric flavan-3-ols. The size of proanthocyanidin molecules can be described by their degree of polymerization (DP) (*8*). PAs containing (epi)catechin or (epi)gallocatechin as subunits are named procyanidins or prodelphinidins, respectively. Anthocyanins and PAs are among the major phytochemicals in berries (*7*, *9*), both of which have been shown to be effective antioxidants (*10*, *11*). These two classes of flavonoids account for a major fraction of the total flavonoids ingested in the Western diet and are of interest in nutrition and medicine because of potential protective effects against diseases (*12*, *13*). The average daily intake of anthocyanins per person has been estimated to be ∼200 mg in the United States (*14*), Our studies have suggested that the average intake of proanthocyanidins is 58 mg/day in the United States. (*9*). In animal studies, consumption of dark-colored berries has been shown to protect against peroxynitrite-induced endothelial dysfunction and chemically induced cancer (*15*−*17*).

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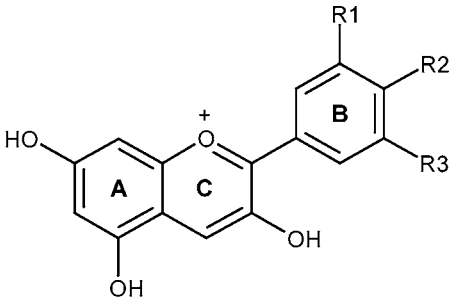


Anthocyanidin	R_1	R_2	R_3	MW
Pelargonidin	H	OH	H	271
Cyanidin	OH	OH	H	287
Delphinidin	OH	OH	OH	303
Peonidin	OMe	OH	H	301
Petunidin	OMe	OH	OH	317
Malvidin	OMe	OH	OMe	331

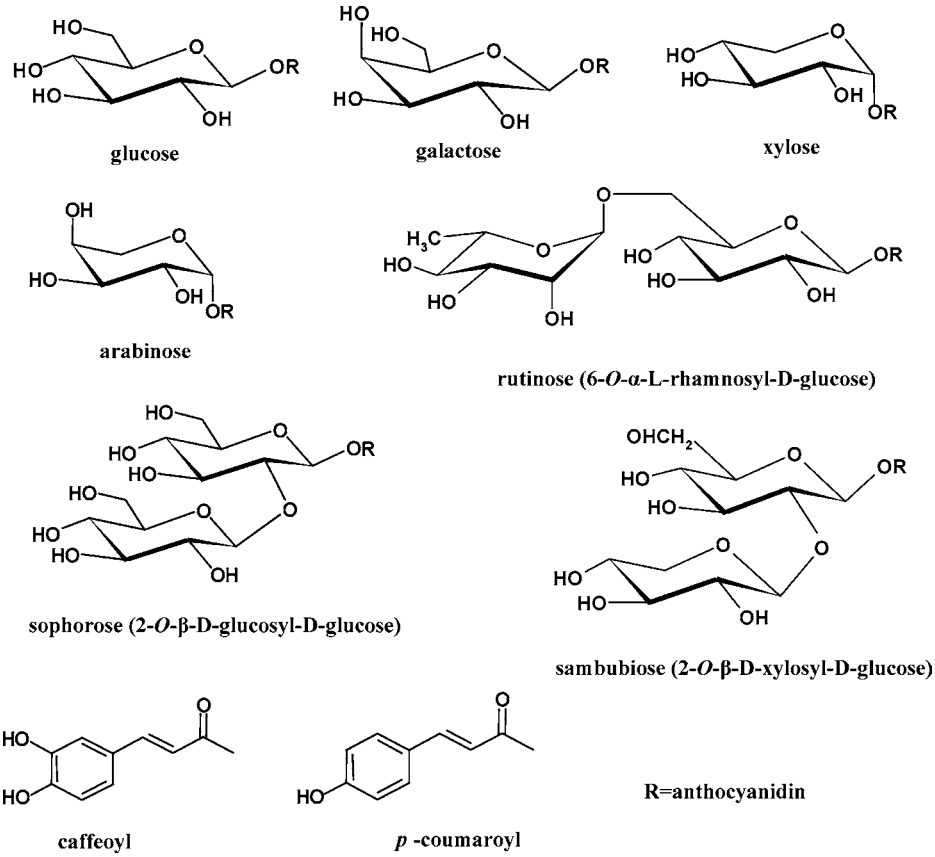


Figure 1. Structures of anthocyanidins, sugar moieties, and acylated substitutes that occur in the berries considered in this paper.

Although some studies have been conducted on *Ribes*, *Aronia*, and *Sambucus* in terms of phenolic compounds and their antioxidant capacities (*18*−*23*), most studies have focused on only one or two of the major classes of compounds, and the minor compounds have been neglected. No data are available on the PA content of *Ribes*, *Aronia*, or *Sambucus*. Additionally, anthocyanins and antioxidant capacity can significantly vary across species and cultivars of berries (*24*).

The objective of this study was to characterize individual anthocyanins, especially the minor anthocyanins, and PAs in different cultivars of black currant, gooseberry, red currant, chokeberry, and elderberry, by HPLC-ESI-MS/MS. Quantitative analysis of individual anthocyanins and PAs was performed as well. Furthermore, lipophilic and hydrophilic antioxidant capacities and concentrations of total phenolics were measured.

MATERIALS AND METHODS

Chemicals and Apparatus. 2,2′-Azobis(2-amidinopropane) dihydrochloride (AAPH) was purchased from Wako Chemicals USA (Richmond, VA). 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) was obtained from Aldrich (Milwaukee, WI). Randomly methylated β-cyclodextrin (Trappsol) (Pharm grade) (RMCD) was obtained from Cyclodextrin Technologies Development Inc. (High Springs, FL). Fluorescein (Na salt) was obtained from Aldrich (Milwaukee, WI). Folin−Ciocalteu's phenol reagent, sodium carbonate, and gallic acid were all purchased from Sigma (St. Louis, MO). All

other solvents were purchased from Fisher (Fair Lawn, NJ). Extraction of samples was performed on an ASE 200 accelerated solvent extractor (Dionex Corp., Sunnyvale, CA). $ORAC_{FL}$ analyses were carried out on a FLUOstar Galaxy plate reader (BMG Labtechnologies, Durham, NC). Fluorescence filters with an excitation wavelength of 485 nm and an emission wavelength of 520 nm were used. Microplates (48 wells) were purchased from VWR (St. Louis, MO). Total phenolics were analyzed on the ANALETTE model 9006 chemistry analyzer (Precision Systems Inc., Natick, MA).

Standards. The 3-*O*-β-glucoside standards of pelargonidin, cyanidin, peonidin, delphinidin, petunidin, and malvidin (six mixed anthocyanin standards, HPLC grade) and cyanidin 3-glucoside (HPLC grade) were obtained from Polyphenols Laboratories (Sandnes, Norway). A composite proanthocyanidin oligomer standard containing monomers through decamers was purified from cocoa. A polymer proanthocyanidin fraction with a mean DP of 36.1 was purified from blueberries and used as a polymer standard (*9*).

Plant Materials and Sample Preparation. Gooseberries (*Ribes grossularia*), black currants (*Ribes nigrum*), and red currants (*Ribes rubrum*) were obtained from Wisley Gardens, Royal Horticultural Society, England. Of the gooseberry varieties, cvs. Leveller (yellow variety), Careless (green variety), and Whinham (red variety) are the leading varieties in England, Europe, and Holland, respectively. The cv. Marigold (green/yellow) may be a potential dessert variety. Varieties of black currant studied were selected because of recognized characteristics in the industry. Cv. Titania is the major cultivar grown in the United States, whereas Titania and cv. Ben Nevis are common in Canada. Cv. Ben Alder is perhaps one of the better flavored varieties, and cv. Ben Lomond is recognized as having good processing characteristics. Elderberry (*Sambucus nigra*) and chokeberry (*Aronia melanocarpa*) were provided by Artemis International, Fort Wayne, IN. Fresh berry samples were frozen and stored frozen at −20 °C until transferred to the Arkansas Children's Nutrition Center, at which time the frozen samples were freeze-dried and ground into powder. The powder was kept at −70 °C until analyzed.

Extraction of dried berries was performed on an ASE 200 accelerated solvent extractor as previously reported (*6*, *25*). Briefly, 1 g of each sample was mixed with 5 g of sea sand (Unimin Corp., Le Sueur, MN), transferred to a 22 mL extraction cell, and extracted with hexane/dichloromethane (1:1) followed by acetone/water/acetic acid (70:29.5:0.5; Ace/H_2O/AcAc) extraction. The extracts from hexane/dichloromethane were used to measure lipophilic $ORAC_{FL}$ capacity after further dilution. Ace/H_2O/AcAc extracts were used to measure the hydrophilic $ORAC_{FL}$ value and total phenolics after dilution into the concentration range of the standards.

For anthocyanin analysis, 1 g of sample was weighed and put into a 15 mL screw-cap tube. The sample was extracted with 15 mL of methanol/water/acetic acid (85:15:0.5; v/v, MeOH/H_2O/AcAc). The sample was then vortexed for 30 s followed by sonication for 5 min. The tube was shaken twice during sonication to resuspend the sample. The tube was then kept at room temperature for 10 min, being vortexed for 30 s after 5 min. The tube was then centrifuged at 4550*g* for 10 min and the supernatant removed. The sample was extracted one more time with 10 mL of MeOH/H_2O/AcAc using the same procedure, and the supernatants were combined. The combined supernatant was transferred into a 25 mL volumetric flask, and MeOH/H_2O/AcAc was added to make up the final volume to exactly 25 mL. The solution was further diluted appropriately and filtered using a 0.22 μm Teflon syringe filter (Cameo 25F, Micron Separations Inc., Westboro, MA) for analysis.

To compare the solvent influence on anthocyanin composition, 4 mL of Ace/H_2O/AcAc extracts of black currant (Ben Alder) and elderberry were put into a SpeedVac (ThermoSavant, Holbrook, NY) to remove acetone. Then the remaining solution was diluted appropriately with acidic methanol and filtered using a 0.22 μm Teflon syringe filter (Cameo, 25F, Micron Separations Inc., Westboro, MA) for anthocyanin analysis.

Extraction and purification of PAs were performed according to a published method (*26*, *27*).

HPLC-MS/MS Analyses of Anthocyanins. Analyses were performed on an HP 1100 series HPLC (Hewlett-Packard, Palo Alto, CA) equipped with a diode array detector. A 250 × 4.6 mm i.d. Zorbax SB-C_{18} column was used for separation. Elution was performed using mobile phase A (5% formic acid aqueous solution) and mobile phase B (methanol). The flow rate was 1 mL/min, and detection was at 520 nm. Two gradient systems that were used were as follows: gradient 1, 5% B, 0−2 min; 5−20% B, 2−10 min; 20% B, 10−15 min; 20−30% B, 15−30 min; 30% B, 30−35 min; 30−45% B, 35−50 min; 45% B, 50−55 min; 45−5% B, 55−65 min, 5% B, 65−68 min; gradient 2, 5% B, 0−2 min; 5−24% B, 2−10 min; 24% B, 10−15 min; 24−35% B, 15−30 min; 35% B, 30−35 min; 35−45% B, 35−50 min; 45% B, 50−55 min; 45−5% B, 55−65 min, 5% B, 65−68 min. Gradient 1 was used to separate anthocyanins in black currant, gooseberry, and chokeberry, whereas gradient 2 was used to separate elderberry and red currant. Low-resolution electrospray mass spectrometry was performed with an Esquire-LC mass spectrometer (MS) (Bruker Daltoniks, Billerica, MA), an ion trap instrument equipped with an electrospray interface. Column effluent was monitored in positive mode of the MS. Major MS parameters were as follows: capillary exit, 3500 V; capillary offset, 500 V; skim 1, 25.4 V; nebulizer, 45 psi; dry gas, 11 L/min; temperature, 345 °C.

For quantification and identification purposes, a mixture of six anthocyanin standards (3-*O*-β-glucosides of delphinidin, cyanidin, petunidin, pelargonidin, peonidin, and malvidin) was used to create a calibration curve of the individual anthocyanins. The results were expressed as anthocyanidin glucoside equivalents.

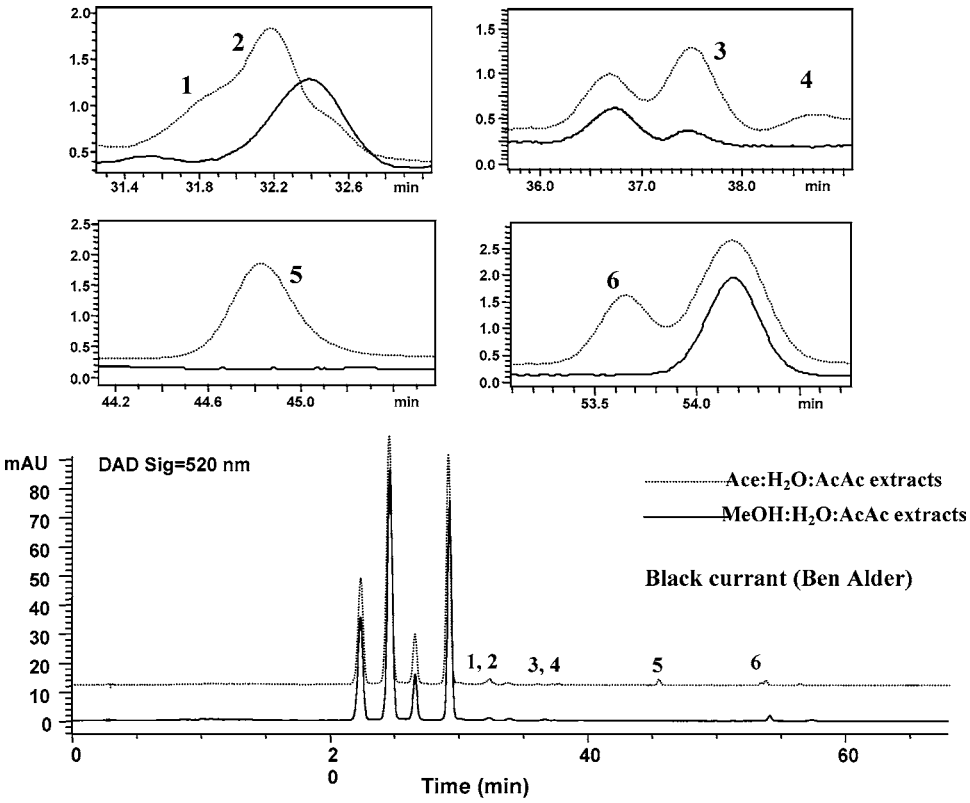
HPLC-MS/MS Analyses of Proanthocyanidins. Methods of quantification and identification of PAs have been described previously (*9*).

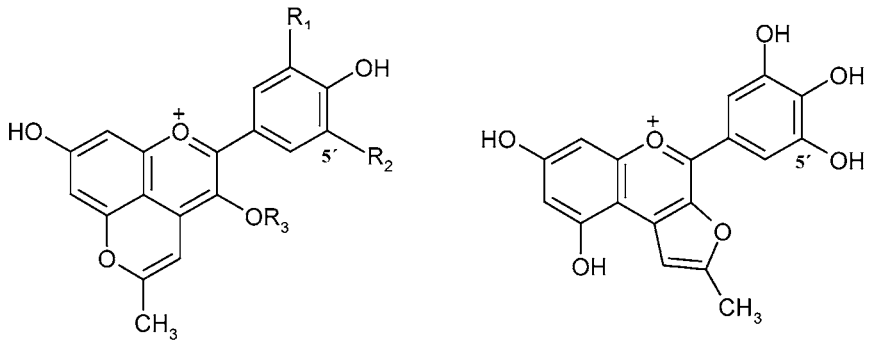
$ORAC_{FL}$ Assay. Both hydrophilic and lipophilic $ORAC_{FL}$ assays were carried out on a FLUOstar Galaxy plate reader, which was equipped with a temperature-controlled incubation chamber and two injection pumps. The temperature of the incubator was set at 37 °C. The procedures were based on our modified $ORAC_{FL}$ method (*28*) and on that reported by Ou and co-workers (*29*, *30*). The data were expressed as micromoles of Trolox equivalents per gram of fresh weight (μmol of TE/g of FW).

Total Phenolic Analysis. The Ace/H_2O/AcAc extracts were subjected to total phenolics measurement by Folin−Ciocalteu reagent according to the method of Wu et al. (*6*). The results were expressed as milligrams of gallic acid equivalents per 100 g of fresh weight (mg of GAE/100 g of FW).

RESULTS AND DISCUSSION

Polyphenol Extraction Strategies. It is difficult to find one solvent system that is universal for all different compounds. Normally different solvent systems are chosen on the basis of the specific analytical task. In this study, the two most commonly used solvent systems for polyphenol analysis and antioxidant capacity measurement were used. One is an acetone/water/acid system, which has been demonstrated to be a good solvent system to extract proanthocyanidins (*31*). The other solvent system was a methanol/water/acid system, which was used to extract anthocyanins. Traditionally, both acetone and methanol have been widely used for anthocyanin analysis. However, two recent papers (*32*, *33*) demonstrated that anthocyanins may undergo unusual facile reactions with acetone to give rise to pyranoanthocyanins. Pyranoanthocyanins can further undergo hydrolysis and rearrangement to generate two furoanthocyanidins. These findings were verified in this investigation by analyzing anthocyanins from black currant and elderberry using Ace/H_2O/AcAc extractions. **Figure 2** shows the six artificial anthocyanins from black currant (Ben Alder) extracts using Ace/H_2O/AcAc. Among them, four pyranoanthocyanins (peaks 1−4) were formed either from cyanidin or from delphinidin glycoside; two furoanthocyanidins (peaks 5 and 6) from delphinidin were the predominant artificial compounds. It is of interest that we could detect only furoanthocyanidins from delphinidin. From elderberry extracts using Ace/H_2O/AcAc, in which cyanidin-based anthocyanins are predominant





Compound **1-4, 5** Compound **6**

Compound	R_1	R_2	R_3	MS	MS/MS
1	OH	OH	Rutinose	649	341
2	OH	OH	Glucose	503	341
3	OH	H	Rutinose	633	325
4	OH	H	Glucose	487	325
5	OH	OH	H	341	---
6	---	---	---	341	---

Figure 2. Comparison of anthocyanin profiles of black currant (cv. Ben Alder) using acetone/water/acetic acid (Ace/H_2O/AcAc, 70:29.5:0.5, v/v) and methanol/water/acetic acid (MeOH/H_2O/AcAc, 85:15:0.5, v/v) extractions. Six artificial anthocyanins (**a**–**f**) were found with Ace/H_2O/AcAc extraction. Type of extraction solvent is represented by a solid line (MeOH/H_2O/AcAc extraction) and dotted line (Ace/H_2O/AcAc extraction) in chromatograms.

and their concentrations are much higher, we could not detect any furoanthocyanidin from cyanidin. This meant that the hydrolytic reaction and rearrangement from pyranoanthocyanin to furoanthocyanidin might be determined by the hydroxyl groups in B-ring of the flavylium structure. It appears that the hydroxyl group at the 5′-position significantly increased the reactivity. Although the quantities of these products formed are small, our results suggest that acetone may not be a good solvent for anthocyanin extraction and analysis.

Characterization and Quantification of Anthocyanins. Identification and peak assignment of anthocyanins was based on the comparison of their retention time and MS data with standards and published data (**Figure 1**; **Table 1**). Under current separation conditions, the elution order of six mixed anthocyanins was delphinidin 3-glucoside, cyanidin 3-glucoside, petunidin 3-glucoside, pelargonidin 3-glucoside, peonidin 3-glucoside, and malvidin 3-glucoside. Quantification of anthocyanins was performed by utilizing the corresponding anthocyanidin 3-glucoside as a standard (**Tables 2** and **3**).

Black Currant. All six cultivars of black currant had similar anthocyanin profiles. A representative chromatogram of anthocyanins from one cultivar of black currant, Ben Alder, is shown

Table 1. Identification of Anthocyanins from Berries Using HPLC-ESI/MS/MS

berry sample	peak	t_R[a] (min)	$[M]^+$ (*m*/*z*)	MS/MS	compound
black currant	1	22.4	465	303	delphinidin 3-glucoside
	2	24.7	611	465/303	delphinidin 3-rutinoside
	3	26.6	449	287	cyanidin 3-glucoside
	4	29.4	595	449/287	cyanidin 3-rutinoside
	5	30.0	479	317	petunidin 3-glucoside
	6	30.9	433	271	pelargonidin 3-glucoside
	7	32.4	625	479/317	petunidin 3-rutinoside
	8	33.8	463	301	peonidin 3-glucoside
	9[c]	33.9	435	303	delphinidin 3-xyloside
	10	34.0	579	433/271	pelargonidin 3-rutinoside
	11	36.6	609	463/301	peonidin 3-rutinoside
	12[c]	39.5	419	287	cyanidin 3-xyloside
	13	54.2	611	303	petunidin 3-(6-coumaroyl)-glucoside
	14	57.4	595	287	cyanidin 3-(6-coumaroyl)-glucoside
gooseberry	18[c]	24.0	449	287	cyanidin-3-galactoside
	3	26.3	449	287	cyanidin 3-glucoside
	4	29.2	595	449/287	cyanidin 3-rutinoside
	8[c]	33.4	463	301	peonidin 3-glucoside
	11[c]	35.7	609	463/301	peonidin 3-rutinoside
	12[c]	38.9	419	287	cyanidin 3-xyloside
	15[c]	44.2	595	287	cyanidin glycoside
	16[c]	50.4	611	287	cyanidin 3-(6-caffeyl)-glucoside
	17[c]	52.0	595	287	cyanidin glycoside
	14[c]	56.1	595	287	cyanidin 3-(6-coumaroyl)-glucoside
chokeberry	18	24.0	449	287	cyanidin 3-galactoside
	3	26.6	449	287	cyanidin 3-glucoside
	19[c]	27.9	433	271	pelargonidin 3-galactoside
	20	29.2	419	287	cyanidin 3-arabinoside
	21[c]	31.1	517	355	NI[b]
	22[c]	31.6	565	287	cyanidin + rhamnose + pentose
	23[c]	33.2	403	271	pelargonidin 3-arabinoside
	12	39.5	419	287	cyanidin 3-xyloside
elderberry	24	14.6	743	581/449/287	cyanidin 3-sambubioside-5-glucoside
	25	14.6	611	449/287	cyanidin 3,5-diglucoside
	26	21.1	581	287	cyanidin 3-sambubioside
	3	21.6	449	287	cyanidin 3-glucoside
	4[c]	24.0	595	449/287	cyanidin 3-rutinoside
	6[c]	25.6	433	271	pelargonidin 3-glucoside
	27[c]	26.1	565	271	pelargonidin 3-sambubioside
red currant	28[c]	16.1	597	303	delphinidin 3-sambubioside
	29	17.4	611	287	cyanidin 3-sophoroside
	30	18.7	757	287	cyanidin 3-glucosylrutinoside
	26	20.2	581	287	cyanidin 3-sambubioside
	3	20.7	449	287	cyanidin 3-glucoside
	31	21.4	727	581/287	cyanidin 3-xylosylrutinoside
	4	22.8	595	449/287	cyanidin 3-rutinoside

[a] Retention times of elderberry and red currant were based on gradient 2, and the retention times of all others were based on gradient 1. [b] Not identified. [c] Identified in this berry for the first time.

in **Figure 3A**. A total of 14 anthocyanins were detected across all black currant cultivars. By comparing their MS data with those recently published (*34*), 12 of them were identified (peaks 1−8, 10, 11, 13, 14). MS data of peak 9 showed a molecular ion of 435 ($[M]^+$ *m*/*z* 435) and a fragment ion of 303 (MS/MS, *m*/*z* 303). The fragment loss of 132 revealed this anthocyanin to be a delphinidin 3-pentoside. Under our current separation conditions, the retention time of delphinidin 3-arabinoside should be shorter than that of cyanidin 3-glucoside. However, the retention time of peak 9 (33.9 min) was longer than that of peak 3 (26.6 min) (cyanidin 3-glucoside). Thus, peak 9 was identified as delphinidin 3-xyloside. Peak 12 had the same MS data ($[M]^+$ *m*/*z* 419, MS/MS, *m*/*z* 287) as cyanidin 3-arabinoside published previously (*34*), but the t_R of peak 12 was 39.5 min, which is longer than that of cyanidin 3-arabinoside. The sequence of elution of anthocyanidin glycosides under our conditions is galactose, glucose, arabinose, and xylose. This indicated that peak 12 was cyanidin 3-xyloside. Xylosides of delphinidin and cyanidin were not identified earlier (*34*), whereas malvidin 3-glucoside and malvidin 3-rutinoside, which were identified earlier (*34*), were not detected in this group of berries. In the cultivars of black currant studied, individual anthocyanin concentrations varied slightly (**Table 2**). Our data on the concentrations of anthocyanins in black currant are much higher than those in a recently published paper that utilized the pH differential method (*22*).

Gooseberry. Among the six cultivars of gooseberries, Whinham, Lancashine, and Dan's Mistake had either pink or light red berry color and were found to have similar anthocyanin profiles containing a total of nine anthocyanins. Because there are few published data available on the anthocyanin composition of gooseberry, the peak identification and assignment were primarily based on the comparison of their MS data and retention times with those of standards and the anthocyanins in black currant cultivars. A representative chromatogram of anthocyanins in the gooseberry cv. Whinham is shown in **Figure 3B**. Black currant and Whinham gooseberry share six anthocyanins, which are peaks 3, 4, 8, 11, 12, and 14 (**Table 1**).

Table 2. Anthocyanin Concentrations in Six Cultivars of Black Currants (Milligrams per 100 g of FW)

		black currant cultivars					
peak	anthocyanin	Ben Alder	Ben Nevis	Ben Lomond	Ben Tirran	Titania	Ukraine
1	delphinidin 3-glucoside	112.54	106.74	113.21	74.34	63.18	54.20
2	delphinidin 3-rutinoside	275.17	311.42	286.69	215.85	204.72	180.30
3	cyanidin 3-glucoside	28.56	21.26	26.76	22.18	12.48	12.50
4	cyanidin 3-rutinoside	134.94	138.83	136.69	113.11	73.38	70.44
5	petunidin 3-glucoside	T[a]	T	T	T	T	T
6	pelargonidin 3-glucoside	ND[b]	ND	T	ND	ND	ND
7	petunidin 3-rutinoside	2.80	3.08	4.15	4.15	2.78	1.86
8	peonidin 3-glucoside	T	ND	ND	ND	ND	ND
9	delphinidin 3-xyloside	T	ND	T	ND	T	ND
10	pelargonidin 3-rutinoside	2.22	2.11	2.15	2.13	1.27	1.26
11	peonidin 3-rutinoside	0.77	0.93	1.67	1.38	0.69	0.40
12	cyanidin 3-xyloside	T	T	T	T	T	ND
13	petunidin 3-(6-coumaroyl)-glucoside	3.60	2.26	1.58	14.41	1.92	1.34
14	cyanidin 3-(6-coumaroyl)-glucoside	1.24	T	0.65	4.43	T	0.43
	total	561.8	586.6	573.5	452.0	360.4	322.7

[a] Trace. [b] Not detected.

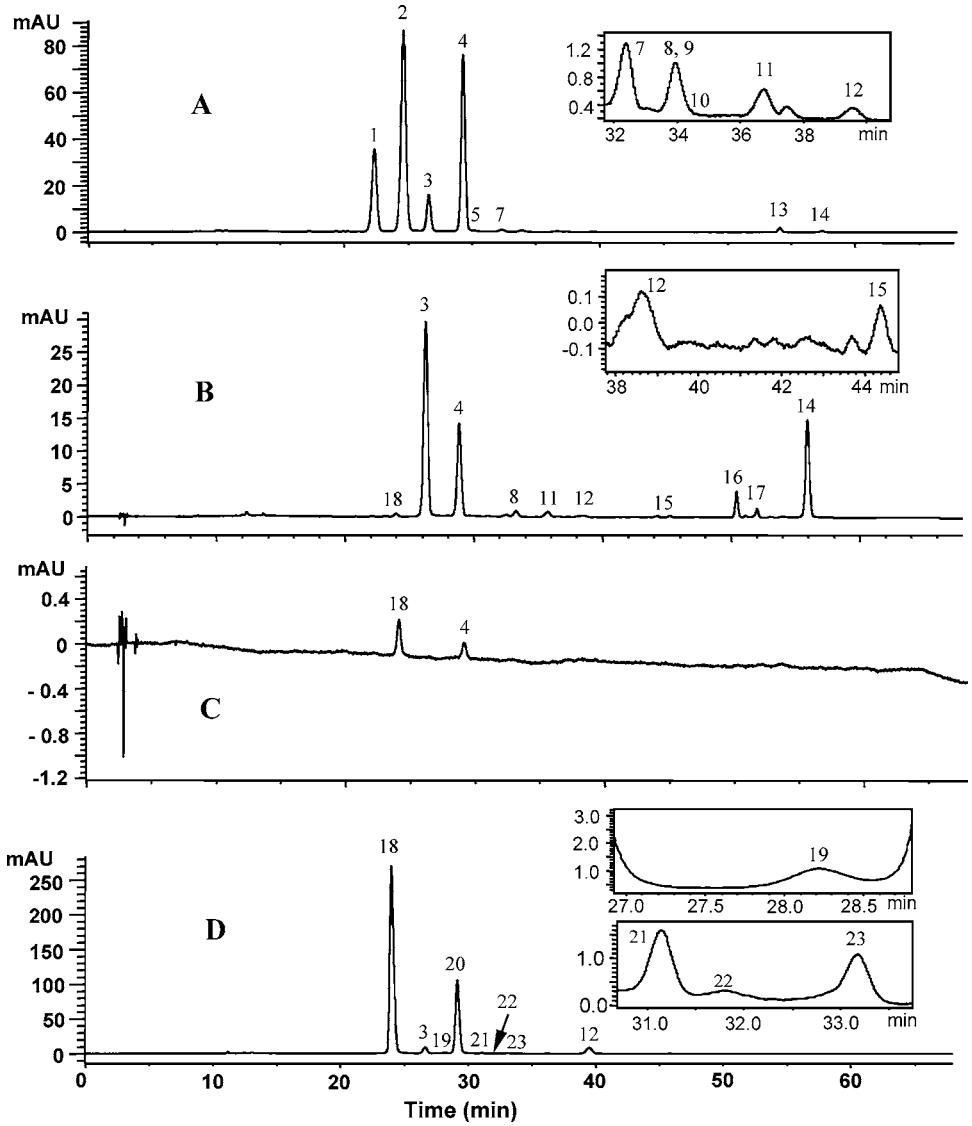


Figure 3. Reverse-phase HPLC chromatograms of anthocyanins detected at 520 nm from (**A**) black currant (cv. Ben Alder), (**B**) gooseberry (cv. Whinham), (**C**) gooseberry (cv. Careless), and (**D**) chokeberry. Elution gradient 1 was used to separate anthocyanins. Refer to **Table 1** for the identification of each numbered peak.

Peak 16 in gooseberry had a molecular weight of 611 ($[M]^+$ *m*/*z* 611) and a fragment ion *m*/*z* of 287. The fragment loss was 324, which could be accounted for by a caffeoyl glucoside. The UV spectrum of this peak showed that it had a weak absorption maximum at 327 nm, which agrees with the MS data indicating a caffeic acid residue (*35*). Thus, this anthocyanin was tenta-

Table 3. Anthocyanin Concentrations in Different Cultivars of Gooseberry, Chokeberry, Elderberry, and Red Currant (Milligrams per 100 g of FW)

		gooseberry cultivars						
peak	anthocyanin	Whinham	Lancashine	Dan's Mistake	Careless	chokeberry	elderberry	red currant
3	cyanidin 3-glucoside	4.91	5.42	1.44	0.049	37.6	739.8	0.16
4	cyanidin 3-rutinoside	2.32	3.36	0.46			4.40	1.61
6	pelargonidin 3-glucoside						1.80	
8	peonidin 3-glucoside	0.13	0.07	0.05				
11	peonidin 3-rutinoside	0.15	0.13	0.05				
12	cyanidin 3-xyloside	T[a]	ND[b]	ND		51.5		
15	cyanidin glycoside	T	ND	ND				
16	cyanidin 3-(6-caffeoyl)-glucoside	0.45	0.24	ND				
17	cyanidin glycoside	0.15	0.07	0.07				
14	cyanidin 3-(6-coumaroyl)-glucoside	2.26	1.18	0.13				
18	cyanidin 3-galactoside				0.025	989.7		
19	pelargonidin 3-galactoside					T		
20	cyanidin 3-arabinoside					399.3		
21	NI					2.4[c]		
22	cyanidin + rhamnose + pentose					T		
23	pelargonidin 3-arabinoside					2.3		
24	cyanidin 3-sambubioside-5-glucoside						82.6	
25	cyanidin 3,5-diglucoside							
26	cyanidin 3-sambubioside						545.9	3.39
27	pelargonidin 3-sambubioside						T	
28	delphinidin 3-sambubioside							0.10
29	cyanidin 3-sophoroside							0.11
30	cyanidin 3-glucosylrutinoside							0.49
31	cyanidin 3-xylosylrutinoside							6.93
	total	10.37	10.46	2.20	0.074	1480.0	1374.4	12.09

[a] Trace. [b] Not detected. [c] Calculated as cyanidin 3-glucoside equivalent.

tively identified as cyanidin 3-(6-caffeoyl)-glucoside. Peaks 15 and 17 had the same ionization pattern ($[M]^+$, *m*/*z* 595; fragment, *m*/*z* 287) as peak 14, cyanidin 3-(6-coumaroyl)-glucoside, but their exact structures could not be determined due to limited information. Another gooseberry cultivar, Careless, was found to have an anthocyanin profile that differed from the three cultivars above. It contained only two anthocyanins in very low concentrations (**Figure 3C**). Peak 18 had the same mass spectral data as cyanidin 3-glucoside, but its retention time was ~24 min, which was shorter than that of cyanidin 3-glucoside. Thus, this anthocyanin was identified as cyanidin 3-galactoside. Peak 4 was identified as cyanidin 3-rutinoside on the basis of its mass spectral data and retention time. Total concentrations of anthocyanins in Whinham, Lacashine, Dan's Mistake, and Careless are shown in **Table 3**.

Chokeberry. Eight anthocyanins were detected in chokeberry (**Figure 3D**), of which the four major anthocyanins were identified as cyanidin 3-galactoside, cyanidin 3-glucoside, cyanidin 3-arabinoside, and cyanidin 3-xyloside on the basis of their MS data compared to published data (*20*, *21*, *36*). These four anthocyanins correspond to peaks 18, 3, 20, and 12, respectively. Four minor anthocyanins were detected in chokeberry for the first time. Among them, peak 19 had a molecular ion of 433 ($[M]^+$, *m*/*z* 433) and a fragment ion of *m*/*z* 271. The MS data indicated that it was a glucoside or galactoside of pelargonidin, with a retention time of 27.9 min, which is shorter than that of the pelargonidin 3-glucoside standard (~30.9 min). Thus, this anthocyanin was identified as pelargonidin 3-galactoside. MS data of peak 21 indicated that this anthocyanin was an unusual anthocyanidin hexoside, with a molecular ion *m*/*z* 517 and a fragment ion *m*/*z* 355. It could not be identified due to limited information. Peak 22 was a cyanidin glycoside, and the difference between molecular weight and the fragment was 278, which can be accounted for by a rhamnose plus a pentose moiety. To our knowledge, there are no published data reporting this structure in chokeberry. The exact structure could not be determined because of limited information. Peak 23 had a molecular ion of 403 ($[M]^+$ *m*/*z* 403) and a fragment ion of *m*/*z* 271, which was identified as a pelargonidin 3-pentoside. Considering that its retention time (33.2 min) was shorter than that of cyanidin 3-xyloside (39.5 min), it was identified as pelargonidin 3-arabinoside. The quantification of individual anthocyanins in chokeberry is shown in **Table 3**. Among them, peak 22, the aglycon of which was not identified, was quantified as cyanidin 3-glucoside equivalents.

Elderberry. Seven anthocyanins were detected in elderberry (**Figure 4E**). Four of them are well-known anthocyanins (*37*): cyanidin 3-sambubioside-5-glucoside (peak 24), cyanidin 3,5-diglucoside (peak 25), cyanidin 3-sambubioside (peak 26), and cyanidin 3-glucoside (peak 3). Three minor peaks were identified as cyanidin 3-rutinoside (peak 4), pelargonidin 3-glucoside (peak 6), and pelargonidin 3-sambubioside (peak 27), by comparing their MS data and relative retention time with those of anthocyanins discussed above. To our knowledge, all three minor anthocyanins were detected in elderberry for the first time. Remarkably, this is the first time that pelargonidin has been identified in elderberry. The quantification of the individual anthocyanins in elderberry is shown in **Table 3**.

Red Currant. One cultivar of red currant, Red Lake, which was analyzed for anthocyanins, contained a total of seven anthocyanins, which were identified (**Figure 4F**). Among them, six corresponded to those that were reported by other investigators (*38*, *39*), namely, peak 29, cyanidin 3-sophoroside; peak 30, cyanidin 3-glucosylrutinoside; peak 26, cyanidin 3-sambubioside; peak 3, cyanidin 3-glucoside; peak 31, cyanidin 3-xylosylrutinoside; and peak 4, cyanidin 3-rutinoside. Peak 28, the retention time of which was shorter than those of all other peaks, had a delphinidin aglycon (fragment *m*/*z* 303) and a molecular weight of 597. The fragment loss was 304, which could be accounted for by a sambubiose residue. Thus, this anthocyanin was identified as delphinidin 3-sambubioside. This is the first time that the delphinidin derivative has been found in red currant. The concentration of individual anthocyanins in red currant is shown in **Table 3**.

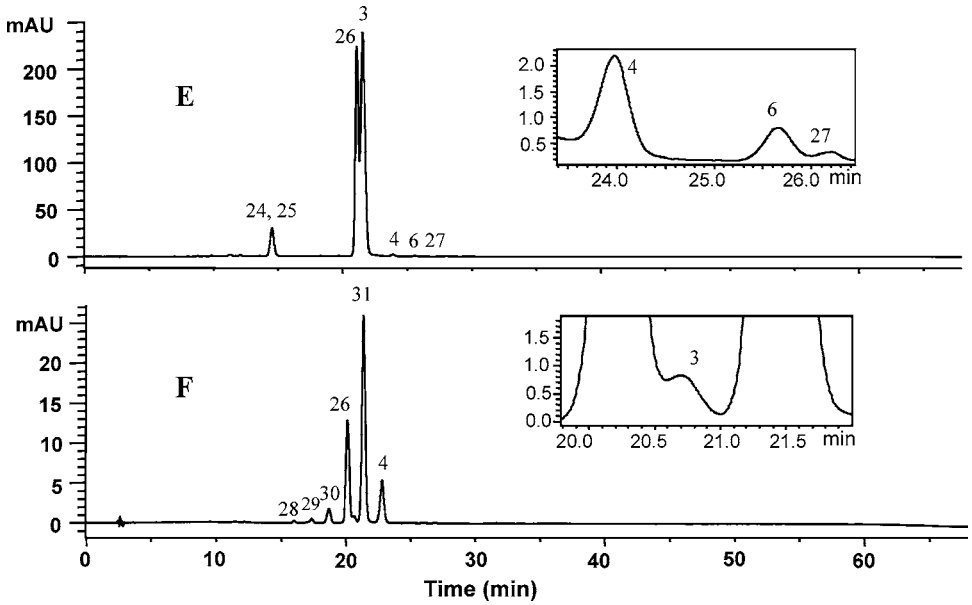


Figure 4. Reverse-phase HPLC chromatograms of anthocyanins detected at 520 nm from (**E**) elderberry and (**F**) red currant (cv. Red Lake). Elution gradient 2 was used to separate anthocyanins. Refer to **Table 1** for the identification of each numbered peak.

Characterization and Quantification of Proanthocyanidins. All cultivars of black currants had similar profiles of PAs (**Figure 5A**). Mass spectrometric data indicated that black currants contained both procyanidins and prodelphinidins. Polymeric PAs (DP > 10) appeared as a major peak in the chromatograms. Previous data (*26*) indicated that the mean degree of polymerization of PAs in black currant was 38.7 with catechin, gallocatechin, and their epimers as subunits (*26*). Total PAs in six cultivars of black currant ranged from 120.6 to 165.8 mg/100 g of FW (**Table 4**). Polymers accounted for >80% of the total PA amount.

Gooseberries and red currant (**Figure 5B**) had profiles of PAs similar to that for black currant but lower concentrations. They contained procyanidins and prodelphinidins, and the predominant PAs were in the polymeric form. Chokeberry contained only procyanidins. The oligomeric procyanidin peaks in chokeberry were well separated and easily distinguishable (**Figure 5C**). The concentration of PAs in chokeberry is among the highest in foods (*9*), which may explain their potent astringent taste. Unlike other berries, elderberry contained no detectable higher oligomers and polymers (**Figure 5D**).

Variation in Anthocyanin and Proanthocyanidin Profiles. In black currant, the same profile of the four major anthocyanins occurred in the different cultivars, even though the individual concentrations varied. The detectable minor anthocyanins were different across cultivars. Cyanidin 3-glucoside and cyanidin 3-rutinoside were the major anthocyanins in all three gooseberry cultivars. The other anthocyanins varied greatly from cultivar to cultivar. Cyanidin 3-(6-coumaroyl)-glucoside was one of the major anthocyanins in Whinham but only a minor anthocyanin in Dan's Mistake.

Antioxidant Capacity and Amounts of Phenolic Compounds. Results for lipophilic and hydrophilic antioxidant capacities ($ORAC_{FL}$) as well as total phenolics in all berries are presented in **Table 5**. The sum of lipophilic and hydrophilic $ORAC_{FL}$ was regarded as total $ORAC_{FL}$. Comparable antioxidant capacity measurements on other fruits and berries can be found in our recent publication (*6*). Similar to the other berries that we have analyzed, the lipophilic antioxidant capacity is quite low (*6*). However, hydrophilic antioxidant capacities for black

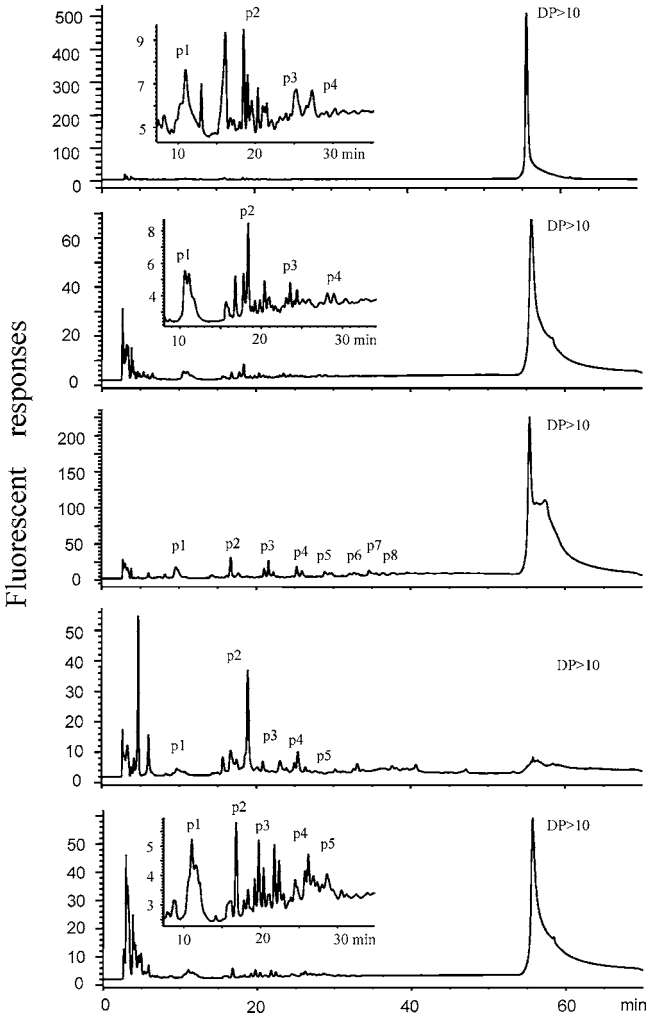


Figure 5. Normal-phase HPLC fluorescence trace of the proanthocyanidins from (**A**) black currant, (**B**) gooseberry, (**C**) chokeberry, (**D**) elderberry, and (**E**) red currant. Labels p1–p8 on the peaks indicate the degree of polymerization of proanthocyanidins in the peaks.

Table 4. Proanthocyanidin Concentrations in Different Cultivars of Black Currant and Gooseberry, Chokeberry, Elderberry, and Red Currant (Milligrams per 100 g of FW)[a]

	monomers	dimers	trimers	4–6-mers	7–10-mers	>10-mers	total	type
black currant								
Ben Alder	0.81	3.34	2.43	9.75	10.86	138.6	165.8	pc, pd
Ben Nevis	0.85	3.10	2.37	9.35	10.85	122.8	149.3	pc, pd
Ben Lomond	0.79	3.56	2.50	9.59	10.12	100.5	127.0	pc, pd
Ben Tirran	0.69	2.62	1.91	7.89	9.37	101.9	124.4	pc, pd
Titania	0.78	2.97	2.15	7.36	8.69	98.7	120.6	pc, pd
Ukraine	0.73	2.38	1.68	6.70	8.54	110.6	130.7	pc, pd
gooseberry								
Whinham	0.41	2.01	1.38	5.69	6.45	63.9	79.8	pc, pd
Lancashine	0.65	2.80	1.82	6.92	8.55	113.3	134.0	pc, pd
Dan's Mistake	0.58	1.25	1.03	4.79	5.47	77.3	90.4	pc, pd
Marigold	0.60	1.52	1.12	5.16	6.41	55.8	70.6	pc, pd
Careless	0.36	1.00	0.73	3.64	4.49	35.4	45.6	pc, pd
Leveller	0.43	1.15	0.89	4.34	5.28	53.9	65.9	pc, pd
chokeberry	5.17	12.48	10.29	40.32	52.87	542.6	663.7	pc
elderberry	1.44	10.62	5.63	10.80	ND	ND	23.3	pc
red currant								
Red Lake	1.26	1.97	1.54	6.87	7.90	41.2	60.8	pc, pd

[a] Monomers, dimers, and trimers are listed separately. Tetramers through hexamers are pooled together as 4–6-mers. Polymers with DP > 10 are quantified collectively and listed as >10-mers. pc, procyanidins; pd, prodelphinidins.

Table 5. Lipophilic and Hydrophilic $ORAC_{FL}$, Total Phenolics of Black Currant, Gooseberry, Chokeberry, Elderberry, and Red Currant (Based on FW)

sample	moisture (%)	L-$ORAC_{FL}$[a] (μmol of TE/g)	H-$ORAC_{FL}$[b] (μmol of TE/g)	TAC[c] (μmol of TE/g)	TP[d] (mg of GAE/g)	H-$ORAC_{FL}$/TP[e]
black currant						
Ben Alder	80.2	0.84	100.6	101.4	13.3	7.6
Ben Nevis	78.5	0.75	90.6	91.3	14.1	6.4
Ben Lomond	77.0	0.68	92.3	92.9	12.2	7.6
Ben Tirran	78.9	0.75	86.6	87.3	13.3	6.5
Titania	78.6	1.15	49.0	50.1	8.4	5.8
Ukarine	72.1	0.68	53.7	54.4	10.8	5.0
gooseberry						
Whinham		0.15	39.2	39.4	5.2	7.5
Lancashine		0.15	41.3	41.4	6.0	6.9
Dan's Mistake		0.28	37.1	37.4	6.3	5.9
Marigold		0.45	33.7	34.1	5.5	6.2
Careless		0.35	20.4	20.8	3.4	6.0
Leveller		0.43	26.4	26.8	4.6	5.8
chokeberry	71.8	2.42	158.2	160.6	20.1	7.9
elderberry	82.5	1.97	145.0	147.0	19.5	7.5
red currant	78.1	1.27	32.6	33.9	5.4	6.0

[a] Lipophilic $ORAC_{FL}$. [b] Hydrophilic $ORAC_{FL}$. [c] Total antioxidant capacity. [d] Total phenolics. [e] Ratio of hydrophilic $ORAC_{FL}$ to total phenolics expressed as μmol of TE/mg of GAE.

currant, chokeberry, and elderberry are among the highest that we have measured in fresh fruits or berries (*6*).

The concentrations of total phenolics were found to correlate with the antioxidant capacities in berries (*24*, *40*, *41*). However, different types of phenolic compounds may contribute differently to total antioxidant capacity for a given food. The relationships between total $ORAC_{FL}$ and total anthocyanins, total PAs, total phenolics, or total anthocyanins plus total PAs of all samples are presented in **Figure 6**. The best linear relationship was observed between total $ORAC_{FL}$ and total phenolics ($R^2 = 0.96$), followed by total $ORAC_{FL}$ versus total anthocyanins ($R^2 = 0.95$) and total $ORAC_{FL}$ versus total anthocyanin plus total proanthocyanidins ($R^2 = 0.91$). The results are in accordance with our previous data (*24*). However, it is important to remember that these high correlations were obtained only within a certain category of samples. One should be cautious in attempting to extend the conclusion to other diverse groups of samples. Our previous studies have shown that the ratio of total $ORAC_{FL}$ to total phenolics in a wide range of samples varied from 1.7 to 156.4 (*6*), indicating that the relationship between $ORAC_{FL}$ and total phenolics is low across a diverse set of samples. There was a weak correlation ($R^2 = 0.37$) between total $ORAC_{FL}$ and total PAs. Remarkably, as shown in **Figure 6B**, if chokeberry and elderberry were removed from the regression, a much higher correlation between total $ORAC_{FL}$ and total PAs would be observed. These two berries are actually two extreme examples; in chokeberry we found very high concentrations of both anthocyanins and PAs, whereas in elderberry we found very high concentrations of anthocyanins but very low concentrations of PAs. Because the major PAs in these berries are polymers, it appears that on a weight basis polymeric PAs do not contribute as much of the antioxidant capacity as anthocyanins. In addition, PA polymers do not react strongly with the Folin−Ciocalteu reagent, resulting in relatively low total phenolic concentrations in some berries with high PA concentrations.

The berries evaluated in this study represent some interesting and unique differences in phytochemical composition. Some of the extremes in anthocyanin concentrations are represented, with none in some gooseberry cultivars to some of the highest anthocyanin concentrations found in common berries in chokeberry and elderberry. Among black currant cultivars are some berries that have delphinidin aglycon present as the predominant

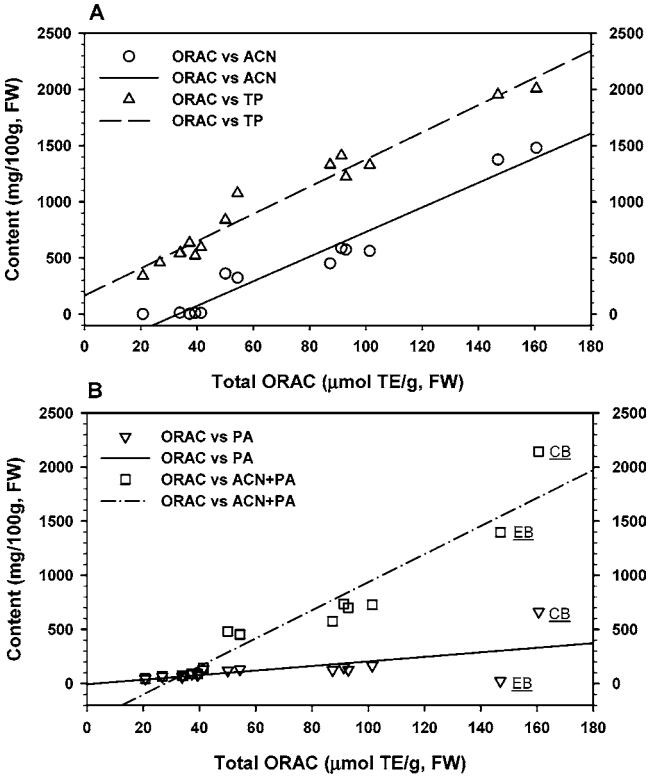


Figure 6. Relationship between total $ORAC_{FL}$ and concentrations of anthocyanins (ACN) or phenolics (TP) (**A**: $ORAC_{FL}$ vs TP, $y = 7.959x - 10.62$, $R^2 = 0.964$; $ORAC_{FL}$ vs ACN, $y = 0.087x + 35.46$, $R^2 = 0.948$) and relationship between total $ORAC_{FL}$ and concentrations of proanthocyanidins (PA) or anthocyanin plus proanthocyanidins (ACN+PA) (**B**: $ORAC_{FL}$ vs PA, $y = 0.175x + 43.98$, $R^2 = 0.369$; $ORAC_{FL}$ vs ACN+PA, $y = 0.0709x + 31.074$, $R^2 = 0.911$). Points in (**B**) labeled as CB or EB refer to chokeberry and elderberry, respectively.

anthocyanin. Elderberry contains an appreciable amount of cyanidin as a diglucoside (sambubioside), which is not common in most berries. All of these differences may have important implications that may become evident from studies of health effects, and as we learn more about the absorption/metabolism of anthocyanins with different aglycons or with anthocyanins containing the complex sugar moieties.

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