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Identification of Flavonoid and Phenolic Antioxidants in Black Currants, Blueberries, Raspberries, Red Currants, and Cranberries†

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The antioxidant capacity (AOC) of black currant, blueberry, raspberry, red currant, and cranberry extracts was determined using the FRAP assay. In addition, the vitamin C content of the berries was determined and phenolic and polyphenolic compounds in the extracts were analyze by reversed-phase HPLC-PDA-MS^3 and by reversed-phase HPLC-PDA with an online antioxidant detection system. A complex spectrum of anthocyanins was the major contributor to the AOC of black currants and blueberries, whereas the lower AOC of red currants and cranberries was due mainly to a reduced anthocyanin content. Raspberries also had a lower anthocyanin content than black currants and blueberries, but there was only a slight decline in the AOC because of the presence of the ellagitannins sanguin H-6 and lambertianin C, which were responsible for 58% of the HPLC-AOC of the berries. Vitamin C was responsible for 18−23% of the HPLC-AOC of black currants, red currants, and cranberries and for 11% of that of raspberries but did not contribute to the AOC of the blueberry extract that was examined. Seven percent of the HPLC-AOC of the cranberry extract was attributable to procyanidin dimers. However, the contribution of polymeric proanthocyanidins to the AOC of the five berries was not determined as when analyzed by reversed-phase HPLC these high molecular weight flavan-3-ols are either retained by the column or eluted as a broad unresolved band.

KEYWORDS: Berries; flavonoids; phenolics; HPLC-MS^3 and online antioxidant detection

INTRODUCTION

Dietary patterns characterized by relatively high intakes of fruits and vegetables are consistently associated with reductions in the incidence of noncommunicable diseases such as coronary heart disease, stroke, cancer, and various chronic disease (*1*). Law and Morris (*2*), for instance, carried out a meta-analysis combining the results of 11 prospective cohort studies and found that people eating ~5 or more 80 g servings/day of fruits and vegetables had a reduced risk of myocardial infarction that was about 15% lower than that of those consuming < 5 servings daily. A further study reported that consumption of 600 g of fruits and vegetables per day can decrease the risk of coronary heart disease by 31% and ischemic stroke by 19% (*3*).

Fruits and vegetables contain several health-promoting factors including fiber and high concentrations of phenolic acids, flavonoids, vitamins, and minerals. Phenolic acids and flavonoids are phytochemicals that, although not essential for survival, may over the long term be one of the factors that contribute to the protective effects of a fruit- and vegetable-rich diet. The phenolic acids potentially involved in these beneficial effects include gallic acid and hydroxycinnamates including coumaric acid, caffeic acid, and derivatives such as chlorogenic acid (*4*). The main flavonoids of interest are anthocyanins, flavan-3-ols, and their polymeric condensation products, flavanones, flavonols, and flavones (*4*). To varying degrees these compounds are potent antioxidants in vitro (*5*), being able to inhibit lipid peroxidation (*6*) and protect low-density lipoproteins against oxidation (*7*). They can also reduce platelet aggregation (*8*) and enhance vasodilation (*9*).

There is only extremely limited evidence on the relative protective efficacy of specific fruits and vegetables. However, in this context, berries are of interest as a result of a study in Finland by Knekt et al. (*10*) in which the intake of berries, alongside other factors, was associated with a 60% decline in heart disease and stroke. A high consumption of berries is a particular feature of the Finnish diet (*11*). Berries, including raspberries, blueberries, black currants, red currants, and cranberries, are a rich source of dietary antioxidants (*12*, *13*). In this paper we report the total antioxidant capacity (AOC) of these berries and, through the use of HPLC-PDA-MS^3 and HPLC-PDA with an online antioxidant detection system, the extent to which vitamin C, phenolic compounds, and flavonoids contribute to the AOC of the different fruits.

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MATERIALS AND METHODS

Berries. Blueberries (*Vaccinium corymbosum*), cranberries (*Vaccinium oxycoccus*), raspberries (*Rubus idaeus*), black currants (*Ribes nigrum*), and red currants (*Ribes rubrum*) were purchased from supermarkets in the west end of Glasgow. Samples weighing 5 g were extracted with 10 mL of methanol/formic acid (99:1, v/v) using an Ultra-Turrax T25 homogenizer (IKA Werke, Staufen, Germany) for 1 min and centrifuged for 20 min at 4000*g*. The pellets were re-extracted, and supernatants were pooled before being reduced to dryness in vacuo. The residues were resuspended in 10 mL of methanol/formic acid (99:1, v/v). Aliquots were stored at −80 °C prior to analysis.

Chemicals. Methanol and acetonitrile were obtained from Rathburn Chemicals (Walkerburn, Scotland). Anthocyanins, (+)-catechin, (−)-epicatechin, quercetin-3-glucoside, myricetin, ellagic acid, kaempferol-3-glucoside, hydroxybenzoic acid, β-carotene, α-tocopherol, Trolox, and gallic acid were purchased from Extrasynthese (Genay, France), whereas ascorbic acid, metaphosporic acid, ferrous sulfate, ferrous chloride, TPTZ, Folin reagent, homocysteine, sodium acetate, myristyltrimethylammonium bromide, sodium hydroxide, and ferric ammonium sulfate were obtained from Sigma (Poole, Dorset, U.K.). ABTS diammonium salt was provided by Merck (Darmstadt, Germany). EDTA and acetic acid were from BDH Chemicals Ltd. (Poole, U.K.), sodium carbonate was from Riedel de Haehn GmbH (Seelze, Germany), and formic and acetic acid were obtained from Fisher Scientific (Loughborough, U.K.).

Antioxidant Assays. The AOC of the berry extracts was measured using the ferric reducing antioxidant power (FRAP) assay (*14*).

HPLC-PDA-MS^2. Samples were analyzed on a Surveyor HPLC system comprising a HPLC pump, a PDA detector, scanning from 250 to 700 nm, and an autosampler cooled to 4 °C (Thermo Fisher Corp., San Jose, CA). Analyses were carried out at 40 °C using a 250 × 4.6 mm i.d., 4 μm, Synergi RP-Max column (Phenomenex, Macclesfield, U.K.) eluted with a 60 min gradient of 5−25% acetonitrile in 1% aqueous formic acid at a flow rate of 1.0 mL/min. After passing through the flow cell of the PDA detector, the column eluate split and 0.2 mL/min was directed to an LCQ Advantage ion trap mass spectrometer fitted with an electrospray interface (Thermo Fisher Corp.). Analyses utilized both the negative and positive ion modes. Samples were analyzed in the mass spectrometer using full-scan data-dependent MS^2 scanning from *m*/*z* 100 to 2000. Capillary temperature was 150 °C, sheath gas and auxiliary gas were 40 and 20 units, respectively, and the source voltage was 3 kV. Compounds that could not be identified by MS^2 were further fragmented to produce MS^3 spectra. The system was controlled by Xcalibur software (Thermo Fisher Corp.).

For quantification purposes, all phenolic acids were expressed as 4-hydroxybenzoic acid, caffeic acid, or 5-*O*-caffeoylquinic acid equivalents; all flavan-3-ols and their polymers as (−)-epicatechin equivalents; anthocyanins conjugates as cyanidin-3-glucoside equivalents, quercetin conjugates as quercetin-3-glucoside or quercetin-3-rutinoside equivalents; kaempferol conjugates as kaempferol-3-glucoside equivalents; myricetin conjugates as myricetin equivalents; ellagic acid conjugates as ellagic acid equivalents; and ellagitannins as gallic acid equivalents.

HPLC-PDA-with Online Antioxidant Detection. The antioxidant activity of individual HPLC peaks was measured using an online HPLC antioxidant detector system (*15*) based on the TEAC assay of Re et al. (*16*). Antioxidants in the HPLC eluate react postcolumn with preformed ABTS$^{\bullet+}$, and the induced bleaching is measured as a negative peak at 720 nm. The stock solution of ABTS$^{\bullet+}$ was made by adding 0.5 mL of a 70 mM $K_2S_2O_8$ solution to 50 mL of 2 mM ABTS. The mixture was stored overnight in the darkness at room temperature to convert ABTS to

Table 1. Total Antioxidant Activity of Berries Measured by FRAP Assays[a]

berry	FRAP (μmol of Fe^{2+}/g)
black currants	51.6 ± 1.2
blueberries	30.0 ± 1.9
raspberries	27.7 ± 1.1
red currants	24.6 ± 0.5
cranberries	18.6 ± 0.3

[a] Data presented as mean values ± SE (*n* = 3) per gram of fresh weight.

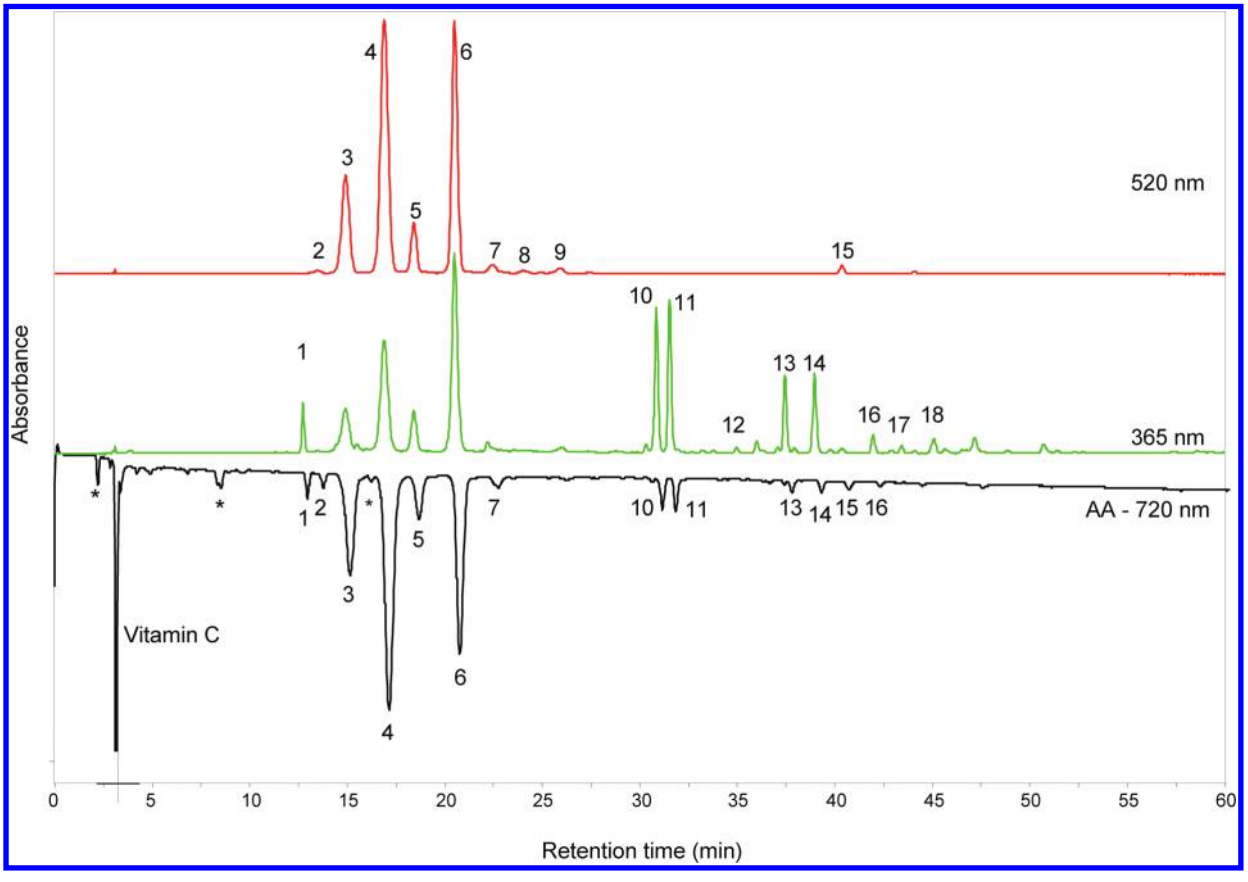

Figure 1. HPLC-PDA traces at 520 and 365 nm and online antioxidant detection (720 nm) of vitamin C and phenolic compounds in black currants. For identification of numbered peaks, see **Table 2** and the Supporting Information.

Table 2. Antioxidant Activity of Phenolic Compounds and Vitamin C in Black Currants[a]

peak	t_R (min)	compound	quantity (nmol/g)	antioxidant activity (nmol of Trolox/g)	antioxidant activity (%)
	3.2	vitamin C	2328 ± 99	1094 ± 101	17.5 ± 1.6
1	12.9	caffeic acid-*O*-glucoside	80 ± 1	76 ± 12	1.2 ± 0.2
2	13.5	delphinidin-3-*O*-galactoside	52 ± 1	60 ± 14	1.0 ± 0.2
3	14.8	delphinidin-3-*O*-glucoside	839 ± 7	886 ± 158	14.2 ± 2.5
4	17.0	delphinidin-3-*O*-rutinoside	2233 ± 37	2049 ± 336	32.8 ± 5.4
5	18.3	cyanidin-3-*O*-glucoside	327 ± 5	261 ± 61	4.2 ± 1.0
6	20.3	cyanidin-3-*O*-rutinoside	1693 ± 1	1181 ± 236	18.9 ± 3.8
7	22.4	petunidin-3-*O*-rutinoside peonidin-3-*O*-galactoside	103 ± 2	77 ± 15	1.2 ± 0.2
8	24.0	malvidin-3-*O*-galactoside peonidin-3-*O*-glucoside	71 ± 1	nd	
9	25.9	peonidin-3-*O*-rutinoside	126 ± 17	nd	
10	31.3	myricetin-3-*O*-rutinoside	135 ± 3	119 ± 17	1.9 ± 0.3
11	31.8	myricetin-*O*-glucuronide	138 ± 2	116 ± 21	1.9 ± 0.3
12	35.0	myricetin-3-*O*-(6″-malonyl)glucoside	29 ± 1	nd	
13	37.5	quercetin-3-*O*-rutinoside	77 ± 2	40 ± 7	0.6 ± 0.1
14	39.1	quercetin-3-*O*-glucoside	83 ± 3	40 ± 9	0.6 ± 0.1
15	40.5	delphinidin-3-*O*-(6″-*p*-coumaroyl)glucoside	77 ± 1	43 ± 8	0.7 ± 0.1
16	42.5	quercetin-3-*O*-(6″-malonyl)glucoside	17 ± 1	19 ± 3	0.3 ± 0.1
17	43.9	kaempferol-3-*O*-rutinoside	12 ± 0	nd	
18	45.2	kaempferol-3-*O*-galactoside	23 ± 1	nd	
		unidentified peaks		189 ± 8	3.0 ± 0.1

[a] Data expressed as mean values ± standard error ($n = 3$). t_R, retention time in minutes; nd, not detected. Peak numbers and retention times refer to HPLC traces in **Figure 1**. For identification of compounds see Table S1 and text in the Supporting Information.

$ABTS^{\bullet+}$. This $ABTS^{\bullet+}$ stock solution was then mixed with 0.1 M phosphate buffer (1:8, v/v) and adjusted to pH 8.0. The HPLC-PDA system used was as described in the previous section. After passing through the flow cell of the PDA detector, column mobile phase was directed to a "T", where it was mixed with the $ABTS^{\bullet+}$ solution delivered at a flow rate of 0.5 mL/min by a LC-10-AD *VP* pump connected to a vacuum degasser (Shimadzu, Kyoto, Japan). The mixture then passed through a 1.5 m × 0.4 mm loop, after which absorbance was measured at 720 nm (Nemphlar Bioscience, Lanark, U.K.). The antioxidant potential of HPLC peaks, expressed as Trolox equivalent antioxidant capacity (TEAC), was quantified by reference to a 10−400 μmol of Trolox standard calibration curve.

Analysis of Vitamin C. Vitamin C was analyzed by HPLC using a Nucleosil ODS 5 mm, 250 × 4.6 mm (i.d.), column eluted isocratically at a flow rate of 0.6 mL/min with a mobile phase at 30 °C comprising 0.05 mM sodium hydroxide, 25 mM myristyltrimethylammonium bromide, and 60 mM acetic acid in 7.5% aqueous acetonitrile containing 100 mg/L homocysteine and 200 mg/L EDTA. Detection was at 262 nm (*17*).

RESULTS

Total FRAP Antioxidant Activities. The total AOCs were high for all five berries (**Table 1**). Overall, black currants, blueberries, and raspberries had the highest AOC with lower values obtained for red currants and cranberries. Moyer et al. (*18*) report FRAP values ranging from 18.5 to 61.4 μmol of Fe^{2+}/g and from 13.1 to 45.2 μmol of Fe^{2+}/g for blueberries and raspberries, respectively. The levels obtained by FRAP assay for blueberries and raspberries presented in **Table 1**, 30.0 and 27.7 μmol of Fe^{2+}/g, respectively, are in keeping with these figures, although the overall AOC of individual produce will undoubtedly vary due to numerous factors including variety, degree of ripeness, season, and storage conditions.

Black Currants. HPLC-PDA analysis at 520 and 365 nm (**Figure 1**) revealed 18 peaks in the black currant extract, which were identified on the basis of their MS fragmentation profiles (**Table 2**, also see Supporting Information). Eleven delphinidin-, cyanidin-, malvidin-, petunidin-, and peonidin-based anthocyanins were detected, with the main components being delphinidin-3-*O*-glucoside (peak 3, 839 nmol/g of fresh weight), delphinidin-3-*O*-rutinoside (peak 4, 2233 nmol/g), and cyanidin-3-*O*-rutinose (peak 6, 1693 nmol/g). In addition to anthocyanins, the black currants contained vitamin C (2328 nmol/g) and smaller quantities of a caffeic acid-*O*-glucoside and several kaempferol and quercetin conjugates (**Table 2**). HPLC with online antioxidant detection revealed that the three main anthocyanins, along with vitamin C, were the major contributors to the AOC of the extract with delphinidin-3-*O*-rutinoside accounting for 32.8% of the total activity (**Figure 1**; **Table 2**). The flavonols myricetin-3-*O*-rutinoside (peak 10) and myricetin-3-*O*-glucuronide (peak 11) were each responsible for 1.9% of the total AOC, with the quercetin and kaempferol and quercetin conjugates making, at best, a minimal contribution (**Table 2**).

Blueberries. Fifteen anthocyanins were detected in blueberries, many of which exhibited substantial antioxidant activity (**Figure 2**; **Table 3**; also see the Supporting Information). Most antioxidant activity was attributed to peak 1 (delphinidin-3-*O*-galactoside), peak 3, which comprised both cyanidin-3-*O*-galactoside and delphinidin-3-*O*-arabinoside, peak 4 (petunidin-3-*O*-galactoside), peak 7 (malvidin-3-*O*-galactoside), and peak 10 (malvidin-3-*O*-arabinoside). Peak 13, which contained 5-*O*-feruloylquinic acid and traces of a quercetin-*O*-diglucoside, also exhibited a sharp antioxidant peak, as did peak 16, quercetin-3-*O*-glactoside (**Figure 2**; **Table 3**). The low vitamin C content of the blueberries did not contribute to their AOC.

Raspberries. Data on raspberries are presented in **Figure 3** and **Table 4**. Eight anthocyanins were detected in five 520 nm. Peak 1, cyanidin-3-*O*-sophoroside, was the major anthocyanin followed by the three compounds contained in peak 2, cyanidin-3-*O*-(2″-*O*-glucosyl)rutinoside, cyanidin-3-*O*-sambubioside, and cyanidin-3-*O*-glucoside. The extract also contained the ellagitannins lambertianin C and sanguin H-6 along with trace amounts of ellagic acid derivatives and quercetin conjugates. In this case, anthocyanins present at a total concentration of 885 nmol/g contributed 16.5% to the overall AOC, whereas vitamin C (1014 nmol/g) contributed 10.5%. In contrast to the black currant and blueberry extracts, the ellagitannins lambertianin C (peak 6) and sanguiin H-6 (peak 7) were the main contributors to the AOC, being responsible for > 58% of the total.

Red Currants. The main components in the red currant extract were vitamin C and peak 4, which contained both cyanidin-3-*O*-rutinoside and cyanidin-3-*O*-(2″-*O*-xylosyl)rutinoside (**Figure 4** and **Table 5**). The anthocyanin peaks contributed ca. 21% to the total AOC, whereas vitamin C was responsible for 47.5% of the total AOC of the extract with a number of unidentified components accounting for a further 23.5%. The extract also contained

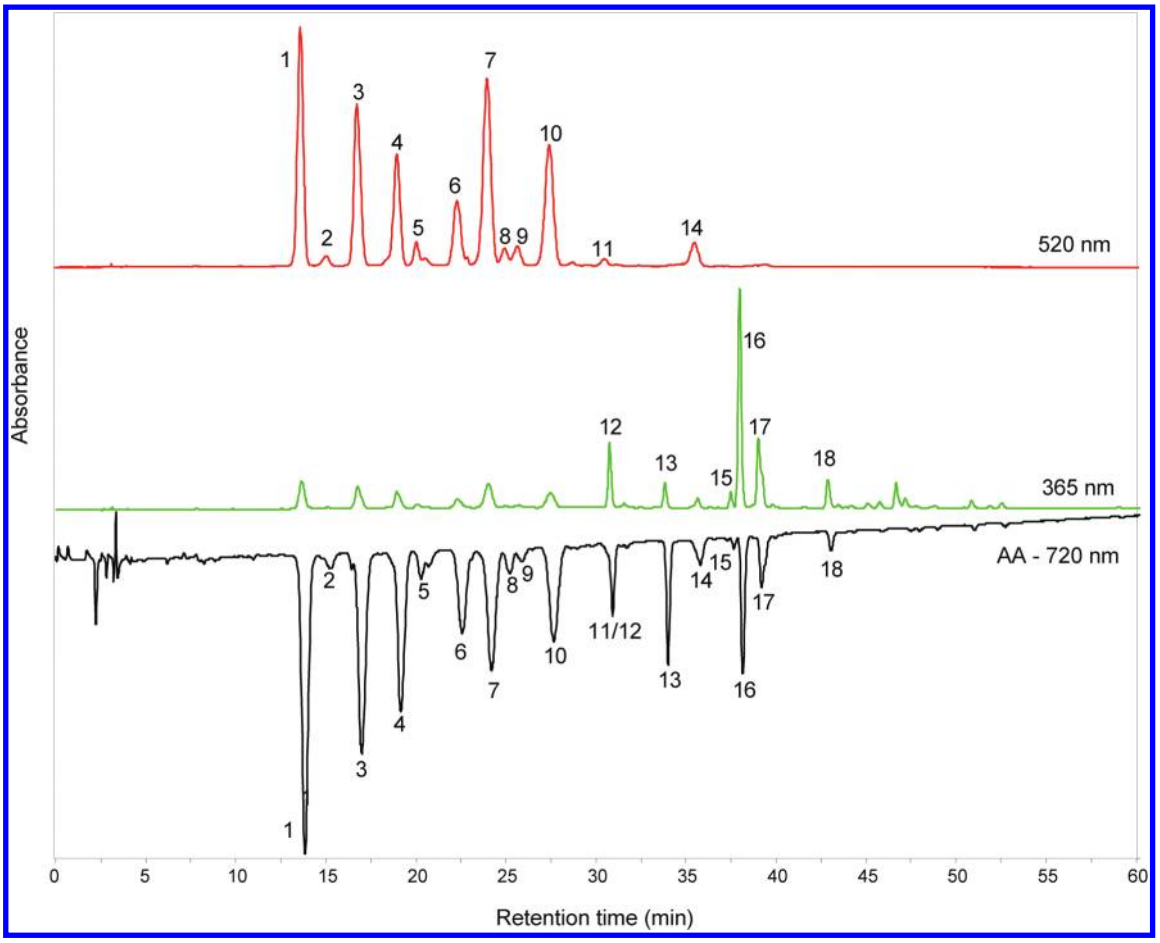

Figure 2. HPLC-PDA traces at 520 and 365 nm and online antioxidant detection (720 nm) of phenolic compounds in blueberries. For identification of numbered peaks see **Table 3** and the Supporting Information.

Table 3. Antioxidant Activity of Phenolic Compounds and Vitamin C in Blueberries[a]

peak	t_R (min)	compound	quantity (nmol/g)	antioxidant activity (nmol of Trolox/g)	antioxidant activity (%)
	3.2	vitamin C	115 ± 4	nd	
1	13.5	delphinidin-3-*O*-galactoside	729 ± 64	1135 ± 44	20.4 ± 0.8
2	14.8	delphinidin-3-*O*-glucoside	67 ± 1	61 ± 2	1.1 ± 0.0
3	16.7	cyanidin-3-*O*-galactoside delphinidin-3-*O*-arabinoside	590 ± 5	860 ± 39	15.4 ± 0.7
4	19.4	petunidin-3-*O*-galactoside	402 ± 35	680 ± 41	12.2 ± 0.7
5	20.2	cyanidin-3-*O*-arabinoside	119 ± 4	142 ± 4	2.5 ± 0.4
6	22.4	petunidin-3-*O*-arabinoside peonidin-3-*O*-galactoside	282 ± 19	421 ± 42	7.6 ± 0.8
7	24.0	malvidin-3-*O*-galactoside	996 ± 100	654 ± 96	11.7 ± 1.7
8	24.7	malvidin-3-*O*-glucoside delphinidin-3-*O*-(6″-*O*-acetyl)glucoside	212 ± 8	90 ± 19	1.6 ± 0.3
9	25.7	peonidin-3-*O*-arabinoside	96 ± 5	25 ± 1	0.4 ± 0.0
10	27.4	malvidin-3-*O*-arabinoside	888 ± 77	510 ± 33	9.1 ± 0.6
11	30.4	petunidin-3-*O*-(6″-*O*-acetyl)glucoside	81 ± 6	164 ± 4	2.9 ± 0.1
12	30.9	myricetin-3-*O*-galactoside	114 ± 11		
13	33.8	quercetin-*O*-diglucoside 5-*O*-feruloylquinic acid	16 ± 3	239 ± 1	4.3 ± 0.0
14	35.5	malvidin-3-*O*-(6″-*O*-acetyl)glucoside	348 ± 15	83 ± 2	1.5 ± 0.0
15	37.6	quercetin-3-*O*-rutinoside	31 ± 1	18 ± 2	0.3 ± 0.0
16	38.1	quercetin-3-*O*-galactoside	368 ± 36	303 ± 17	5.4 ± 0.3
17	39.2	quercetin-3-*O*-glucoside	155 ± 15	144 ± 6	2.6 ± 0.1
18	43.0	quercetin-3-*O*-arabinoside quercetin-3-*O*-(6″-*O*-acetyl)glucoside	75 ± 2.3	45 ± 2	0.8 ± 0.0

[a] Data expressed as mean values ± standard error (n = 3). t_R, retention time; nd, not detected. Peak numbers and retention times refer to HPLC traces in **Figure 2**. For identification of compounds see Table S2 and text in the Supporting Information.

a number of myricetin, kaempferol, and quercetin conjugates but their contribution to the overall AOC, like that of peak 1 (4-hydroxybenzoic acid-*O*-hexoside) and peak 3 (a caffeic acid-*O*-glucoside), was relatively small (**Table 5**).

Cranberries. Information on the phenolic compounds, vitamin C, and their contribution to the AOC of a cranberry extract is presented in **Figure 5** and **Table 6**. The main antioxidant peak corresponds to vitamin C, which was responsible for 22.6% of the AOC. (−)-Epicatechin (peak 5) is the major phenolic compound at 1121 nmol/g, but it contributes only 14% of the overall AOC along with peonidin-3-*O*-galactoside (peak 6). The anthocyanins constitute the second major group with peaks 2, 4, 6, 7, 8,

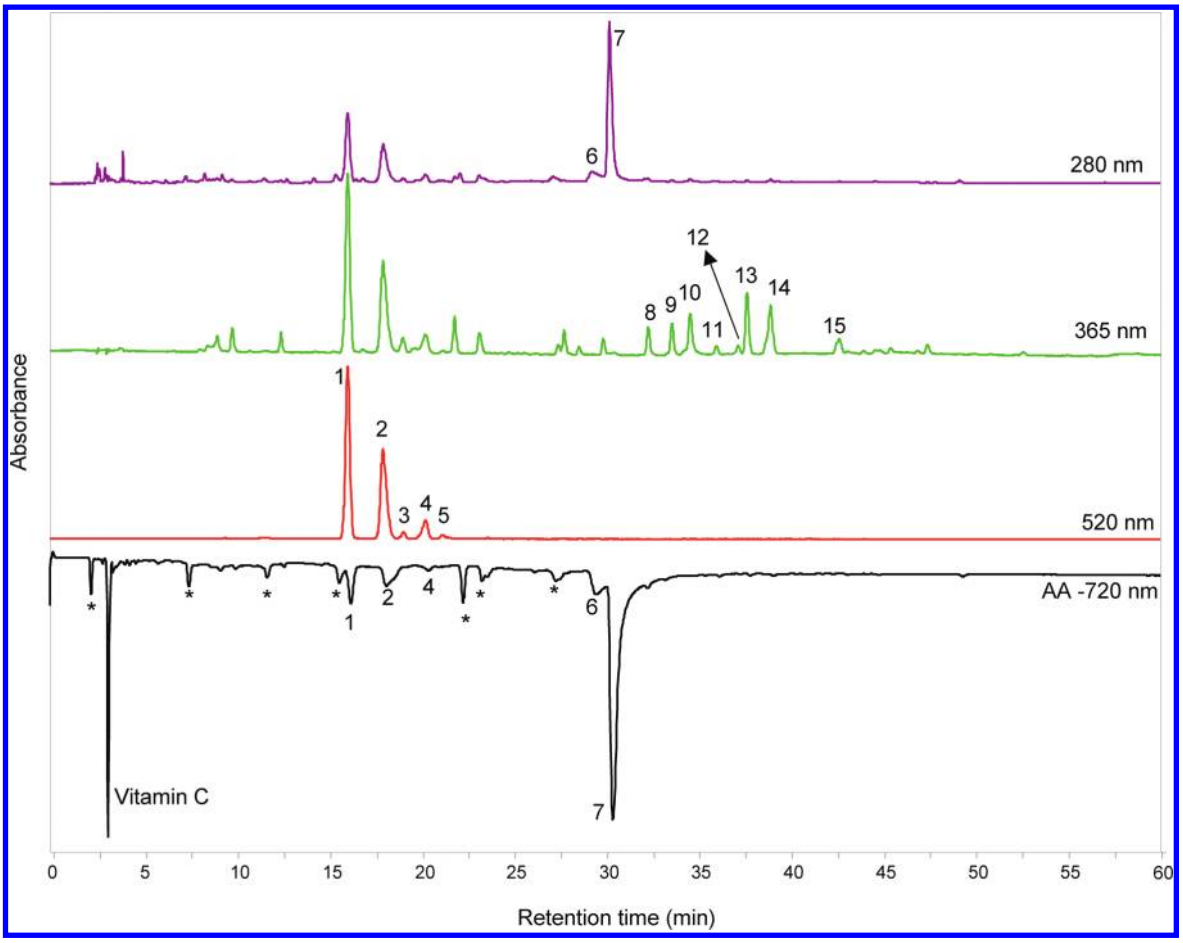

Figure 3. HPLC-PDA traces at 280, 365, and 520 nm and online antioxidant detection (720 nm) of vitamin C and phenolic compounds in raspberries. For identification of numbered peaks see **Table 4** and the Supporting Information.

Table 4. Antioxidant Activity of Phenolic Compounds and Vitamin C in Raspberries[a]

peak	t_R (min)	compound	quantity (nmol/g)	antioxidant activity (nmol of Trolox/g)	antioxidant activity (%)
	3.1	vitamin C	1014 ± 30	681 ± 27	10.5 ± 0.4
1	16.1	cyanidin-3-*O*-sophoroside	375 ± 28	454 ± 41	7.0 ± 0.6
2	18.0	cyanidin-3-*O*-(2′′-*O*-glucosyl)rutinoside cyanidin-3-*O*-sambubioside cyanidin-3-*O*-glucoside	307 ± 11	526 ± 15	8.1 ± 0.23
3	18.9	pelargonidin-3-*O*-sophoroside	44 ± 3	nd	
4	20.3	cyanidin-3-*O*-rutinoside	85 ± 4	93 ± 9	1.4 ± 0.1
5	21.2	pelargonidin-3-*O*-glucoside pelargonidin-3-*O*-(2′′-*O*-glucosyl)rutinoside	74 ± 1	nd	
6	29.6	lambertianin C	322 ± 41	886 ± 12	13.6 ± 0.2
7	30.8	sanguiin H-6	1030 ± 107	2905 ± 360	44.7 ± 5.6
8	32.7	ellagic acid-*O*-pentoside	7.9 ± 1.2	nd	
9	33.5	ellagic acid-*O*-pentoside	10.1 ± 1.3	nd	
10	34.4	ellagic acid	11 ± 1	nd	
11	35.8	quercetin-*O*-galactosylrhamnoside	7.5 ± 0.1	nd	
12	37.1	quercetin-3-*O*-(2′′-*O*-glucosyl)rutinoside	6.7 ± 0.1	nd	
13	37.7	quercetin-3-*O*-galactoside	25 ± 2	nd	
14	39.2	quercetin-3-*O*-glucoside	28 ± 4	nd	
15	42.5	ellagic acid-4-*O*-acetylxyloside	5.1 ± 1.8	nd	
		unidentified peaks		951 ± 79	14.6 ± 0.3

[a] Data expressed as mean values ± standard error ($n = 3$). t_R, retention time in minutes; nd, not detected. Peak numbers and retention times refer to HPLC traces in **Figure 3**. For identification of compounds see Table S3 and text in the Supporting Information.

and 9 adding up to 725 nmol/g and contributing 39% of the total AOC of cranberries. A total of 456 nmol/g of flavonols were present, and they were responsible for 10% of the overall AOC.

DISCUSSION

Black currants had the highest AOC in the FRAP assay followed by blueberries, raspberries, and red currants, and the lowest was cranberries (**Table 1**). Detailed analysis of the

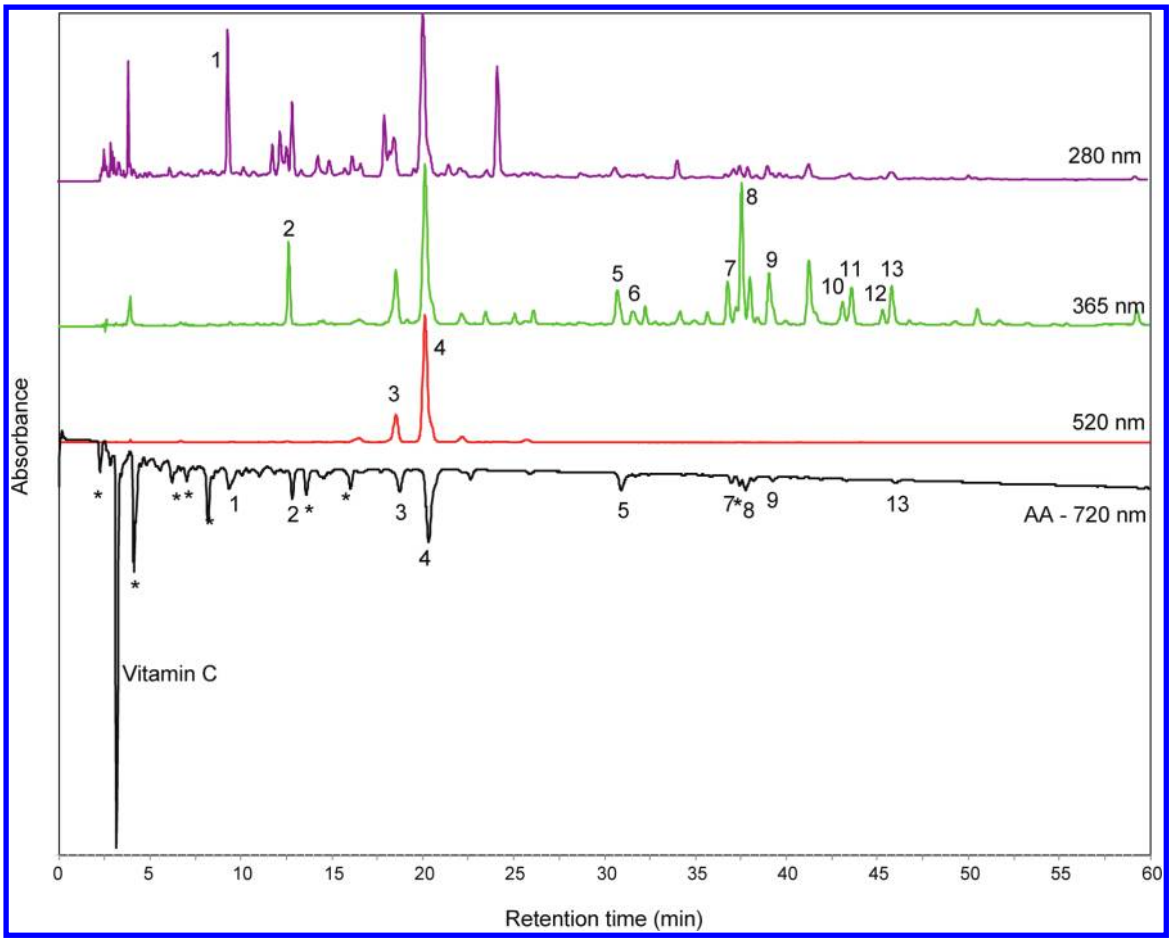

Figure 4. HPLC-PDA traces at 280, 365, and 520 nm and online antioxidant detection (720 nm) of vitamin C and phenolic compounds in red currants. For identification of numbered peaks see **Table 5** and the Supporting Information.

Table 5. Antioxidant Activity of Phenolic Compounds and Vitamin C in Red Currants[a]

peak	t_R (min)	compound	quantity (nmol/g)	antioxidant activity (nmol of Trolox/g)	antioxidant activity (%)
	3.2	vitamin C	313 ± 41	407 ± 28	47.5 ± 3.2
1	9.1	4-hydroxybenzoic acid-*O*-hexoside	73 ± 1	16 ± 0	1.9 ± 0.0
2	12.8	caffeic acid-*O*-glucoside	16 ± 1	25 ± 1	2.9 ± 0.1
3	18.0	cyanidin-3-*O*-sambubioside	81 ± 1	23 ± 1	2.7 ± 0.1
4	20.3	cyanidin-3-*O*-rutinoside cyanidin-3-*O*-(2″-*O*-xylosyl)rutinoside	247 ± 1	156 ± 7	18.2 ± 1.4
5	31.1	myricetin-3-*O*-rutinoside	4.6 ± 0.0	2.5 ± 0.0	0.3 ± 0.0
6	31.6	myricetin-*O*-rhamnoside	3.7 ± 0.1	nd	
7	37.4	quercetin-3-*O*- rutinoside	23 ± 0	9.4 ± 0.2	1.1 ± 0.0
8	38.0	quercetin-3-*O*- galactoside	8.6 ± 0.1	8.3 ± 0.1	1 ± 0.0
9	39.1	quercetin-3-*O*-glucoside	11 ± 1	4.9 ± 0.3	0.6 ± 0.1
10	43.0	quercetin-3-*O*-(6″-*O*-malonyl)glucoside	4.6 ± 0.0	nd	
11	43.6	kaempferol-*O*-rutinoside	5.1 ± 0.1	nd	
12	45.2	kaempferol-3-*O*-galactoside	3.2 ± 0.1	nd	
13	45.9	kaempferol-3-*O*-glucoside	5.4 ± 0.2	4.2 ± 1.1	0.5 ± 0.2
		unidentified peaks		201 ± 5	23.4 ± 0.8

[a] Data expressed as mean values ± standard error (n = 3). t_R, retention time; nd, not detected. Peak numbers and retention times refer to HPLC traces in **Figure 4**. For identification of compounds see Table S4 and text in the Supporting Information.

compounds in each berry revealed that black currants contained highest levels of anthocyanins, with 5521 nmol/g, whereas blueberries contained 4810 nmol/g, cranberries, 725 nmol/g, and red currants, 328 nmol/g. The black currant anthocyanins were responsible for 73% of the total AOC, whereas vitamin C contributed 18% (**Table 7**). In contrast, the lower level of vitamin C in blueberries did not contribute to the antioxidant capacity of the berries, which was dominated by the anthocyanins, which were responsible for 84% of the AOC detected by HPLC with online antioxidant, whereas flavonols supplied 14%. The anthocyanin contents of the red currant (328 nmol/g) and cranberry (725 nmol/g) were lower than that of the black currants and blueberries. Unidentified HPLC peaks were responsible for 23% of the AOC of red currants, whereas in cranberries procyanidin dimers supplied 12% and flavonols 10% (**Table 7**).

Raspberries contained also a relatively lower quantity of anthocyanins (885 nmol/g) that contributed 16% of the AOC, whereas 11% originated from a vitamin C content of 1014 nmol/g.

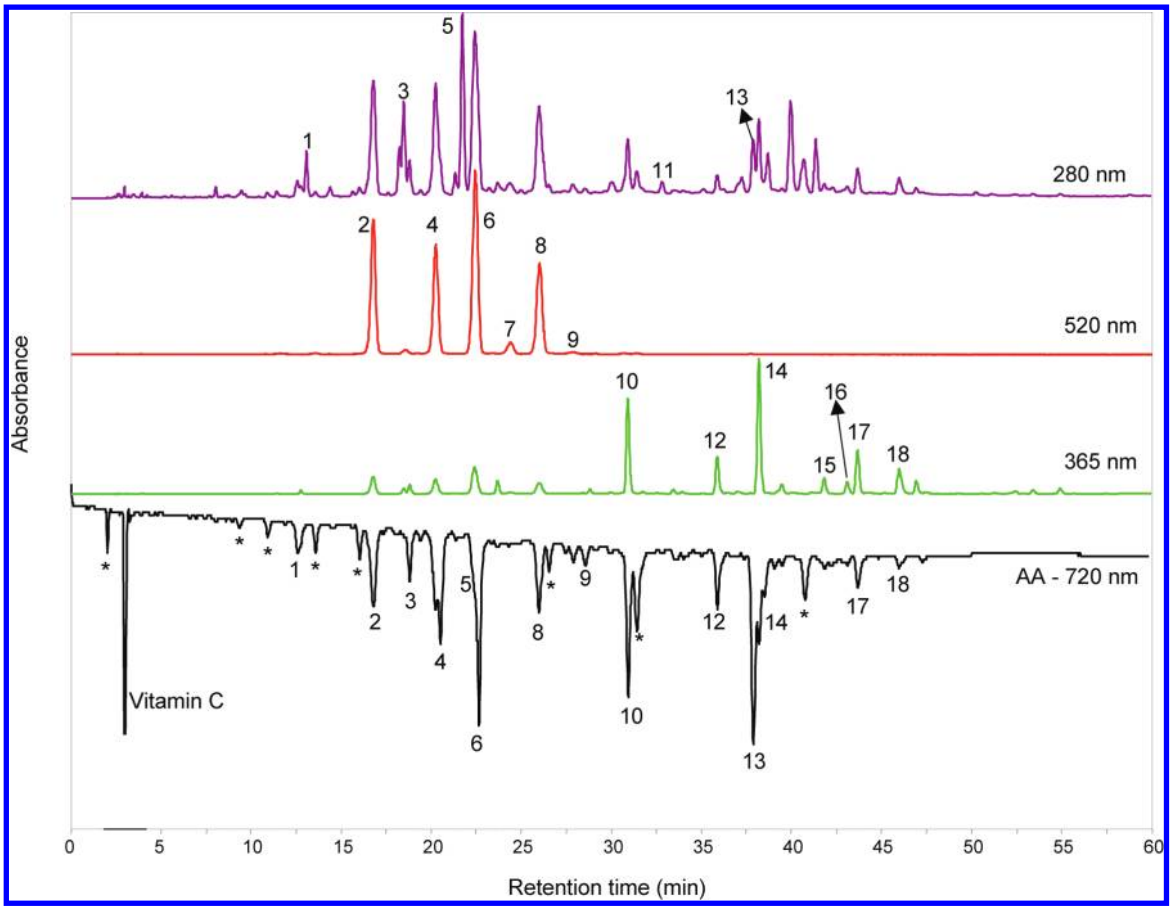

Figure 5. HPLC-PDA traces at 280, 365, and 520 nm and online antioxidant detection (720 nm) of vitamin C and phenolic compounds in cranberries. For identification of numbered peaks see **Table 6** and the Supporting Information.

Table 6. Antioxidant Activity of Phenolic Compounds and Vitamin C in Cranberries[a]

peak	t_R (min)	compound	quantity (nmol/g)	antioxidant activity (nmol of Trolox/g)	antioxidant activity (%)
	3.0	vitamin C	1107 ± 3	487 ± 10	22.6 ± 0.0
1	13.1	procyanidin dimer	405 ± 59	38 ± 2	1.8 ± 0.1
2	16.8	cyanidin-3-*O*-galactoside	160 ± 24	154 ± 6	7.1 ± 0.3
3	18.4	*p*-coumaric acid-*O*-hexoside	119 ± 12	36 ± 2	1.7 ± 0.1
4	20.3	cyanidin-3-*O*-arabinoside	138 ± 20	262 ± 6	12.2 ± 0.3
5	21.7	(−)-epicatechin	1121 ± 185	tr	tr
6	22.4	peonidin-3-*O*-galactoside	243 ± 34	310 ± 16	14.4 ± 0.7
7	24.2	peonidin-3-*O*-glucoside	34.3 ± 5	nd	
8	25.7	peonidin-3-*O*-arabinoside	124 ± 15	102 ± 2	4.7 ± 0.1
9	27.4	malvidin-3-*O*-arabinoside	26 ± 1	18 ± 4	0.8 ± 0.2
10	30.9	myricetin-3-*O*-galactoside	112 ± 17	139 ± 7	6.5 ± 0.3
11	32.7	procyanidin dimer	91 ± 14	nd	
12	35.8	myricetin-3-*O*-arabinoside	42 ± 6	40 ± 2	1.9 ± 0.1
13	37.7	procyanidin dimer	498 ± 64	226 ± 20	10.5 ± 0.9
14	38.2	quercetin-3-*O*-galactoside	184 ± 28	tr	
15	41.4	quercetin-3-*O*-(2′′-*O*-xylosyl)pyranoside	23 ± 3	nd	
16	43.2	quercetin-3-*O*-arabinosylpyranoside	19 ± 2	nd	
17	43.8	quercetin-3-*O*-arabinosylfuranoside	58 ± 11	32 ± 1	1.5 ± 0.0
18	46.1	quercetin-3-*O*-rhamnoside	18 ± 3	15 ± 0	0.7 ± 0.0
		unidentified peaks		296 ± 29	13.7 ± 1.0

[a] Data expressed as mean values ± standard error ($n = 3$). t_R, retention time; nd, not detected; tr, trace. Peak numbers and retention times refer to HPLC traces in **Figure 5**. For identification of compounds see Table S5 and text in the Supporting Information.

Unlike the other berries, raspberries were a rich source of ellagitannins in the form of lambertianin C and sanguiin H-6, which together were responsible for 58% of the AOC (**Table 7**). This is in keeping with the findings of Beekwilder et al. (*19*), who analyzed raspberries with similar HPLC-online antioxidant detection and found that in var. Tulamen anthocyanins were responsible for 17% and ellagitannins for 54% of the detectable AOC. Raspberries also contained ellagic acid and three sugar conjugates at a total concentration of 34 nmol/g, which was not sufficient to induce a measurable response in the online ABTS antioxidant detector system (**Figure 3**; **Tables 4** and **7**).

Table 7. Total Content and Contribution to the Antioxidant Capacity of Vitamin C and Different Groups of Phenolics Detected in Berries[a]

compound	black currant	blueberry	raspberry	red currant	cranberry
vitamin C	2328 (18)	115 (0)	1014 (11)	313 (47)	1107 (23)
anthocyanins	5521 (73)	4810 (84)	885 (16)	328 (21)	725 (39)
ellagitannins			1352 (58)		
ellagic acid derivatives			34 (0)		
(−)-epicatechin					1121 (0)
procyanidin dimers					994 (12)
chlorogenic acid	80 (1)	8 (2)		89 (5)	119 (2)
flavonols	514 (5)	751 (14)	67 (0)	69 (4)	456 (10)
unidentified	(3)		(15)	(23)	(14)

[a] Data expressed in nmol/g of fresh weight; numbers in parentheses are percentages of the total antioxidant activity.

(−)-Epicatechin and procyanidin dimers were detected in cranberries (**Figure 5**; **Table 6**), and the dimers, but not the monomer, made a contribution to the AOC of the berries (**Table 7**). Higher molecular weight polymeric procyanidins most probably will have contributed to the FRAP AOC of the unchromatographed berry extracts (**Table 1**). According to Gu et al. (*20*) raspberries contain 0.3 mg/g polymeric procyanidins and cranberries, 4.2 mg/g. They will not, however, have contributed to the AOC determined by HPLC as when analyzed by reversed-phase HPLC polymeric proanthocyanidins are either retained by the column or eluted as a broad unresolved band. The analysis of proanthocyanidins comprising up to 10 flavan-3-ol units can be achieved using normal-phase HPLC (*21*); however, the dichloromethane/methanol/aqueous acetic acid mobile phase employed with this system is not compatible with the ABTS reagent used with the online HPLC system. A further potential source of error with analysis of proanthocyanidins is that estimates can vary substantially depending upon the method of extraction (*20*, *22*, *23*).

Although the berries analyzed in this study were unknown varieties and no details were available about their postharvest regimen, they each contained a characteristic qualititative profile of flavonoids and phenolic compounds in keeping with previously published research (*4*). The berries were purchased from a local supermarket and, therefore, reflect what is available for consumption by a local population. It would have been useful to know the cultivars and where the berries were grown. However, this in itself would not provide information on the extent to which the levels of flavonoids and phenolic compounds vary from year to year, depending upon annual variations in climate. Also, in any growing season, these levels are likely to be influenced by the prevailing environmental conditions encountered within a time window of weeks, if not days, prior to harvest.

Supporting Information Available: Identification of compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

LITERATURE CITED

(1) Margetts, B.; Buttriss, J. Epidemiology linking consumption of plant foods and their constituents with health. In *Plants: Diet and Health*; Goldberg, G., Ed.; Blackwell Publishing: Oxford, U.K., 2003; pp 49−64.

(2) Law, M. R.; Morris, J. K. By how much does fruit and vegetable consumption reduce the risk of ischemic heart disease. *Eur. J. Clin. Nutr.* **1998**, *52*, 549–556.

(3) Lock, K.; Pomerleau, J.; Causer, L; Altmann, D. R.; McKee, M. The global burden of disease attributable to low consumption of fruit and vegetables: implications for a global strategy on diet. *Bull. WHO* **2005**, *83*, 100–108.

(4) Crozier, A.; Yokota, T.; Jaganath, I. B.; Marks, S. C.; Saltmarsh, M.; Clifford, M. N. Secondary metabolites in fruits, vegetables, beverages and other plant based dietary components. In *Plant Secondary Metabolites: Occurrence, Structure and Role in the Human Diet*; Crozier, A., Clifford, M. N., Ashihara, H., Eds.; Blackwell: Oxford, U.K., 2006; pp 208−302.

(5) van Acker, S. A. B. E.; de Groot, M. J.; van den Berg, D. J.; Tromp, M. N. J. L.; den Kelder, G.; van der Vijgh, W. J. F.; Bast, A. A quantum chemical explanation of the antioxidant activity of flavonoids. *Chem. Res. Toxicol.* **1996**, *9*, 1305–1312.

(6) Vinson, J. A.; Dabbagh, Y. A. Effect of green and black tea supplementation on lipids, lipid oxidation and fibrinogen in the hamster: mechanisms for the epidemiological benefits of tea drinking. *FEBS Lett.* **1998**, *433*, 44–46.

(7) Fuhrman, B.; Lavy, A.; Aviram, M. Consumption of red wine with meals reduces the susceptibility of human plasma and low-density-lipoprotein to lipid-peroxidation. *Am. J. Clin. Nutr.* **1995**, *61*, 549–554.

(8) Renaud, S.; Delorgeril, M. Wine, alcohol, platelets, and the French paradox for coronary heart-disease. *Lancet* **1992**, *339*, 1523–1526.

(9) Fitzpatrick, D. F.; Hirschfield, S. L.; Coffey, R. G. Endothelium-dependent vasorelaxing activity of wine and other grape products. *Am. J. Physiol.* **1993**, *265*, H774–H778.

(10) Knegt, P.; Jarvinen, R.; Reunanen, A.; Maatela, J. Flavonoid intake and coronary heart disease in Finland: a cohort study. *Br. Med. J.* **1996**, *312*, 478–481.

(11) Kahkonen, M. P.; Hopla, A. I.; Heinonen, M. Berry phenolics and their antioxidant activity. *J. Agric. Food Chem.* **2001**, *49* 4076–4082.

(12) Halvorsen, B. L.; Carlsen, M. H.; Phillips, K. M.; Bohn, H. K.; Holte, K.; Jacobs, D. R.; Blomhoff, R. Content of redox-active compounds (i.e. antioxidants) in food consumed in the United States. *Am. J. Clin. Nutr.* **2006**, *84*, 95–135.

(13) Haleem, M. K.; Barton, K. L.; Borges, G.; Crozier, A.; Anderson, A. S. Increasing antioxidant intake from fruits and vegetables: practical strategies for the Scottish population. *J. Hum. Nutr. Diet.* **2008**, *21*, 539–546.

(14) Benzie, I. F. F.; Strain, J. J. Ferric reducing/antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. *Methods Enzymol.* **1999**, *299*, 15–27.

(15) Stewart, A. J.; Mullen, W.; Crozier, A. On-line HPLC analysis of the antioxidant activity of phenolic compounds in green and black tea. *Mol. Nutr. Food Res.* **2005**, *49*, 52–60.

(16) Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biol. Med.* **1999**, *26*, 1231–1237.

(17) Ross, M. A. Determination of ascorbic acid and uric acid in plasma by high-performance liquid chromatography. *J. Chromatogr., B* **1994**, *657*, 197–200.

(18) Moyer, R. A.; Hummer, K. E.; Finn, C. E.; Frei, B.; Wrolstad, R. E. Anthocyanins, phenolics and antioxidant capacity in diverse small fruits: *Vaccinium*, *Rubus*, and *Ribes*. *J. Agric. Food Chem.* **2002**, *50*, 519−525.

(19) Beekwilder, J.; Jonker, H.; Meesters, P.; Hall, R. D.; van der Meer, I.; de Vos, R. Antioxidants in raspberry: on-line analysis links

antioxidant activity of a diversity of individual metabolites. *J. Agric. Food Chem.* **2005**, *53*, 3313–3320.

(20) Gu, L.; Hammerstone, J. F.; Beecher, G.; Holden, J.; Haytowitz, D.; Gebhardt, S.; Prior, R. L. Concentration of proanthocyanidins in common foods and estimates of consumption. *J. Nutr.* **2004**, *134*, 613–617.

(21) Hammerstone, J. F.; Lazarus, S. A.; Mitchell, A. E.; Rucker, R.; Schmitz, H. H. Identification of procyanidins in cocoa (*Theobroma cacao*) and chocolate using high-performance liquid chromatography/mass spectrometry. *J. Agric. Food Chem.* **1999**, *47*, 490–496.

(22) Hellström, J. K.; Törröen, A. R.; Mattila, P. H. Proanthocyanidins in common food products of plant origin. *J. Agric. Food Chem.* **2009**, *56*, 7617–7624.

(23) Khanal, R. C.; Howard, L. R.; Prior, R. L. Procyanidin composition of selected fruits and fruit byproducts is affected by extraction method and variety. *J. Agric. Food Chem.* **2010**, DOI: 10.1021/jf9015398.

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