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Flavonoid constituents and their contribution to antioxidant activity in cultivars and hybrids of rabbiteye blueberry (*Vaccinium ashei* Reade)

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ABSTRACT

Fruit from 42 blueberry (*Vaccinium* spp.) cultivars, including 36 rabbiteye cultivars (*Vaccinium ashei* Reade), three *V. ashei* hybrid derivatives, and three northern highbush (*Vaccinium corymbosum* L.) standards were evaluated for antioxidant capacity, individual flavonoid content, and the contribution of each identified phenolic compound to total antioxidant activity. Considerable variation was found in flavonoid content, antioxidant activity, and their contribution to total antioxidant activity among cultivars. Among 42 blueberry cultivars, the rabbiteye 'Early May' contained the highest amount of chlorogenic acid, myricetin 3-arabinoside, quercetin derivatives, and delphinidin-, cyanidin-, petunidin-, and malvidin-basis anthocyanins. 'Early May' cultivar also had the highest antioxidant activity (88.2 μmol TE/g fw). 'Owen', 'Bluegem', 'Clara', Climax', and 'Centurion' were among the other rabbiteye cultivars that also had high levels of flavonoids and antioxidant activities. In contrast, the pink-fruited *V. ashei* hybrid, 'Pink Lemonade', had the lowest content of flavonoids and lowest antioxidant activity. The mean flavonoid content and antioxidant activity of rabbiteye cultivars was higher than those among northern highbush and *V. ashei* hybrids. The antioxidant activity of *V. ashei* hybrid derivatives was derived mainly from chlorogenic acid, myricetin, and quercetin, which contributed 62.5% of total antioxidant activity, whereas anthocyanins (malvidin, petunidin, delphinidin, and cyanidin) were the main contributors to the antioxidant activity of rabbiteye cultivars (76.2%) and northern highbush standards (76.8%). Blueberry cultivars identified to have high phenolic content and high antioxidant activity could be used as parents for future blueberry breeding programmes to develop new blueberry cultivars with higher antioxidant activity and further improve human health.

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1. Introduction

This study evaluated the flavonoid content and contribution of individual flavonoid compounds to the total antioxidant activity in rabbiteye (RE) blueberry (*Vaccinium ashei* Reade), *V. ashei* hybrid derivatives (RE-D), and northern highbush (NHB) blueberry standards (*Vaccinium corymbosum* L.). Rabbiteye blueberry is a species of blueberry native to the Southeastern United States and is commercially grown across the South. Northern highbush blueberry is native to North America and produced in temperate regions throughout North America, particularly the Pacific Northwest, the Great Lakes region, and the Atlantic states of the United States, and has been commercially produced for many years. RE-D have been developed from the crosses of *V. ashei* with *Vaccinium constablaei* Gray, *Vaccinium darrowii* Camp, and *V. corymbosum* for later flowering time and earlier fruit ripening than their *V. ashei* parents. These crosses have also yielded more cold-hardy RE-D, possibly suitable for growing under northern conditions.

Blueberries are one of the richest sources of antioxidant photochemicals among fruits and vegetables. These antioxidants are capable of performing a number of functions, including acting as free radical-scavengers, peroxide decomposers, singlet and triplet oxygen quenchers, enzyme inhibitors, and synergists (Larson, 1988). Antioxidants can also delay or prevent the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidising chain reactions. Growing evidence suggests that eating more fruits and vegetables can aid in preventing stomach, lung, mouth, esophagus, colon, and rectal cancers. The incidence of other chronic diseases, such as coronary heart disease, atherosclerosis,

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and stroke, may also be reduced through increased fruit and vegetable consumption. Fruits and vegetables may also play a preventive role in birth defects, cataract formation, hypertension, asthma, diverticulosis, obesity, and diabetes (Ness & Powles, 1997; Satué-Gracia, Heinonen, & Frankel, 1997; Steinmetz & Potter, 1996; World Cancer Research Fund, 1997).

Significant variations of antioxidant activities, anthocyanins, and phenolics were observed among the different *Vaccinium* species (Ehlenfeldt & Prior, 2001; Howard, Clark, & Brownmiller, 2003; Prior et al., 1998; Taruscio, Barney, & Exon, 2004; Wang & Jiao, 2000). The high antioxidant activities in fruits are largely attributed to phenolic compounds, such as anthocyanins and other flavonoid compounds. The overall activity is generally considered to be dependent on their structure and relative content in berries. However, synergistic interactions between phenolics and other components of berry fruits have also been observed to influence the antioxidant activity of berry fruits (Lila & Raskin, 2005). The antioxidant activities of phenolic compounds are mainly due to their redox properties which can play an important role in absorbing and neutralising free radicals, quenching singlet and triplet oxygen or decomposing peroxide (Larson, 1988).

The flavonoid content and antioxidant activity of berry crops, including blueberries, are becoming targeted traits by berry breeders. For decades, breeding programmes had predominantly focused on creating varieties with improved commercial traits, such as large berry size, berry colour, small scars, taste, flavour, firmness, and productivity (Moore, 1965). Recently, breeding programmes have turned their focus to fruit quality, especially to improve nutritional value and to obtain cultivars with enhanced levels of bioactive substances (Scalzo, Politi, Pellegrini, Mezzetti, & Battino, 2005). The genetic options to achieve new genotypes able to provide berries with the proper balance of bioactive compounds for improving consumer health have been reported (Battino et al., 2009). It has been shown that fruit nutritional quality is a heritable trait and can be improved through breeding practices (Capocasa, Scalzo, Mezzetti, & Battino, 2008). Therefore, phytonutrient levels in parental varieties are important for breeding selection. Several studies have evaluated the antioxidant capacity, flavonoid compounds, and vitamin content of genotype selections to be used in breeding various crop varieties, including peaches, nectarines, tomatoes, potatoes, as well as berries (Battino et al., 2009; Cantin, Moreno, & Gogorcena, 2009; Saha et al., 2010; Shiroma-Kian, Tay, Manrique, Giusti, & Rodriguez-saona, 2008; Slimestad & Verheul, 2009). The purpose of this study was to evaluate individual flavonoid contents and contributions of each identified flavonoid to scavenging peroxyl radical capacity of 42 blueberry (*Vaccinium* spp.) cultivars, including 36 RE cultivars (*V. ashei*), three RE-D, and three NHB (*V. corymbosum*) standards. Information obtained from this study would be helpful for breeding programmes to create progenies with improved antioxidant levels.

2. Materials and methods

2.1. Chemicals

2′,2′-Azobis (2-amidinopropane) dihydrochloride (AAPH) was obtained from Wako Chemicals USA Inc. (Richmond, VA). Fluorescein disodium, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox, 97%), kaempferol (90%), and quercetin (98%) were purchased from Sigma (St. Louis, MO). Acetonitrile, methanol, acetone, and water were of HPLC grade and were purchased from Baxter (Muskegon, MI). All anthocyanins and their aglycones (purities for all standards were $\geqslant$95.0%) were obtained from Indofine Chemical Co., Inc. (Somerville, NJ). Other authentic standards were obtained from Sigma Chemical Co. and Fisher Scientific (purities were $\geqslant$95.0%).

2.2. Fruit sample handling and preparation

Blueberry fruit (*Vaccinium* species) used in this study were grown at USDA-ARS plots at the Marucci Center for Blueberry and Cranberry Research and Extension, Chatsworth, NJ, USA. Fully-ripe (100% blue) blueberries were hand-harvested at bush maturity from 45% to 100% with the most typical value being approximately 60%. Approximately 500–900 g of fruit were harvested per genotype from test plots of two plants, and 42 different genotypes were sampled and used for this study. This included 36 RE (*V. ashei*) blueberry cultivars, along with three RE-D and three NHB blueberry standards. The RE blueberry cultivars used were 'Alapaha', 'Aliceblue', 'Austin', 'Baldwin', 'Beckyblue', 'Bluebelle', 'Bluegem', 'Bonita', 'Brightwell', 'Briteblue', 'Callaway', 'Centurion', 'Chaucer', 'Choice', 'Clara', 'Climax', 'Coastal', 'Delite', 'Early May', 'Ethel', 'Garden Blue', 'Homebell', 'Ira', 'Menditoo', 'Montgomery', 'Myers', 'Owen', 'Powderblue', 'Premier', 'Satilla', 'Southland', 'Suwanee', 'Tifblue', 'Walker', 'Windy', and 'Woodard'. The three RE-D evaluated were 'Pearl River' (60% *V. ashei*: 40% northern highbush), 'Snowflake' (75% *V. ashei*: 25% *V. constablaei*), and 'Pink Lemonade' [a pink-fruited, ornamental selection (50% *V. ashei*: 17% *V. darrowii*: 33% *V. corymbosum*)] (Ehlenfeldt & Finn, 2007)]. The three NHB standards used were 'Bluecrop', 'Duke', and 'Elliott' (all *V. corymbosum* with 6.3%, 0%, and 3.9% *Vaccinium angustifolium* Ait., respectively). The fruit were sorted to eliminate damaged, shrivelled, and unripe fruit, and to select for uniform size and colour. Berries were initially frozen in a −70 °C freezer, then transported to Beltsville with freezer packs in a cooler, and ultimately stored at −80 °C until they were used for analysis.

2.3. HPLC analysis of blueberry anthocyanins and phenolic compounds

Triplicate composite 5 g samples, cut from 20 fruit each per cultivar, were extracted three times with 10 ml of 80% acetone, containing 0.2% formic acid, using a Polytron (Brinkmann Instruments, Inc., Westbury, NY), and then centrifuged at 14,000*g* for 20 min at 4 °C. The supernatants were combined, with final volumes 30 ml, and transferred to vials, stored at −80 °C, and used for HPLC analysis and antioxidant activity assay.

Twenty-five millilitres from the above acetone–formic acid extracts were concentrated to 1 ml, using a Buchler Evapomix (Fort Lee, NJ), in a water bath at 30 °C. The concentrated samples were dissolved in 4 ml of acidified water (3% formic acid) and then passed through a C_{18} Sep-Pak cartridge (Waters), which was previously activated with 3 ml of methanol, followed by 6 ml of water and then 6 ml of 3% aqueous formic acid. Anthocyanins and other phenolics were then recovered with 2.0 ml of acidified methanol containing 3% formic acid. The methanol extract was passed though a 0.45 μm membrane filter (Millipore, MSI, Westboro, MA) and 20 μl were analysed by HPLC (Waters, Milford, MA). Samples were injected at ambient temperature (20 °C) into a reverse phase NOVA-PAK C_{18} column (150 × 3.9 mm, particle size 4 μm) with a guard column (NOVA-PAK C_{18}, 20 × 3.9 mm, particle size 4 μm) (Waters Corporation, MA). The mobile phase consisted of 5% aqueous formic acid (A) and HPLC grade acetonitrile (B). The flow rate was 1 ml/min, with a gradient profile consisting of A with the following proportions (v/v) of B: 0–1 min, 4%, 1–10 min, 4–6% B; 10–15 min, 6% B; 15–35 min, 6–18% B; 35–40 min, 18–20% B; 40–42 min, 20–45% B; 42–45 min, 45–100% B; 45–50 min, 100% B. The phenolic compounds in berry extracts were identified by their retention times and UV spectra recorded with a diode array detector and by chromatographic comparison with authentic markers (Gao, Mazza, & Mazza, 1994; Kader, Rovel, Girardin, & Metche, 1996; Wang, Wang, & Chen, 2008; Zheng & Wang, 2003). The results were also confirmed by co-injection with authentic standards. Each standard was dissolved in methanol at a concentration of 1 mg/ml, and five dilute solutions from these stock solutions were used to prepare calibration curves of each standard.

Recoveries were measured by extracting the recovered amounts of pure substances added to frozen blueberries before the experiment. Three replicates from each sample were used for HPLC analyses. The *trans*-resveratrol in the purified extracts was injected into an HPLC system, as described by Wang, Chen, Wang, and Chen (2007). Results of the analyses for resveratrol were expressed as μg of *trans*-resveratrol per gramme of fresh wt.

2.4. Oxygen radical absorbance capacity (ORAC) assay

The ORAC assay was carried out using a high-throughput instrument platform consisting of a robotic eight-channel liquid handling system and a microplate fluorescence reader (Huang, Ou, Hampsch-Woodill, Flanagan, & Prior, 2002). A FL800 microplate fluorescence reader (Bio-Tek Instruments, Inc., Winooski, VT) was used with fluorescence filters for an excitation wavelength of 485 ± 20 nm and an emission wavelength of 530 ± 25 nm. The plate reader was controlled by software KC4 3.0 (revision 29). Sample dilution was accomplished by a Precision 2000 automatic pipetting system managed by Precision Power software (version 1.0) (Bio-Tek Instruments, Inc.). The ORAC values were determined by calculating the net areas under the curve (AUC) of the standards and samples (Huang et al., 2002). The standard curve was obtained by plotting trolox concentrations against the average net AUC of the two measurements for each concentration. Final ORAC values were calculated using the regression equation between trolox concentration and the net AUC and were expressed as micromoles of trolox equivalents per gramme of fresh weight (Huang et al., 2002).

2.5. Statistical analysis

Quantitative values for the variables: resveratrol (resv), chlorogenic acid (chlo), myricetin 3-arabinoside (myr-3-ara), quercetin 3-galactoside (q-3-gal), and quercetin 3-glucoside (q-3-glu), delphinidin 3-galactoside (d-3-gal), delphinidin 3-glucoside, (d-3-glu), cyanidin 3-galactoside (c-3-gal), delphinidin 3-arabinoside (d-3-ara), and cyanidin 3-glucoside (c-3-glu), petunidin 3-galactoside (p-3-gal), petunidin 3-glucoside (p-3-glu), petunidin 3-arabinoside (p-3-ara), malvidin 3-galactoside (mal-3-gal), malvidin 3-glucoside (mal-3-glu), and malvidin 3-arabinoside (mal-3-ara) were analysed as one-factor linear models, using Proc Mixed (SAS Institute, 2010a, 2010b) with cultivar as the factor. . The analyses of variance results were statistically significant ($p < 0.0001$) for all variables. Means comparisons were done with Sidak adjusted *p*-values so that the experiment wise error was 0.05. The variables were also analysed jointly in a multivariate analysis, using the centroid hierarchical cluster method (JMP 9, SAS Institute, 2010a, 2010b). The scree plots results indicated that three clusters were sufficient to group the cultivars. The clusters are shown in a dendrogram tree diagram (Fig. 1).

3. Results and discussion

3.1. Phenolics and anthocyanins in blueberries

Phenolics in berry extracts, separated and identified by using reversed-phase high-performance liquid chromatography (HPLC), are presented in Tables 1–3. Considerable variation was found in phenolic compounds among blueberry cultivars. The berry extracts from 42 blueberry cultivars contained chlo, resv and flavonoids, such as myr-3-ara, q-3-gal, q-3-glu, d-3-gal, d-3-glu, c-3-gal, d-3-ara, c-3-glu, p-3-gal, p-3-glu, p-3-ara, mal-3-gal, and mal-3-ara (Tables 1–3). Resveratrol levels in blueberries were low (0.6–9.3 μg/g fw) compared to other flavonoids. Chlorogenic acid was a major cinnamic derivative found in large amounts in blueberries (84.5–686 μg/g fw). 'Early May' contained the highest amount of chlo among the blueberry cultivars tested in this study, whereas berry extracts from 'Pink Lemonade' (pink-fruited) contained the least. There was an approximately 8.1-fold difference between the highest and lowest values (Table 1). Among RE-D, 'Snowflake' had higher chlo values than had 'Pink Lemonade' and 'Pearl River'. The value in 'Snowflake' was higher than those of the mean of 36 RE cultivars (369 *vs*. 292 μg/g fw). Among the NHB cultivars, 'Elliott' had a higher amount of chlo (331 μg/g fw) than had the other two NHB cultivars 'Bluecrop' (103 μg/g fw) and 'Duke' (130 μg/g fw). The chlo level in 'Elliott' was also higher than the mean of 36 RE cultivars (292 μg/g fw) and the three RE-D (262 μg/g fw) (Table 1).

In RE blueberries, myr-3-ara ranged from 4.2 to 91.4 μg/g fw, representing a 21.8-fold difference between the highest and the lowest amounts. 'Early May' and 'Bluegem' had the highest amounts, followed by 'Windy', 'Woodard', and 'Alapaha', whereas the RE 'Ethel', the RE-D 'Pink Lemonade' and the NHB 'Duke' had the lowest. Relatively high amounts of q-3-gal were found in RE blueberry cultivars of 'Centurion', and 'Suwanee', while the lowest amounts were found in 'Austin' and 'Baldwin'. The highest amount of q-3-glu was found in 'Baldwin', followed by the RE-D 'Snowflake' and 'Pearl River' and RE blueberries 'Owen', 'Clara', and 'Brightwell'. 'Pink Lemonade' had the lowest content (56.4 μg/g fw), reflecting a 5.8-fold difference (Table 1).

Blueberry fruit contained four major anthocyanins: delphinidin, cyanidin, petunidin, and malvidin (Tables 2 and 3). Comparison of 42 blueberry cultivars showed that the main anthocyanin pigments were the same, but there were significant quantitative differences among cultivars. Among RE cultivars, the ranking of anthocyanin types followed the general order of malvidin > delphinidin > cyanidin. The mean values of anthocyanins in μg/g fw were: d-3-gal (118), d-3-glu (86.9), c-3-gal (87.5), d-3-ara (164), c-3-glu (51.7), p-3-gal (175), p-3-glu (155), p-3-ara (234), mal-3-gal (500), mal-3-glu (248), and mal-3-ara (174) (Tables 2 and 3).

D-3-gal content ranged from 12.1 to 202 μg/g fw. The RE cultivar 'Ethel' had the highest amount, whereas the RE-D 'Pearl River' and 'Pink Lemonade' had the lowest. Other cultivars with high d-3-gal contents were the RE cultivars 'Aliceblue', 'Early May', and 'Myers, (Table 2). Another two delphinidin derivatives are d-3-ara and d-3-glu. The amount of d-3-ara ranged from12.3 to 400 μg/g fw, with the RE 'Owen' containing the highest amount, and RE-D 'Pearl River' the lowest (Table 2). d-3-Glu content ranged from 0.2 to 196 μg/g fw. RE 'Early May' had the highest level, followed by 'Owen' and 'Centurion'. RE-D 'Pearl River' and 'Pink Lemonade' had the lowest. The content of c-3-gal ranged from 0.4 to 186 μg/g fw with RE cultivars 'Clara', 'Early May', and 'Tifblue' containing the highest amounts, whereas the RE cultivars 'Bluegem', 'Choice', 'Ethel' and the RE-D 'Pearl River', and 'Pink Lemonade' contained the least. c-3-Glu was lower than c-3-gal, ranging from 1.3 ('Ethel') to 99.3 ('Early May') μg/g fw (Table 2).

P-3-gal values ranged from 2.3 to 313 μg/g fw, with the highest amounts found in the RE cultivars 'Early May' (313 μg/g fw), 'Bluegem' (256 μg/g fw) and 'Aliceblue' (247 μg/g fw), while 'Pink Lemonade' had the lowest (2.3 μg/g fw). There was approximately a 136-fold difference between the highest and lowest values (Table 3). Among RE-D, 'Snowflake' had a higher amount of p-3-gal than had 'Pink Lemonade' and 'Pearl River'. However, the content of p-3-gal in 'Snowflake' was lower than the mean values of 36 REs and three NHB blueberry cultivars (Table 3). Among the NHB cultivars, 'Elliott' had a higher p-3-gal value (225 μg/g fw) than had the other two NHB cultivars, 'Bluecrop' (122 μg/g fw) and 'Duke' (100 μg/g fw), and the mean of 36 RE cultivars (175 μg/g fw) (Table 3).

A wide variation in the content of p-3-glu and p-3-ara was also found among blueberry cultivars, ranging from 5.2 to 227 μg/g fw, and 7.1 to 355 μg/g fw, respectively. This reflects 43.7-fold and 50.0-fold differences, respectively, among these 42 cultivars

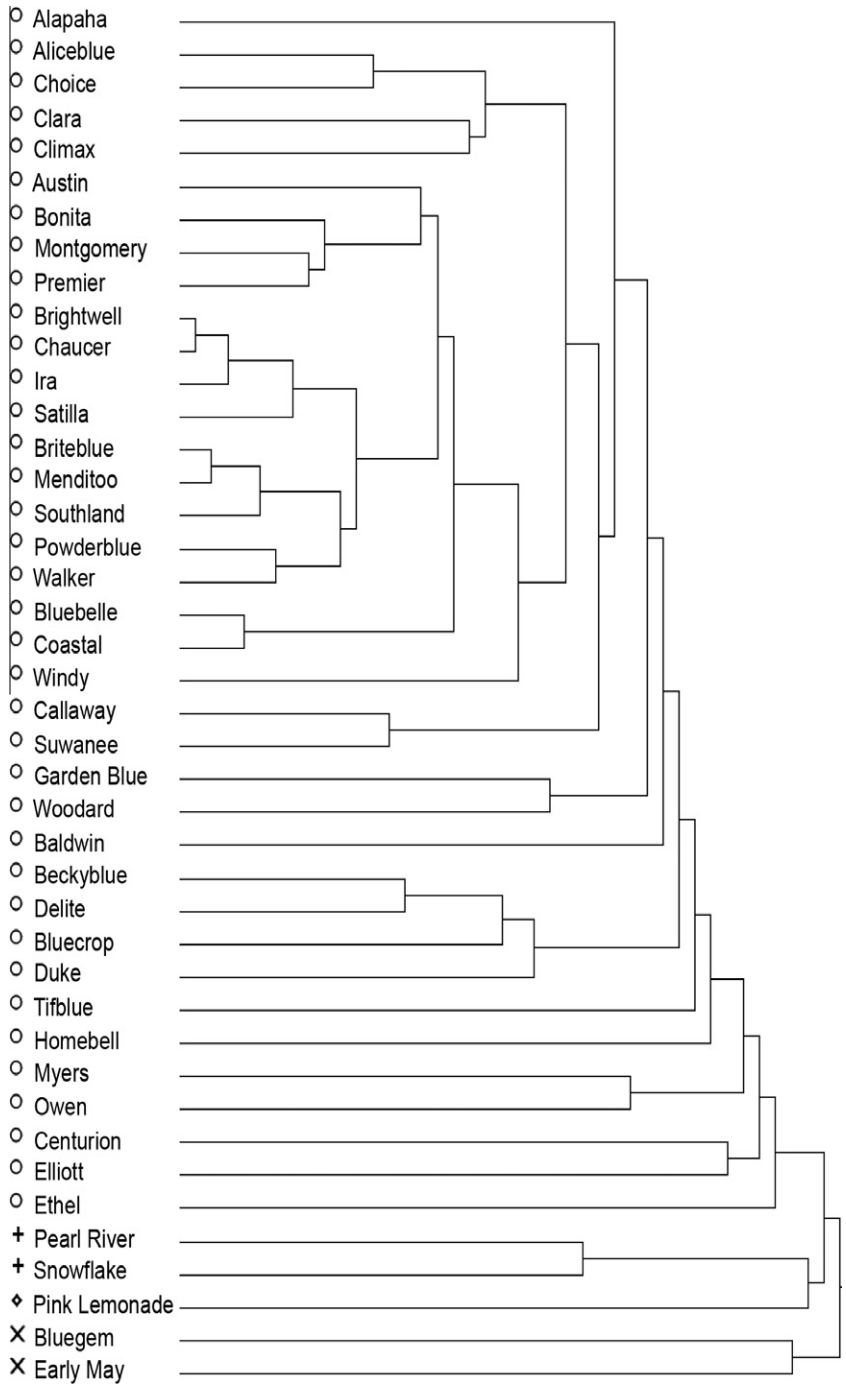

Fig. 1. The centroid hierarchical cluster analysis of the 16 flavonoid variables: resv, chlo, myr-3-ara, q-3-gal, q-3-glu, d-3-gal, d-3-glu, c-3-gal, d-3-ara, c-3-glu, p-3-gal, p-3-glu, p-3-ara, mal-3-gal, mal-3-glu, and mal-3-ara. Cultivars with the same symbol are in the same cluster.

(Table 3). The RE cultivar 'Woodard' had the highest amounts of p-3-glu and p-3-ara, whereas, the RE-D 'Pink Lemonade', and 'Pearl River' had the lowest (Table 3).

The content of malvidin-based anthocyanins of these blueberries is shown in Table 3. M-3-gal content ranged from 12.5 to 840 μg/g fw. The RE cultivar 'Homebell' had the highest amount, followed by 'Woodard', 'Tifblue' and 'Ethel'. The RE-D 'Pink Lemonade' had the lowest. The range for mal-3-glu content was 29.7–598 μg/g fw; the RE 'Bluegem' had the highest level and the RE-D 'Pink Lemonade' had the lowest. In addition to mal-3-glu, mal-3-ara was also a major constituent in blueberries, ranging from 21.3 to 323 μg/g fw, reflecting a 15.2-fold difference among cultivars (Table 3). The RE 'Ethel' had the highest level and the RE-D 'Pink Lemonade' had the lowest.

Among 42 blueberry cultivars, the RE 'Early May' contained the highest amounts of chlo, myr-3-ara, d-3-glu, c-3-gal, c-3-glu, p-3-gal, and p-3-glu. 'Ethel' had the highest levels of d-3-gal, p-3-glu, and mal-3-ara. 'Tifblue' had the highest amounts of c-3-gal and c-3-glu. 'Woodard' contained the highest amounts of p-3-glu, and p-3-ara. 'Centurion' had the highest contents of resv and q-3-gal, while 'Owen' had the highest level of d-3-ara. The RE-D 'Pink Lemonade' had the lowest of all anthocyanin contents (Tables 1–3).

At the species level, RE berries had the highest mean of chlo and myr-3-ara, compared to RE-D and NHB blueberries. The RE-D had

Table 1
Resveratrol (Resv), chlorogenic acid (Chlo), myricetin 3-arabinoside (m-3-ara), quercetin 3-galactoside (q-3-gal), and quercetin 3-glucoside (q-3-glu) content in fruit of 42 blueberry cultivars (36 *V. ashei*, three *V. ashei* derivative hybrids, and three northern highbush standards).

Blueberry cultivar	Resv (μg/g fw)		Chlo (μg/g fw)		Myr-3-ara μg/g fw		Q-3-gal μg/g fw		Q-3-glu μg/g fw	
Vaccinium ashei Reade (rabbiteye)										
Alapaha	1.2	pq[a]	130	v	57.2	d	91.3	lmn	211	fgh
Aliceblue	4.1	f	364	e	38.1	gh	70.0	pqr	191	jkl
Austin	3.1	ij	218	pqr	25.5	pq	6.0	t	199	ij
Baldwin	2.6	lm	261	mn	15.7	u	7.0	t	325	a
Beckyblue	0.8	stu	105	w	12.3	v	96.2	jk	75.2	t
Bluebelle	1.7	o	128	v	26.5	op	105	i	122	q
Bluegem	4.4	e	493	c	87.3	b	91.3	lmn	212	fg
Bonita	0.7	tu	244	no	16.7	st	88.2	mn	157	no
Brightwell	0.6	u	203	qrs	28.1	no	73.0	p	233	cd
Briteblue	3.2	hi	400	d	37.9	gh	60.5	r	192	jkl
Callaway	3.4	h	234	o	34.2	j	228	c	66.9	u
Centurion	7.0	b	257	mn	36.3	hi	303	a	218	ef
Chaucer	2.3	n	272	kl	14.8	uv	115	hi	197	jk
Choice	4.0	f	374	de	32.0	k	110	i	182	lm
Clara	5.4	d	379	de	30.4	m	94.4	ikl	235	cd
Climax	3.3	hi	372	de	37.5	h	110	hi	205	ghi
Coastal	2.8	kl	145	u	24.5	q	92.6	klm	151	o
Delite	0.7	tu	208	qrs	9.6	w	49.9	s	91.3	st
Early May	5.8	c	686	a	91.4	a	93.0	klm	229	cde
Ethel	0.9	rst	293	i	4.2	y	158	f	120	q
Garden Blue	2.9	jk	250	n	30.1	m	88.8	mn	136	p
Homebell	1.3	p	304	hi	26.9	op	95.5	jkl	213	fg
Ira	0.8	stu	302	ii	39.0	g	78.4	op	227	d
Menditoo	3.4	h	328	fg	34.7	ij	86.1	no	198	h–k
Montgomery	2.5	mn	202	s	18.2	r	70.6	pq	179	m
Myers	2.3	n	524	b	38.7	g	71.7	pq	271	b
Owen	0.7	tu	523	b	46.8	f	95.6	jkl	238	c
Powderblue	2.6	lm	313	ghi	28.0	no	144	g	197	jk
Premier	1.1	pqr	271	klm	27.2	o	103	ij	184	lm
Satilla	2.7	klm	314	ghi	17.2	s	92.4	klm	166	n
Southland	2.6	lm	281	k	34.7	ij	61.6	qrs	139	p
Suwanee	0.9	rst	231	opq	29.1	n	243	b	153	o
Tifblue	3.1	ij	219	pq	31.1	l	72.0	pq	125	q
Walker	1.2	pq	261	m	47.1	f	93.5	klm	192	j–m
Windy	2.5	mn	263	m	68.4	c	90.3	lmn	179	m
Woodard	1.0	qrs	170	t	58.8	d	89.3	mn	82.8	st
Mean (rabbiteye)	2.5		292		34.3		101		180	
Vaccinium ashei hybrid derivatives										
Pearl River	1.0	qrs	331	f	35.2	hij	214	d	279	b
Snowflake	1.0	qrs	369	e	32.1	k	174	e	282	ab
Pink Lemonade	0.9	rst	84.5	x	6.7	x	226	cd	56.4	v
Mean (rabbiteye deriv.)	1.0		262		24.7		204		206	
Vaccinium corymbosum L. (northern highbush)										
Bluecrop	3.7	g	103	w	16.2	tu	104	ij	141	p
Duke	0.6	u	130	v	6.9	x	119	h	111	r
Elliott	9.3	a	331	f	50.3	e	93.1	klm	187	klm
Mean (N. highbush)	4.5		188		24.5		105		146	

[a] Means within the same column followed by different letters were significantly different at $p \leqslant 0.05$.

the highest mean of q-3-gal and q-3-glu, compared to the other species (Table 1). RE berries also had the highest means of d-3-glu, d-3-ara, c-3-glu, and petunidin- and malvidin-based anthocyanins, whereas RE-D had the lowest (Tables 1–3).

The multivariate centroid hierarchical cluster analysis indicated that 3–4 clusters were needed to cluster the variables: resv, chlo, myr-3-ara, q-3-gal, q-3-glu, d-3-gal, d-3-glu, c-3-gal, d-3-ara, c-3-glu, p-3-gal, p-3-glu, p-3-ara, mal-3-gal, mal-3-glu, and mal-3-ara (Fig. 1). The centroid method is more robust against outliers than are other methods, such as Ward's method which is a minimum variance method. The clustering suggests that, compositionally, the three NHB cultivars were not distinctly different from the majority of the RE cultivars (34 cultivars), whereas the three RE-D were both distinct from the majority of the RE and were similar within their cluster. This clustering may reflect the influence of the additional species involved in their ancestry (*V. constablaei* and *V. darrowii*). 'Early May' and 'Bluegem' are unusual in that, while they are not genetically different from the other RE cultivars, they resolved into a distinct cluster. The most notable features about these two cultivars, compositionally, are their high total ORAC levels, (ranking 1st and 3rd, respectively) and the high percentages of ORAC activity derived from mal-3-gal and mal-3-glu (Table 4).

3.2. Contributions of identified flavonoids to antioxidant activity in blueberries

The contributions of identified individual flavonoids to total antioxidant activity (sum of ORAC of the identified individual flavonoids for each cultivar) in blueberries are shown in Table 4. The mean percentage contribution of resv to total ORAC values for RE, RE-D and NHB blueberry fruit were all 0.3%, indicating that resv was a minor contributor to antioxidant activity, due to its low level in blueberries. The percentage contribution of chlo to total ORAC values ranged from 2.7% to 8.4%. 'Pearl River', 'Myers', 'Snowflake', and 'Early May' had higher percentage contributions compared to other cultivars. The mean percentage contributions

Table 2
Delphinidin 3-galactoside (d-3-gal), delphinidin 3-glucoside, (d-3-glu), cyanidin 3-galactoside (c-3-gal), delphinidin 3-arabinoside (d-3-ara), and cyanidin 3-glucoside (c-3-glu) contents in fruit of 42 blueberry cultivars (36 *V. ashei*, 3 *V. ashei* derivative hybrids, and three northern highbush standards).

Blueberry cultivar	D-3-gal[a] (μg/g fw)		D-3-glu[a] (μg/g fw)		C-3-gal[a] (μg/g fw)		D-3-ara[a] (μg/g fw)		C-3-glu[a] (μg/g fw)	
Vaccinium ashei Reade (rabbiteye)										
Alapaha	137	g[b]	89.9	ijk	42.2	k	60.7	p	47.8	hi
Aliceblue	187	ab	125	d	36.8	l	270	d	69.3	cde
Austin	141	g	67.0	n	34.5	l	200	f	42.4	i
Baldwin	155	f	51.9	p	74.7	i	281	c	33.1	jk
Beckyblue	51.7	no	44.5	q	61.6	j	38.8	q	13.3	n
Bluebelle	80.2	l	41.5	r	26.0	m	36.8	q	41.7	i
Bluegem	91.5	i–l	103	g	9.8	o	209	ef	81.6	bc
Bonita	100	i	87.6	jkl	63.6	j	190	g	24.4	l
Brightwell	96.9	J	54.1	p	122	e	124	l	30.4	k
Briteblue	142	g	124	de	142	cd	85.4	o	49.5	hi
Callaway	156	ef	112	f	25.8	m	146	j	53.6	g
Centurion	169	bc	143	c	110	f	171	h	66.7	cde
Chaucer	89.9	i–l	55.9	op	110	f	122	l	34.3	jk
Choice	184	ab	84.0	kl	4.0	p	177	h	49.3	hi
Clara	167	c	97.0	hij	185	a	219	e	70.3	cd
Climax	126	h	125	d	131	d	161	i	63.3	def
Coastal	123	h	87.9	jkl	23.8	m	106	mn	63.7	def
Delite	48.3	o	24.9	s	68.6	ij	187	g	10.1	o
Early May	188	ab	196	a	183	a	299	b	99.3	a
Ethel	202	a	97.3	hij	0.3	q	210	ef	1.3	q
Garden Blue	89.8	kl	76.1	m	72.7	i	108	m	60.1	f
Homebell	137	g	10.3	u	165	b	273	cd	31.1	k
Ira	92.6	i–l	71.3	mn	164	b	139	k	47.3	hi
Menditoo	101	i	98.3	hi	128	de	124	l	53.0	g
Montgomery	40.3	p	41.2	r	15.9	n	110	m	35.5	jk
Myers	187	ab	125	d	122	e	211	ef	82.6	bc
Owen	170	bc	161	b	144	c	400	a	67.4	cde
Powderblue	56.2	mn	108	g	111	f	105	n	71.4	cd
Premier	57.0	m	43.8	qr	35.1	l	198	f	36.5	j
Satilla	81.0	jkl	61.8	o	129	de	37.8	q	62.2	def
Southland	159	de	128	d	124	e	156	i	66.6	de
Suwanee	102	i	98.8	hi	25.0	m	140	k	51.1	gh
Tifblue	30.9	q	69.0	mn	181	a	156	i	97.3	ab
Walker	88.8	kl	99.8	h	74.7	i	35.8	q	50.3	gh
Windy	84.2	kl	56.8	op	107	fg	142	jk	51.9	gh
Woodard	124	h	66.9	n	89.3	h	272	cd	50.1	gh
Mean (rabbiteye)	118		86.9		87.4		164		51.7	
Vaccinium ashei hybrid derivatives										
Pearl River	10.2	s	0.2	v	8.3	o	28.6	r	9.8	o
Snowflake	39.4	p	23.2	s	46.8	k	66.9	p	17.2	m
Pink Lemonade	12.1	r	10.2	u	7.2	o	12.3	s	3.2	p
Mean (rabbiteye deriv.)	20.6		11.2		20.8		35.9		10.1	
Vaccinium corymbosum L. (northern highbush)										
Bluecrop	54.0	mn	12.0	t	15.9	n	86.1	o	9.7	u
Duke	168	c	13.1	t	103	g	82.4	o	3.3	v
Elliott	162	d	139	c	161	b	188	g	74.7	c
Mean (N. highbush)	128		54.8		93.4		119		29.2	

[a] Data expressed as microgrammes of cyanidin 3-glucoside equivalents per gramme of fresh wt.
[b] Means within the same column followed by different letters were significantly different at $p \leqslant 0.05$.

of chlo to the total ORAC values for 36 RE, RE-D, and NHB blueberries were 5.3%, 7.0%, and 4.0 %, respectively. This indicates that chlo is one of the important contributors to antioxidant activity. Chlo was a major cinnamic derivative found in large amounts in blueberries (Kader et al., 1996).

In general, blueberries contained higher amounts of quercetin-based flavonols than myricetin-based flavonols (Table 1). Q-3-gal and q-3-glu served as two main constituents for antioxidant activity in blueberry extracts. The ORAC values of myr-3-ara were lower than those of q-3-gal or q-3-glu (Table 4). q-3-Gal contributed 5.3% to total ORAC values in RE blueberries, 27.5% in RE-D, and 6.7% in NHB blueberries. The contribution of q-3-glu to total ORAC values in RE cultivars, RE-D and NHB blueberries were 10.7%, 23.4%, and 10.3 %, respectively. Therefore, the contributions of chlo, myricetin, and quercetin to antioxidant activity in RE-D (62.5%) were higher than those in REs (23.8%) or in NHB blueberries (23.2%).

The sum of ORAC values of 11 anthocyanins identified in blueberries ranged from 2.63 to 65.5 μmol TE/g fw and accounted for 25.1–85.9% of the total ORAC values in this study (Table 4). The percentage contributions of ORAC value of anthocyanins to total antioxidant activity were 76.2%, 37.5%, and 76.8% for RE cultivars, RE-D, and NHB blueberries, respectively. This indicates that anthocyanins contributed significantly to total antioxidant activity in blueberries, especially in RE and NHB blueberries.

Among anthocyanin constituents of blueberries, malvidin-based anthocyanins were responsible for higher antioxidant activity levels (ORAC) than were petunidin-, delphinidin-, or cyanidin-based anthocyanins. It has been shown that anthocyanins are strong antioxidants with free radical-scavenging properties attributed to the phenolic hydroxy groups attached to ring structures (Rice-Evans & Miller, 1996; Wang, Cao, & Prior, 1997). M-3-gal had high ORAC values up to 16.5 μmol TE/g fw. The RE cultivars 'Homebell', 'Ethel', 'Tifblue', and 'Woodard' had high ORAC values, whereas the RE-D 'Pink Lemonade' had low values (Table 4). On the species level, the contribution of m-3-gal to the total ORAC value was 17.9% for RE blueberries, 7.2% for RE-D, and 20.2% for NHB

Table 3
Petunidin 3-galactoside (p-3-gal), petunidin 3-glucoside (p-3-glu), petunidin 3-arabinoside (p-3-ara), malvidin 3-galactoside (mal-3-gal), malvidin 3-glucoside (mal-3-glu), and malvidin 3-arabinoside (mal-3-ara) contents in fruit of 42 blueberry cultivars (36 *V. ashei*, 3 *V. ashei* derivative hybrids, and three northern highbush standards).

Blueberry cultivar	P-3-gal[a] (μg/g fw)		P-3-glu[a] (μg/g fw)		P-3-ara[a] (μg/g fw)		Mal-3-gal[a] (μg/g fw)		Mal-3-glu[a] (μg/g fw)		Mal-3-ara[a] (μg/g fw)	
Vaccinium ashei Reade (rabbiteye)												
Alapaha	39.5	s[b]	97.4	r	215	lm	359	mn	135	t	154	n
Aliceblue	247	bc	189	cd	228	kl	622	cd	390	d	161	k–n
Austin	157	k	177	def	207	lmn	446	k	286	gh	180	i–l
Baldwin	135	m	167	efg	224	kl	537	f	132	t	173	j–l
Beckyblue	59.4	r	117	p	139	s	315	op	67.8	w	76.3	v
Bluebelle	155	k	135	lm	213	lm	296	p	260	i	129	op
Bluegem	256	b	166	efg	299	cd	625	cd	598	a	149	n
Bonita	122	no	140	klm	262	ghi	473	ij	213	lm	147	n
Brightwell	119	o	123	o	184	pq	448	k	108	u	185	hi
Briteblue	177	hi	144	jkl	216	lm	561	f	220	j	103	t
Callaway	183	h	180	cde	186	pq	541	f	287	gh	126	p
Centurion	237	bc	183	cd	252	ij	457	jk	380	d	123	pq
Chaucer	137	m	126	no	179	pq	373	lm	112	u	174	j–l
Choice	180	h	148	i–l	262	ghi	595	de	284	gh	182	h–k
Clara	196	f	199	b	277	d–h	624	cd	264	hi	239	e
Climax	221	cd	164	efg	295	d	591	e	448	c	247	de
Coastal	202	e	138	k–n	177	q	298	p	290	g	88.9	u
Delite	69.8	q	69.8	s	138	s	326	o	172	rs	112	rs
Early May	313	a	221	a	323	b	624	cd	500	b	195	h
Ethel	145	l	215	a	265	f–i	765	b	175	rs	323	a
Garden Blue	234	bc	195	b	315	bc	621	cde	192	pq	293	b
Homebell	182	h	197	b	297	cd	840	a	134	t	238	e
Ira	181	h	129	mno	187	opq	442	k	215	kl	164	klm
Menditoo	170	i	146	i–l	241	jk	435	k	219	jk	184	h–k
Montgomery	127	n	130	l–p	258	hi	487	h	338	e	209	g
Myers	219	cd	165	fg	189	nop	259	q	282	gh	186	hij
Owen	195	f	182	cd	239	k	627	c	211	mn	196	h
Powderblue	161	jk	129	mno	179	pq	353	n	221	j	110	s
Premier	141	lm	151	ijk	279	efg	624	cd	164	s	263	cd
Satilla	191	g	161	gh	237	k	351	n	106	u	218	f
Southland	219	cd	164	efg	198	mno	360	mn	318	ef	116	qr
Suwanee	215	d	137	lm	190	m–q	353	n	386	d	85.8	u
Tifblue	193	fg	98.4	r	287	d–g	765	b	281	gh	148	n
Walker	163	j	107	q	148	s	226	r	162	s	71.6	v
Windy	164	j	157	g–j	288	def	603	de	176	rs	220	f
Woodard	194	f	227	a	355	a	769	b	195	opq	282	bc
Mean (rabbiteye)	175		155		234		500		248		174	
Vaccinium ashei hybrid derivatives												
Pearl River	28.4	t	5.2	u	76.8	u	143	t	49.9	x	49.9	w
Snowflake	125	n	58.1	t	238	k	196	s	78.4	v	110	s
Pink Lemonade	2.3	u	6.2	u	7.1	v	12.5	u	29.7	y	21.3	x
Mean (Rabbiteye deriv.)	52.0		23.2		107		117		52.7		60.4	
Vaccinium corymbosum L. (northern highbush)												
Bluecrop	122	no	109	q	96.8	t	488	hi	201	no	164	klm
Duke	100	p	151	h–k	160	r	380	l	84.7	v	155	n
Elliott	225	cd	186	cd	290	c–f	511	g	281	gh	193	h
Mean (N. Highbush)	149		149		182		460		189		171	

[a] Data expressed as microgrammes of cyanidin 3-glucoside equivalents per gramme of fresh wt.
[b] Means within the same column followed by different letters were significantly different at $p \leqslant 0.05$.

blueberries. The mean ORAC values of mal-3-glu and mal-3-ara, and their percentage contributions to the total ORAC value in RE and NHB blueberries, were higher than those in RE-D (Table 4). Malvidin-based anthocyanins contributed 32.3% of antioxidant activity to total antioxidant activity which was higher than petunidin- (19.5%), delphinidin- (14.5%), or cyanidin-based (6.5%) anthocyanins (Table 4).

Among the 42 blueberry cultivars used in this study, the RE cultivar 'Bluegem' had high ORAC contributions from chlo, myr-3-ara, p-3-gal, and myr-3-glu. 'Early May' had high ORAC contributions from chlo, myr-3-ara, c-3-gal, c-3-glu, d-3-glu, and p-3-gal. 'Ethel' had high amounts of ORAC values from d-3-gal and myr-3-ara; 'Myers' had high ORAC values from chlo and q-3-glu, while 'Owen' had high ORAC values from chlo and d-3-ara. In NHB, 'Bluecrop', 'Duke', and 'Elliott' had high ORAC contributions from malvidin-based and petunidin-based anthocyanins, and q-3-glu. RE-D 'Pearl River' and 'Snowflake' had high ORAC values from q-3-glu. 'Pink Lemonade', (RE-D) had the lowest antioxidant capacity. This may be due to its low content of anthocyanin and phenolics (Tables 1–4).

There was little correlation between antioxidant activity and the levels of individual flavonoid compounds in the 42 blueberries cultivars tested, with *r* values ranging from 0.27 to 0.75. However, there was a better and significant correlation between antioxidant activity (ORAC) and total phenolics with a *r* of 0.89. The correlation between ORAC and total anthocyanin was also substantial with a *r* of 0.73. Therefore, as reported previously (Wang & Lewers, 2007), the individual concentration of a specific phytochemical may not be a good index of antioxidant activity. Hidalgo, Sánchez-Moreno, and Pascual-Teresa (2010) found that flavonoids present in a mixture could interact, and their interactions could have synergistic or antagonistic effects on total antioxidant capacity,

Phenolics and flavonoids exhibited varying antioxidant capacities. The radical-scavenging and antioxidant activities of individual phenolic compounds in blueberries are not only dose-dependent, but also attributable to their composition and chemical structures.

Table 4
Contribution of identified phenolics to total antioxidant activity (ORAC) in fruit of 42 blueberry cultivars (36 *V. ashei*, 3 *V. ashei* derivative hybrids, and three northern highbush standards).[a]

Blueberry cultivar	%Contribution of individual identified phenolic to total ORAC																Total[b] ORAC
	Rev	Chlro	My-3-ara	Q-3-gal	Q-3-glu	D-3-gal	D-3-glu	C-3-gal	D-3-ara	C-3-glu	P-3-gal	P-3-glu	P-3-ara	Mal-3-gal	Mal-3-glu	Mal-3-ara	μmol TE/g fw
Vaccinium ashei Reade (rabbiteye)																	
Alapaha	0.2	3.3	4.1	6.0	16.1	7.5	5.6	2.5	3.2	3.5	1.9	5.3	9.5	17.1	6.8	7.5	41.3 r–u[c]
Aliceblue	0.2	6.1	1.8	2.9	9.2	6.3	4.8	1.6	8.8	3.2	6.9	6.4	6.2	18.4	12.0	5.1	66.7 def
Austin	0.3	5.9	1.6	0.4	12.6	6.3	3.5	1.7	8.6	2.6	5.8	7.9	7.5	16.6	11.6	7.2	50.4 l–o
Baldwin	0.2	5.2	0.9	0.6	18.8	6.4	2.5	3.2	11.0	1.9	4.6	6.8	7.4	19.1	5.0	6.4	55.2 i–l
Beckyblue	0.2	3.8	1.5	9.6	8.9	4.3	4.2	5.3	3.2	1.3	4.7	9.7	9.4	22.9	5.2	5.7	27.1 x
Bluebelle	0.2	3.3	2.2	7.5	10.2	4.8	2.8	1.7	2.2	3.3	7.6	7.9	10.1	15.3	14.0	6.9	38.2 u
Bluegem	0.3	6.9	3.6	3.7	9.4	2.9	3.7	1.8	6.3	3.5	6.6	5.3	7.6	17.1	17.0	4.2	72.0 bc
Bonita	0.3	5.0	1.9	5.0	10.3	4.7	4.6	3.1	8.4	1.6	4.7	6.5	9.8	19.1	9.0	6.1	48.7 nop
Brightwell	0.2	4.2	2.3	4.4	16.1	4.8	3.1	6.2	5.9	2.4	4.9	6.0	7.3	19.2	4.9	8.1	45.9 o–r
Briteblue	0.4	6.7	3.4	3.8	11.1	5.8	5.7	6.0	3.6	2.5	6.3	5.8	7.1	19.9	8.2	3.8	55.5 ijk
Callaway	0.3	4.3	1.9	11.4	4.2	6.5	5.3	1.5	5.9	3.0	6.3	7.5	6.3	19.7	10.9	4.8	54.0 j–m
Centurion	0.3	3.9	1.8	12.1	10.3	5.6	5.4	3.8	5.5	3.1	6.5	6.1	6.8	13.3	11.6	3.7	67.7 cde
Chaucer	0.2	5.6	1.7	6.9	13.9	4.5	4.0	5.7	5.9	2.6	5.7	6.3	7.2	16.4	5.4	7.9	45.0 p–s
Choice	0.3	6.3	1.7	5.1	9.8	7.0	3.7	0.6	6.5	2.9	5.7	5.6	8.5	19.6	9.9	6.8	59.2 hi
Clara	0.4	5.5	1.9	3.8	10.8	5.5	3.6	6.3	6.9	3.5	5.3	6.5	7.3	17.8	7.9	7.0	69.2 bcd
Climax	0.3	5.7	2.3	4.4	9.5	4.2	4.7	4.5	5.1	2.8	6.0	5.4	7.8	16.9	13.3	7.3	69.1 bcd
Coastal	0.2	3.7	1.7	5.8	11.1	6.4	5.2	1.4	5.3	4.4	8.7	7.2	7.5	13.5	13.7	4.2	43.4 q–t
Delite	0.3	6.9	1.5	4.2	8.9	3.4	2.2	4.9	12.4	1.0	4.0	4.8	7.7	19.7	10.8	7.0	32.6 vw
Early May	0.2	7.8	3.9	2.9	8.3	4.8	5.7	5.4	7.4	4.4	6.6	5.7	6.7	14.0	11.7	4.5	88.2 a
Ethel	0.2	5.1	0.7	7.0	6.3	7.4	4.1	0.1	7.4	0.2	4.5	7.9	7.9	24.6	5.9	10.6	61.2 gh
Garden Blue	0.2	4.8	1.8	4.3	7.6	3.6	3.4	3.0	4.1	3.2	7.6	7.7	10.0	21.4	6.9	10.3	47.3 opq
Homebell	0.1	5.1	1.3	4.1	10.4	4.7	0.7	5.9	9.0	1.5	5.2	6.8	8.3	25.2	4.3	7.4	65.5 d–g
Ira	0.2	5.4	2.9	4.6	13.7	4.1	3.5	7.3	5.7	2.8	6.4	5.5	6.5	16.5	8.4	6.3	52.8 j–n
Menditoo	0.3	6.1	1.6	4.9	11.7	4.5	5.2	5.6	5.2	3.2	5.9	6.3	8.2	16.0	8.4	7.0	53.8 j–m
Montgomery	0.2	4.3	1.5	4.2	12.1	2.2	2.3	0.9	5.1	2.3	5.0	6.2	9.9	20.3	14.6	8.9	47.3 f
Myers	0.3	8.3	2.3	3.2	13.9	6.8	5.2	4.7	7.4	4.7	6.6	6.5	6.6	8.2	9.4	6.1	62.2 fgh
Owen	0.2	7.1	2.9	3.8	10.4	5.7	5.6	4.6	11.8	2.8	5.2	5.6	6.0	16.9	6.0	5.4	73.3 b
Powderblue	0.3	5.9	2.7	8.2	12.8	2.9	5.6	5.3	4.7	4.8	6.1	5.9	6.7	14.2	9.3	4.6	49.1 m–p
Premier	0.3	5.1	1.9	5.2	10.9	2.5	2.2	1.8	8.2	2.1	4.9	6.3	9.4	22.9	6.3	9.9	53.7 j–m
Satilla	0.2	6.8	2.4	5.4	11.4	4.3	3.4	6.5	1.8	4.1	7.6	7.8	9.2	14.8	4.8	9.5	46.6 opq
Southland	0.2	5.4	2.6	3.5	8.4	6.7	6.2	5.5	6.4	3.8	7.7	6.9	6.8	13.3	12.3	4.5	53.3 j–n
Suwanee	0.3	4.5	2.1	12.5	9.3	4.4	4.8	1.2	5.8	3.0	7.6	5.9	6.6	13.3	15.1	3.4	52.4 k–n
Tifblue	0.1	3.8	1.7	3.5	7.0	1.6	3.1	7.4	5.9	5.1	6.3	3.9	9.1	26.2	10.1	5.3	57.5 ij
Walker	0.3	6.2	4.5	6.4	15.4	5.4	6.5	4.4	2.3	3.9	7.7	6.5	6.8	11.3	8.5	3.7	39.6 tu
Windy	0.2	4.9	3.9	4.7	10.2	3.5	2.5	4.5	5.6	2.9	5.5	6.4	9.4	21.3	6.5	8.0	55.8 ijk
Woodard	0.2	2.9	2.8	3.9	4.3	4.5	2.8	3.4	9.4	2.5	5.8	8.1	10.3	23.7	6.4	9.1	62.8 e–h
Mean (rabbiteye)	0.3	5.3	2.3	5.3	10.7	4.9	4.1	3.9	6.3	3.0	6.0	6.5	7.9	17.9	9.2	6.5	54.5
Vaccinium ashei hybrid derivatives																	
Pearl River	0.3	8.4	4.6	20.1	30.9	1.9	1.8	1.7	2.3	2.2	1.9	1.5	4.9	9.9	3.7	3.9	28.7 wx
Snowflake	0.3	8.2	5.4	11.7	22.4	2.3	2.1	2.8	3.7	1.4	5.9	3.5	10.8	9.7	4.1	5.6	40.0 stu
Pink Lemonade	0.2	4.3	2.7	50.8	17.0	3.2	2.3	1.5	2.3	0.9	1.5	1.3	1.2	2.2	5.2	3.7	11.9 y
Mean (rabbiteye deriv.)	0.3	7.0	4.3	27.5	23.4	2.5	2.1	2.0	2.8	1.5	3.1	2.1	5.6	7.2	4.3	4.4	26.9
Vaccinium corymbosum L. (northern highbush)																	
Bluecrop	0.3	2.7	1.4	7.7	12.3	3.4	0.9	1.1	5.1	0.9	6.2	6.7	4.8	26.2	11.3	9.0	36.7 uv
Duke	0.2	3.6	1.6	8.6	9.4	10.0	1.0	6.4	4.8	0.4	5.0	9.1	7.8	19.0	4.7	8.4	37.4 uv
Elliott	0.4	5.6	2.8	3.9	9.1	5.6	5.5	5.8	6.3	3.5	6.5	6.4	8.1	15.5	8.9	6.1	65.2 d–g
Mean (N. highbush)	0.3	4.0	1.9	6.7	10.3	6.3	2.5	4.4	5.4	1.6	5.9	7.4	6.9	20.2	8.3	7.8	46.4

[a] See Tables 1–3 for abbreviations of individual phenolic compounds.
[b] Means within the same column followed by different letters were significantly different at $p \leqslant 0.05$.
[c] Sum of ORAC of the individual flavonoids identified for each cultivar.

The antioxidant capacity of polyphenols is dictated by both the structural configurations of the compound and the number of OH groups. The ring orientation determines the ease with which a hydrogen atom from a hydroxyl group can be donated to a free radical, and the ability of the compound to support an unpaired electron (Roginsky, 2003). Thus, the position of the OH groups in relation to one another is a more important factor affecting the antioxidant capacity of phenolics than the number of OH groups (Hosseinian, Li, Hydamaka, Tsopmo, & Lowry, 2007; Roginsky, 2003). The degree of hydroxylation and methoxylation of the B ring in the aglycone moiety, and the nature or extent of glycosylation or acylation present, have also been shown to affect radical-scavenging (Kähkönen, Heinämäki, & Heinonen, 2003; Zheng & Wang, 2003). Hydroxyl groups in the ortho position of the B ring appear to greatly enhance the antioxidant capacity of phenolics (Rice-Evans & Miller, 1996; Shahidi, Janitha, & Wanasundara, 1992). Glycosylation decreases the antioxidant capacity of phenolics by reducing free OH and metal chelation sites (Shahidi et al., 1992). However, the effect of glycosylation on antioxidant capacity will depend upon the solubility of the antioxidant (e.g., water-soluble or lipid-soluble) (Hosseinian, Muir, Westcott, & Krol, 2006; Hosseinian et al., 2007; Rice-Evans & Miller, 1996; Roginsky, 2003). Adding a sugar moiety diminishes the antioxidant capacity of the aglycone. This may be attributed to steric impediment by sugar moieties. For example, the antioxidant capacity of the cyanidin 3, 5-diglucoside is 10 times lower than that of cyanidin 3-rutinoside, which might be due to the sugar attachments at the 3 and 5 positions of the chromane ring (Fukumoto & Mazza, 2000; Seeram, Momin, Nair, & Bourquin, 2001). Seeram et al. (2001) found that inhibition of COX (cyclooxygenase) appeared to decrease with increasing complexity of glycosylation in cyanidin-based anthocyanins (i.e., cyanidin > cyanidin 3-rutinoside > cyanidin 3-glucosylrutinoside).

Myricetin and quercetin are effective antioxidants and are potent quenchers of $ROO^{\cdot}$, $O_2^{\cdot-}$, and $^1O_2$ (Larson, 1988). Quercetin and other polyphenols have been shown to play a protective role in carcinogenesis by reducing the bioavailability of carcinogens. Quercetin, with 3′,4′-dihydroxy substitution in the B-ring and conjugation between the A- and B-rings, has a high antioxidant potential (Cao, Sofic, & Prior, 1997). Flavonols, such as myricetin and quercetin in general, have higher antioxidant activities than have anthocyanins with the same hydroxylation patterns, measured with the ORAC assay (Bors, Heller, Michel, & Saran, 1990; Zheng & Wang, 2003).

This study showed that in the blueberries tested, malvidins were present in the highest amounts and produced the highest levels of antioxidant activity, followed by petunidins, delphinidins, and cyanidins. The mean contents of malvidin-based anthocyanins were 921, 230, and 819 μg/g fw for RE cultivars, RE-D, and NHB standards, respectively. Levels of petunidin-, delphinidin-, and cyanidin-based anthocyanin also were higher in RE cultivars than in either RE-D or NHB blueberries. Delphinidin-based anthocyanin content and antioxidant activity (ORAC) was higher than that of cyanidin-based anthocyanins, but lower than those of malvidin- and petunidin-based anthocyanins (Table 4). The antioxidant activity of RE-D was mainly derived from chlorogenic acid, myricetin, and quercetin, which contributed 62.3% of total antioxidant activity, whereas the antioxidant activities of the RE cultivars and NHB blueberries were derived from anthocyanins (malvidin, petunidin, delphinidin, and cyanidin) which contributed 76.5% of total antioxidant activity (Table 4).

Collectively, the results from this study showed that blueberries had high phenolic content and high antioxidant capacities, but their levels were quite varied among cultivars. A diverse range of total anthocyanin content was also shown in *Rubus* fruit (McGhie, Hall, Ainge, & Mowat, 2002). Significant variation in total antioxidant capacity in blueberry genotypes has also been demonstrated by Connor, Luby, Tong, Finn, and Hancock (2002). The existence of this unexploited variability in cultivated and wild germplasms will be useful for genetic improvement of fruit quality. The scientific knowledge and technical capacities available for improving phytonutrient content in a number of horticultural crops through plant genetic enhancement have been reported by Farnham, Simon, and Stommel (1999). Cultivars with high antioxidant contents and antioxidant enzyme activities should be useful in breeding programmes for the development of more nutritious blueberry cultivars. On this basis, well-focused breeding programmes can create new varieties specifically selected for improved fruit quality and antioxidant potential in blueberries. The ultimate goal is to produce fruit with enhanced levels of flavonoids and other phenolics so that they can provide greater health benefits to the consumers.

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