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Anthocyanins, Phenolics, and Antioxidant Capacity in Diverse Small Fruits: *Vaccinium*, *Rubus*, and *Ribes*

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Fruits from 107 genotypes of *Vaccinium* L., *Rubus* L., and *Ribes* L., were analyzed for total anthocyanins (ACY), total phenolics (TPH), and antioxidant capacities as determined by oxygen radical absorbing capacity (ORAC) and ferric reducing antioxidant power (FRAP). Fruit size was highly correlated ($r = 0.84$) with ACY within *Vaccinium corymbosum* L., but was not correlated to ACY across eight other *Vaccinium* species, or within 27 blackberry hybrids. Certain *Vaccinium* and *Ribes* fruits with pigmented flesh were lower in ACY, TPH, ORAC, and FRAP compared to those values in berries with nonpigmented flesh. ORAC values ranged from 19 to 131 μmol Trolox equivalents/g in *Vaccinium*, from 13 to 146 in *Rubus*, and from 17 to 116 in *Ribes*. Though ACY may indicate TPH, the range observed in ACY/TPH ratios precludes prediction of ACY from TPH and vice versa for a single genotype. In general, TPH was more highly correlated to antioxidant capacity than ACY was. This study demonstrates the wide diversity of phytochemical levels and antioxidant capacities within and across three genera of small fruit.

KEYWORDS: Anthocyanins; phenolics; ORAC; FRAP; blueberry; blackberry; black currant; rabbiteye; highbush; lowbush; marionberry; jostaberry; gooseberry

INTRODUCTION

Increasing epidemiological evidence associates diets rich in fruits and vegetables with reduced risk of heart disease, cancer, and other chronic diseases (*1*, *2*). A major benefit from such a diet may be increased consumption of antioxidants (*3*), including carotenoids, ascorbate, tocopherols, and phenolics. One phenolic fraction, the flavonoids, are potent in vitro antioxidants (*4*−*6*) and include compounds such as flavones, isoflavones, flavonones, catechins, and the red, blue, and purple pigments known as anthocyanins (*7*). Wang et al. (*8*) observed that compounds other than vitamin C are major sources of antioxidant capacity in fruits. Blueberries (*Vaccinium* L. species), blackberries (*Rubus* L. hybrids), and black currants (*Ribes nigrum* L.) are rich sources of dietary anthocyanins and antioxidants (*8*−*10*). Many cultivars and native species of these berries exist, some with substantially higher antioxidant levels than others (*11*, *12*). Plant anthocyanin levels vary according to season and growing location (*11*, *13*, *14*), confounding attempts to compare reported values within or across species and genera. Differing laboratory methods of extraction and analysis may also contribute to variance in reported levels of anthocyanins, phenolics, and antioxidants.

Prior et al. (*11*) compared total anthocyanins (ACY), total phenolics (TPH), and oxygen radical absorbing capacity (ORAC) of four *Vaccinium* species and 23 genotypes. Our objectives were similar, but our methodology and study group were broader. Our objectives were to (1) determine the ACY, TPH, and two measures of antioxidant capacity, ORAC and FRAP, in 107 genotypes of dark-colored, small fruits with representatives of nine *Vaccinium* L., seven *Rubus* L., and five *Ribes* L. species; (2) determine whether these measurements are correlated; (3) determine whether berry size is correlated with ACY, TPH, ORAC, or FRAP; and (4) compare the ACY, TPH, ORAC, and FRAP in first vs last ripe *Vaccinium corymbosum* cv. Summit.

MATERIALS AND METHODS

Safety. There is an explosion hazard as liquid nitrogen becomes gaseous. For proper venting procedures during liquid nitrogen milling of frozen materials, refer to Rodriguez-Saona and Wrolstad (*15*).

Sampling Procedures. Ripe fruit samples, as judged by flavor and color, were harvested during summer 2000 from two Willamette Valley

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sites: Oregon State University North Willamette Experiment Station (Aurora, OR) and the U.S. Department of Agriculture, Agricultural Research Service, National Clonal Germplasm Repository (Corvallis, OR). Approximately 60 g of fruit was collected from 1 to 4 clones of each genotype. The highbush blueberry, *V. corymbosum* L. cv. Summit, was picked very early and very late in its season. Fruit was placed immediately on ice in the field and frozen at −10 °C later that same day. Care was taken to avoid unripe, damaged, or overripe fruit.

Samples were prepared according to Rodriguez-Saona and Wrolstad (*15*). About 40 g of berries were counted as each sample was weighed to determine average berry size. The frozen fruits were further cooled in liquid nitrogen; then they were cryogenically milled in a stainless Waring blender jar containing a lid modified with a chimney. Chilled tubes were filled with milled fruit powder and weighed, and then the powder was extracted with acetone, followed by two additional extractions with 70:30 acetone/water. The pooled supernatants were partitioned with two volumes of chloroform. The nonpolar phase was discarded, and the aqueous extracts were stored at −10 °C or at −70 °C if for antioxidant analysis.

Determination of Total Anthocyanins (ACY). Anthocyanin quantitation was performed by the pH differential method of Giusti and Wrolstad (*16*). Samples were diluted 1:150 in pH 1.0 and pH 4.5 buffers, then measured at 520 and 700 nm in a Shimadzu 300 UV−Visible spectrophotometer. ACY was based on a cyanidin 3-glucoside molar extinction coefficient of 26,900 and a molecular weight of 449.2. Resultant values were expressed in terms of mg of anthocyanin/100 g of fresh-frozen fruit.

Determination of Total Phenolics (TPH). The Folin−Ciocalteu method (*17*) was used to determine total soluble phenolics (TPH). Extracts were diluted 1:500 or 1:1000 before incubation at 40 °C. Absorption was measured at 755 nm. TPH was expressed as mg of gallic acid/100 g of fresh-frozen fruit.

Determination of Antioxidant Capacity. Antioxidant capacity was determined by ORAC and FRAP assays at the Linus Pauling Institute, Oregon State University. The ORAC assay was performed as described by Cao et al. (*18*) and adapted for use in a 96-well microplate fluorometer (model Cytofluor 4000, PerSeptive Biosystems, Framingham, MA). ORAC values, derived from triplicate analyses, are expressed as μmol Trolox equivalents (TE) per g of fresh-frozen fruit. Trolox is a water-soluble tocopherol analogue used as a reference compound for antioxidant capacity. The FRAP assay (*19*) was adapted for use in a 96-well microplate spectrophotometer (ThermoMax, Molecular Devices, Foster City, CA). FRAP values, derived from triplicate analyses, are expressed as μmol of ferric iron reduced per g of fresh-frozen fruit.

Statistical Analysis. Correlation and regression analyses were performed using Microsoft Excel Data Analysis. Differences at $p = 0.05$ were considered significant.

RESULTS AND DISCUSSION

ACY, TPH, ORAC, and FRAP. Wide ranges of ACY, TPH, ORAC, and FRAP were observed within each genus (**Tables 1**, **2**, and **3**), consequently the genus means were not significantly different. Having chosen dark fruited, highly pigmented genotypes, we were not surprised to observe that many values of ACY, TPH, ORAC, and FRAP for genotypes in our tests were higher than that reported generally for fruits and vegetables (*8*). Species effects were apparent. Black raspberries, *Rubus occidentalis* L. cvs. Munger, Jewel, and Earlysweet, (**Table 2**) had the highest ACY (627, 607, and 464 mg ACY/100 g, respectively), and FRAP (169, 184, and 206 μmol/g, respectively) levels of tested fruits, and high levels of TPH and ORAC (**Tables 1**, **2**, and **3**). Wild selections of rabbiteye blueberry, *Vaccinium ashei*, from Florida and Georgia (**Table 1**) had the highest ORAC (131, 129, and 122 μmol TE/g) and higher levels of ACY and FRAP than did many other fruits. *Ribes valdivianum*, a black fruited currant from Chile, and *Ribes nigrum* cvs. Consort and Willoughby (**Table 3**) had the highest TPH of all fruits tested (1790, 1342, 1122 mg TPH/100 g, respectively).

Table 1. Total Anthocyanin Content (ACY), Berry Size, Total Phenols (TPH), Antioxidant Activity (ORAC and FRAP), and Total Anthocyanins/Phenolics in 30 *Vaccinium* Genotypes

genotype	ACY mg/100 g[a]	berries/ 100 g	TPH mg/100 g[a]	ORAC μmol TE/g	FRAP μmol/g	ACY/ TPH
			V. angustifolium Aiton			
Brunswick	208 ± 4.0	324	692 ± 3.7	87.8	97.9	0.30
			V. ashei Reade (= *V. virgatum* Aiton)			
Bluegem	242 ± 6.0*	161	717 ± 1.6	110.8	140.1	0.34
CVAC 200.003	383 ± 9.4*	287	870 ± 20*	130.7	127.1	0.44
CVAC 1161.001	484 ± 1.8	142	961 ± 15	129.4	161.4	0.50
CVAC 1170.001	515 ± 3.6	218	952 ± 0.5	122.8	157.3	0.54
means	406 ± 87	202	875 ± 80	123.4	146.5	0.46
			V. constablaei Gray x *V. ashei*			
Little Giant	259 ± 4.2	321	583 ± 14	24.6	59.0	0.44
			V. corymbosum L. and hybrids			
Bluecrop (N)[b]	84 ± 1.0	91	304 ± 15	50.0	34.4	0.28
Brigitta Blue (N)	103 ± 1.7*	56	246 ± 5.4*	18.6	18.5	0.42
Duke (N)	173 ± 8.1#	64	274 ± 18	32.6	42.3	0.63
G-224 (N)	91 ± 1.7	39	249 ± 19	19.4	27.1	0.37
G-344 (S)	101 ± 1.5*	59	171 ± 12	25.9	30.4	0.59
Rubel (N)	269 ± 3.8	129	435 ± 1.1	49.6	74.6	0.62
Summit[c] (S)	73 ± 1.4	40	211 ± 13	28.0	30.6	0.35
Summit II[d] (S)	119 ± 1.5	100	369 ± 0.3	50.9	39.5	0.32
CVAC 1057.001	239 ± 4.5	267	507 ± 14	72.3	59.9	0.47
CVAC 23.001	322 ± 4.6	356	757 ± 37	96.8	107.3	0.43
CVAC 24.001	224 ± 1.2	314	381 ± 24	78.0	64.6	0.59
CVAC 25.001	303 ± 2.9	453	740 ± 13	58.8	83.5	0.41
CVAC 35.001	304 ± 6.4	368	624 ± 8.2	58.1	68.6	0.49
CVAC 45.001	279 ± 2.6	316	520 ± 4.1	65.5	77.6	0.54
CVAC 5.001	430 ± 5.3	311	868 ± 17	79.6	120.6	0.50
means	208 ± 78	198	444 ± 155	52.3	58.6	0.47
			V. membranaceum Douglas ex Torr.			
V. membranaceum	116 ± 4.3	247	423 ± 7.1	42.9	53.8	0.27
CVAC 370	131 ± 4.7	298	412 ± 5.3	52.0	54.2	0.32
CVAC 255.003	153 ± 1.1	137	269 ± 16	35.2	42.6	0.57
CVAC 255 bulk	110 ± 1.0	99	225 ± 5.1	26.8	27.9	0.49
CVAC 425	110 ± 0.5	165	347 ± 19	36.5	40.1	0.32
means	124 ± 13	189	335 ± 62	38.7	43.7	0.39
			V. myrtilloides Michx.			
CVAC 19.001	298 ± 1.5	530	656 ± 8.1	73.0	94.9	0.45
			V. ovalifolium Smith			
V. ovalifolium	266 ± 2.4	408	678 ± 4.6	48.0	80.4	0.39
			V. ovatum Pursh			
CVAC 329.001	336 ± 9.8	487	641 ± 4.8	86.0	100.2	0.52
CVAC 144.001	357 ± 3.0	447	842 ± 23	69.8	115.1	0.42
			V. parvifolium Smith			
CVAC 381	34 ± 1.0	179	228 ± 20	78.0	64.6	0.15
overall means	230 ± 89	239	521 ± 172	62.5	74.1	0.43

[a] Mean ± SEM ($n = 2$, unless: # for $n = 3$, or * for $n = 4$). [b] N = Northern Highbush; S = Southern Highbush. [c] Harvested 7/10/2000. [d] Harvested 8/24/2000.

Vaccinium. The red huckleberry, *V. parvifolium*, had the lowest ACY (34 mg ACY/100 g), yet its ORAC and FRAP values were higher than those of many other *Vaccinium* genotypes. *Vaccinium* samples ranged from 34 to 515 mg ACY/100 g; ORAC values in *Vaccinium* ranged from 19 to 131 μmol TE/g (**Table 1**).

Within highbush blueberry (*V. corymbosum* L.) seedlings and cultivars, ACY varied from 73 to 430 mg/100 g, compared to reported values of 25−495 mg/100 g (*7*), 93−235 mg/100 g (*11*), or 39−331 mg/100 g (*20*). Bilberry, *V. myrtillus*, was unavailable for our analyses.

Values of ACY, TPH, ORAC, and FRAP for the cultivated highbush blueberry, *V. corymbosum* cvs. Bluecrop, Duke, and Rubel, and *V. constablaei* × *ashei* hybrid cv. Little Giant, were consistent with those in other reports (*11*, *21*). Reported ORAC for Bluecrop′ was 17.0 (*11*) or 60.1 μmol TE/g (*21*); we report a value of 50.0 μmol TE/g. ORAC values for the blueberry

Table 2. Total Anthocyanin Content (ACY), Berry Size, Total Phenolics (TPH), Antioxidant Activity (ORAC and FRAP), and Total Anthocyanins/Phenolics in 37 *Rubus* Species and Cultivars

genotype	ACY mg/100 g[a]	berries/ 100 g	TPH mg/100 g[a]	ORAC μmol TE/g	FRAP μmol/g	ACY/ TPH
			Rubus species blackberries			
R. cyri Juz.	143 ± 3.8*	42	545 ± 14	46.2	71.4	0.26
R. georgicus Focke	89 ± 2.9	57	561 ± 8.2	41.6	96.3	0.16
R. insularis F. Aresch.	170 ± 3.5	27	472 ± 14	51.4	97.1	0.36
R.ursinus (Cham. & Schltdl.) G4-19	206 ± 1.0	87	678 ± 13	78.8	93.5	0.30
R.ursinus G4 bulk	211 ± 0.2	108	629 ± 7.2	60.4	79.8	0.34
means	164 ± 36	64.2	577 ± 56.1	55.7	87.6	0.28
			Rubus hybrid blackberries			
ORUS 1112-2 (Siskiyou × OSC 1717)	94 ± 0.3	13	412 ± 5.5	36.4	86.7	0.23
ORUS 1122-1 (Olallie × ORUS 728-3)	181 ± 3.7#	10	620 ± 10	56.7	80.4	0.29
ORUS 1316-1 (ORUS 817R-6 × ORUS 1122-1)	106 ± 1.5	12	381 ± 16	40.6	75.5	0.28
ORUS 1324-1 (ORUS 834-5 × ORUS 1045-14)	124 ± 0.1	11	458 ± 19	26.7	46.5	0.27
ORUS 1369-3 (ORUS 828-42 × ORUS 1122-1)	161 ± 1.7	14	454 ± 7.8	38.6	63.3	0.35
ORUS 1382-2 (ORUS 1117-11 × ORUS 728-3)	217 ± 9.0*	15	650 ± 25*	70.5	91.2	0.33
ORUS 1431-1 ((Black Douglass × Lumpy) × Walt)	130 ± 4.2*	18	609 ± 28*	53.5	93.3	0.21
ORUS 1439-1 (Black Douglass × (Long Black × Mono))	106 ± 0.2	17	381 ± 5.6	36.1	53.8	0.28
ORUS 1452-1 (Black Douglass × Kotata)	128 ± 0.1	21	481 ± 2.3	52.4	69.4	0.27
ORUS 1714 (Chester × Cherokee)	168 ± 3.1	26	428 ± 6.3	47.8	60.5	0.39
ORUS 1719A (*R. caucasicus* × Chester)	148 ± 0.6	35	641 ± 30	60.5	87.2	0.23
ORUS 1719F (*R. caucasicus* × Chester)	156 ± 2.0	26	395 ± 5.6	39.0	40.6	0.39
ORUS 1719H (*R. caucasicus* × Chester)	134 ± 1.4	24	560 ± 3.3	45.2	73.7	0.24
ORUS 1719K (*R. caucasicus* × Chester)	109 ± 1.1	34	441 ± 0.6	40.4	63.2	0.25
ORUS 1722 (*R. caucasicus* × Cherokee)	80 ± 0.1	26	355 ± 15	33.3	56.8	0.23
ORUS 1723 (*R. caucasicus* × Cherokee)	152 ± 0.0	37	433 ± 12	45.0	48.9	0.35
ORUS 1726-1 (*R. georgicus* × Cherokee)	143 ± 0.2	29	509 ± 11	70.6	92.7	0.28
ORUS 1880 (*R. georgicus* × Cherokee)	97 ± 0.5	12	394 ± 10	43.8	48.8	0.25
NZ 9128R-1	113 ± 0.6#	13	378 ± 4.0#	35.1	47.9	0.30
NZ 9351-4	169 ± 3.6	13	444 ± 1.4	51.2	64.7	0.38
NZ 9629R-1	215 ± 0.5	13	545 ± 5.8	52.7	78.0	0.39
Cherokee [erect]	123 ± 4.5	13	407 ± 20	37.9	58.7	0.30
Chester [semierect]	164 ± 1.1	15	361 ± 8.1	47.5	56.9	0.45
Marion [trailing]	230 ± 2.1	21	560 ± 5.3	69.5	98.1	0.41
Navaho [erect]	126 ± 2.5	17	304 ± 6.2	38.8	58.9	0.41
Siskiyou [trailing]	133 ± 3.7	11	543 ± 10	47.3	106.1	0.24
Triple Crown [semierect]	113 ± 3.9	10	275 ± 0.3	35.1	43.4	0.41
means	141 ± 27	18.7	460 ± 71.8	46.4	68.3	0.31
			Rubus species raspberries			
R. innominatus S. Moore	52 ± 0.6	64	126 ± 0.3	13.1	19.9	0.41
R. niveus Thunb.	230 ± 2.2	56	402 ± 0.7	45.2	69.4	0.57
			Rubus occidentalis L. and hybrid black raspberries			
Earlysweet	464 ± 7.8	74	897 ± 32	100.3	205.6	0.52
Jewel	607 ± 2.5	52	1079 ± 34	146.0	184.1	0.56
Munger	627 ± 8.3&	71	890 ± 30	104.6	169.1	0.70
means	566 ± 63	66	955 ± 76	117.0	186.3	0.60
overall means	179 ± 89	31	505 ± 127	52.4	79.2	0.34

[a] Mean ± SEM ($n = 2$, unless: # for $n = 3$, * for $n = 4$, or & for $n = 5$).

hybrid Little Giant′ have been reported as 25.5 (*11*) and 20.8 μmol TE/g (*20*); we report 24.6 μmol TE/g. Wild highbush (*V. corymbosum* L.) genotypes from North Carolina and Maine had much higher ACY, TPH, ORAC, and FRAP than did highbush cultivars (**Table 1**), indicating that this species has a full range of ACY and antioxidant capacity levels as judged by ORAC and FRAP and should not arbitrarily be thought of as lower than other blueberry species.

Rubus. The wild, red form *R. innominatus* from China had the lowest ACY, TPH, ORAC, and FRAP of the *Rubus* genotypes tested. Selected hybrid blackberry cultivars such as Triple Crown, Cherokee, Navaho, and Siskiyou had intermediate levels. Total ACY for blackberry samples ranged from 80 to 230 mg/100 g (**Table 2**). This compares to a range (70−201 mg/100 g) and a mean (137 mg/100 g) reported by Fan-Chiang (*22*) for 52 blackberry samples collected from the United States and international sources. Fan-Chiang used the same extraction and spectrophotometric procedures as we did. Marion, a complex that is predominantly *R. ursinus* (*22*) had 230 mg ACY/100 g, the highest of the blackberries we tested (**Table 2**). This compares to values of 144, 167, and 197 mg/100 g reported by Fan-Chiang (*23*) for the respective 1996, 1997, and 1998 seasons. Clearly, seasonal and maturity influences can have a marked effect on anthocyanin pigment content. Total *Rubus* samples ($n = 37$) ranged from 52 to 627 mg ACY/100 g; ORAC in *Rubus* ranged from 13 to 146 μmol TE/g (**Table 2**). Wang and Lin (*24*) report ORAC values for Chester Thornless and Triple Crown′ cultivar blackberries as 22.2 and 20.3, whereas we report 35.1 and 47.5 μmol TE/g. As reported by the Oregon Raspberry and Blackberry Commission (*25*), ORAC values for Marion′ blackberry and Munger′ black raspberry cultivars (*R. occidentalis*) were 28 and 77, respectively; we report 69.5 and 104.6 μmol TE/g for these. The black raspberry cv. Jewel (*R. occidentalis*) had the highest ORAC value, 146 μmol TE/g, observed over all 108 samples analyzed in this study.

Ribes. The gooseberry *R. uva-crispa* L. cv. Captivator had the lowest ACY of any genotype of the study (**Table 3**). Extracts from the gooseberries *R. uva-crispa* cv. OT 126 and *R.*

Table 3. Total Anthocyanin Content (ACY), Berry Size, Total Phenolics (TPH), Antioxidant Activity (ORAC and FRAP), and Total Anthocyanins/Phenolics in 40 *Ribes* Genotypes

genotype	ACY mg/100 g[a]	berries/100 g	TPH mg/100 g[a]	ORAC μmol TE/g	FRAP μmol/g	ACY/TPH
			R. uva-crispa L. (= *R. grossularia* L.) Gooseberry			
Captivator	14 ± 0.4	27	191 ± 17	17.0	25.2	0.07
			R. x nidigrolaria Bauer Jostaberries			
ORUS 10	43 ± 4.0	51	301 ± 21	26.7	45.9	0.14
ORUS 9	71 ± 1.2	47	304 ± 13	22.0	31.8	0.23
ORUS 7	78 ± 2.7	52	302 ± 12	29.0	35.9	0.26
ORUS 6	89 ± 0.4	45	302 ± 5	28.5	38.7	0.29
ORUS 8	89 ± 2.4	39	338 ± 12	34.3	42.6	0.26
means	74 ±	47	309 ± 11	28.1	39.0	0.24
			R. nigrum L. and hybrids			
Alagan	169 ± 6.0	121	694 ± 33	92.0	106.7	0.24
Baldwin	186 ± 2.0*	99	807 ± 4.7	54.0	64.6	0.23
Beloruskaja sladkaja	157 ± 0.8*	116	910 ± 7.9	51.3	101.2	0.17
Ben Conan	162 ± 4.2	66	498 ± 15	47.4	74.1	0.33
Ben Lomond	261 ± 5.2*	91	933 ± 36*	45.5	88.8	0.28
Ben Nevis	252 ± 6.2*	60	815 ± 25	44.9	77.1	0.31
Blackdown	216 ± 1.0*	128	812 ± 33*	38.0	78.4	0.27
Boskoop	240 ± 2.7*	125	796 ± 3.6	69.2	92.8	0.30
Consort	411 ± 12*	176	1342 ± 28	93.1	114.0	0.31
Coronet	231 ± 0.6	178	704 ± 4.0	48.4	91.6	0.33
Crusader	319 ± 0.6	158	727 ± 10	50.5	89.4	0.44
Dosz Siberjoczk	259 ± 2.0	201	928 ± 7.7	44.9	79.0	0.28
Hystawneznaja	156 ± 1.2*	107	791 ± 11	49.2	89.4	0.20
Kantata	180 ± 4.8*	87	552 ± 5.6	42.5	62.8	0.33
Kantata 50	207 ± 2.4	115	551 ± 30	36.9	67.2	0.38
Kirovchanka	263 ± 10	118	675 ± 31	65.5	118.2	0.39
Kosmiczeskaja	221 ± 16	88	763 ± 27	62.2	108.7	0.29
Minaj Smyriov	158 ± 1.2*	126	808 ± 18	44.5	84.1	0.20
Neosyspujastaja	220 ± 7.1	165	554 ± 24	52.7	67.3	0.40
Nikkala XI	257 ± 0.8	105	650 ± 14	62.7	99.4	0.40
Ojebyn	165 ± 3.5*	102	830 ± 33[#]	54.9	61.5	0.20
Pinot Deboir	208 ± 2.7	132	710 ± 13	47.7	88.8	0.29
Polar	213 ± 3.1*	132	752 ± 19	59.4	95.5	0.28
Risager	181 ± 5.8	119	797 ± 24	54.4	108.7	0.23
Silvergieters Zwarte	346 ± 10[$]	130	1053 ± 16	65.1	105.8	0.33
Slitsa	128 ± 1.7	109	632 ± 0.8	64.0	62.4	0.20
Strata	298 ± 0.6	134	883 ± 23	57.5	100.3	0.34
Titania	281 ± 3.9*	92	890 ± 28*	54.7	100.4	0.32
Tsema	180 ± 6.0	90	800 ± 8.7	52.1	102.4	0.23
Tunnaja	275 ± 4.9*	107	900 ± 33	77.7	99.0	0.31
Wassil	199 ± 6.9	94	742 ± 4.1	58.9	95.5	0.27
Willoughby	275 ± 3.9*	131	1122 ± 26*	73.4	145.9	0.25
means	229 ± 44	117	799 ± 121	57.1	92.0	0.29
			R. odoratum Wendl.			
Crandall	273 ± 1.0*	74	958 ± 33	68.0	107.8	0.28
			R. valdivianum Phil.			
R. valdivianum	358 ± 1.5*	907	1790 ± 59*	115.9	219.3	0.20
overall means	207 ± 61	126	748 ± 209	53.9	86.7	0.28

[a] Mean ± SEM ($n = 2$, unless: [#] for $n = 3$, * for $n = 4$, or [$] for $n = 6$).

oxyacanthoides L. cv. Jahn's Prairie displayed anthocyanin−phenolic degradation, even when the acetone extract was heated to boiling as recommended for samples with high polyphenol oxidase activity (*15*). Thus, ACY, TPH, and antioxidant capacities were not measured for these gooseberries.

Ribes × *nidigrolaria* ORUS selections ACY values were higher than those for Captivator, but were lower than *R. nigrum* cultivars (**Table 3**). Our ACY values for *R. nigrum* cvs. Ojebyn, Ben Lomond, and Titania were lower than those reported from Poland (*26*). Our *Ribes* samples ranged from 14 to 411 mg ACY/100 g; Banaszczyk and Pluta (*26*) observed *R. nigrum* cv. Ben Alder to have 467 mg ACY/100 g. Our ORAC values in *Ribes* ranged from 17 to 116 μmol TE/g (**Table 3**).

Antioxidant Capacity: ORAC and FRAP. The range of our ORAC values may be higher than those previously reported (*20*, *21*, *24*) because of the following:

(1) The goal of our extraction method (*15*), is to determine the maximal amount of ACY, TPH, and antioxidant capacity that exist in a plant sample. The liquid nitrogen milling and acetone extraction procedures minimize enzyme activity and oxidation, especially after thawing or grinding has damaged the fruit. Some published extraction methods contain steps in which berries are allowed to thaw or incubate at room temperature before steps are taken to limit enzyme activity and oxidation. *Rubus* samples are especially susceptible to berry damage during picking, transportation, and thawing before extraction. A portion of the relatively large *Rubus* seeds were also ground during high-speed milling and thus contributed to TPH and antioxidant capacity.

(2) Berries were picked in small lots and placed on ice in the field immediately, then frozen the same day, usually within 2 h.

Table 4. Correlation Coefficients (*r*) of Size, Total Anthocyanins (ACY), Total Phenolics (TPH), and Antioxidant Capacity (ORAC and FRAP)[a]

	Size:ACY	ACY:TPH	ACY:ORAC	ACY:FRAP	TPH:ORAC	TPH:FRAP
Vaccinium ($n = 31$)	0.55**	0.93**	0.73**	0.88**	0.79**	0.93**
V. corymbosum ($n = 15$)	0.84**	0.93**	0.78**	0.96**	0.81**	0.94**
Vaccinium, 8 species ($n = 16$)	0.29	0.93**	0.69**	0.85**	0.76**	0.92**
Rubus ($n = 37$)	0.45**	0.83**	0.90**	0.85**	0.92**	0.90**
Rubus hybrids (blackberries) ($n = 27$)	0.003	0.57**	0.70**	0.38*	0.73**	0.75**
Ribes ($n = 40$)	0.46**	0.82**	0.71**	0.74**	0.81**	0.88**
Ribes nigrum ($n = 32$)	0.41*	0.63**	0.38*	0.46**	0.44**	0.55**
all samples ($n = 108$)	0.43**	0.73**	0.79**	0.80**	0.84**	0.84**

[a] * = $p < 0.05$; ** = $p < 0.005$.

(3) The highest ORAC values observed in the *Vaccinium* population belonged to wild plants and seedlings, not the cultivars.

(4) Significant location and year-to-year effects on antioxidant activity have been reported in nine highbush blueberry cultivars (*13*). ACY levels of eight of the hybrid blackberries in this study ranged from 62 to 165% of the values reported by Fan-Chiang (*23*). The same extraction method was used in both studies; but the fruit samples of Fan-Chiang (*23*) were collected during the 1996, 1997, or 1998 growing seasons.

Correlations. *ACY and TPH*. Anthocyanins comprise a significant fraction of the TPH in *Vaccinium* and *Rubus* (ACY/TPH ratios, **Tables 1**, **2**, and **3**). The ACY/TPH ratio was lowest (0.28) in *Ribes*, not due to less ACY than the other two genera but to markedly higher TPH. Correlations of ACY to TPH were highest for *Vaccinium*, but were significant ($p = 0.005$) for all three genera (**Table 4**). Though ACY may indicate TPH, the range observed in ACY/TPH ratios (**Tables 1**−**3**) precludes prediction of ACY from TPH and vice versa, for a single genotype.

Influence of Anthocyanin and Phenolic Levels on Antioxidant Capacity. ACY averaged 34% of TPH in the 108 samples we studied, thereby contributing significantly to the overall antioxidant capacity (*4*, *6*). Ascorbate levels contributed <1% to the total antioxidant capacity in highbush and lowbush blueberries (*21*) and <10% in a diversity of *Rubus* species (*12*), and are not reported for the samples in this study. Correlation coefficients between ACY or TPH and ORAC or FRAP (**Table 4**) are significant across the *Vaccinium*, *Rubus*, and *Ribes* sample sets and their subsets. ACY and TPH correlated highly with ORAC values, though in every case ($n = 7$) TPH:ORAC correlations were slightly higher than ACY:ORAC correlations. TPH:FRAP correlations were higher in six of seven cases compared to ACY:FRAP correlations (**Table 4**).

Rubus innominatus (**Table 2**) and Captivator cv. gooseberry (**Table 3**) are lowest in ACY and TPH within their respective genus, leading to the lowest ORAC and FRAP values as well. Despite the lowest ACY and low TPH, the red-fruited *V. parvifolium* (**Table 1**) has average ORAC and FRAP values for the 30 *Vaccinium* genotypes tested. Deighton et al. (*12*) reported an extreme case of decoupled ACY and FRAP when the yellow-fruited *Rubus lambertianus* Ser., devoid of ACY but high in TPH, displayed a FRAP value higher than several other red or black fruited *Rubus* species, many of which contained significant ACY. We primarily chose from among the most highly pigmented small fruits available, which may have influenced the significant correlations observed between ACY and TPH, as well as between ACY and total antioxidant capacities as measured by ORAC or FRAP.

The correlation of TPH:ORAC for *Ribes nigrum* ($r = 0.44$) is much lower than that for *Vaccinium* and *Rubus* species (**Table 4**). This low *Ribes* TPH:ORAC correlation may be due to the high ascorbic acid content of black currant fruit (*26*) that could contribute to ORAC but could not be measured by TPH. This could also account, in part, for the regression line slope differences in **Figure 1**.

The high correlation between ORAC and FRAP for all 108 samples ($r = 0.84$) suggests that either of these two measurements have validity for determining antioxidant activity with these fruits. The mechanisms of these two assays are distinct: the ability to trap a free radical with ORAC vs ferric ion reduction with FRAP. It should be pointed out, however, that in vitro measurement of antioxidant capacity may or may not reflect what happens in vivo. Very little is known about the absorption and metabolism of these compounds.

Regression Analysis. Scatterplots of TPH vs antioxidant capacity (as FRAP) are presented in **Figure 1** for *Vaccinium*, *Rubus*, or *Ribes* genotypes. The spread of values within each genus can be observed, as well as two clusters of highest FRAP values: *Vaccinium ashei* Reade (rabbiteye blueberries) and *Rubus occidentalis* L. (black raspberries). See **Table 1** (*Vaccinium*) and **Table 2** (*Rubus*) for individual values. *Ribes valdivianum* is not included in **Figure 1** *Ribes*, as the TPH for this species is 63% more than the next highest value, *Ribes nigrum* cv. Willoughby (**Table 3**).

Berry Size Correlations. The highest correlation ($r = 0.82$, $p = 0.005$) between berry size and ACY (**Table 4**) was observed for highbush blueberry genotypes ($n = 15$). In highbush blueberries, pigments reside exclusively in the skin. For a given volume of fruit, the amount of skin or surface area increases as berry size decreases; leading to the general observation that smaller highbush blueberries contain more anthocyanins per unit volume (*11*). Yet, ACY and berry size was not correlated ($r = 0.29$; $n = 16$) across eight other *Vaccinium* species and hybrids (**Table 4**). Perhaps if a range of clones differing in berry size *within* each species were examined, a significant correlation with total ACY could be observed. The relatively large berries of CVAC 1161 and CVAC 1170, rabbiteye blueberries (*V. ashei* Reade), contained the highest ACY within the 31 *Vaccinium* samples (**Table 1**). Therefore, larger berries of one *Vaccinium* species may still contain more ACY as compared to smaller berries of a different species. We made no attempts to compare blueberry skin thickness or berry shape, other potential sources of divergent ACY levels, though we do note that the large-fruited highbush cultivars had less spherical berries than the smaller-fruited highbush species material in our study.

The lack of a berry size-to-ACY correlation ($r = 0.003$, $n = 27$) for blackberry hybrids (**Table 4**) was anticipated, as druplet skin is quite thin and flesh is very dark. The current trend in *Rubus* breeding toward larger berry size appears to have had little effect on ACY, for the full range of ACY is encompassed both by cultivars and advanced breeding selections (**Table 2**).

ACY in black currant cultivars (*Ribes nigrum*, $n = 32$) was significantly affected by berry size ($r = 0.41$, $p = 0.05$), but to

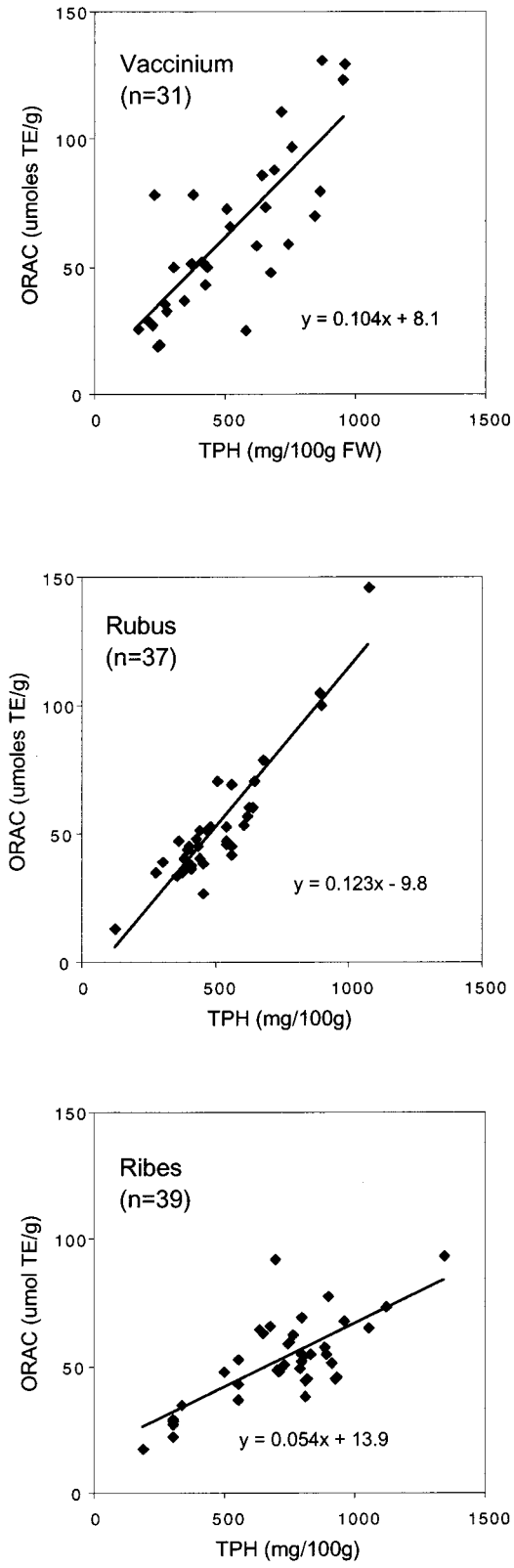

Figure 1. Influence of total phenolic content (TPH) on antioxidant capacity (as ORAC) of *Vaccinium*, *Rubus, and Ribes* samples. The *Ribes* regression ($n = 39$) does not include *R. valdivianum*.

a much lesser degree than in highbush blueberries. Black currants have undergone selection for hundreds of years (*27*), whereas cultivars of highbush blueberries were developed much more recently (*28*). *Ribes valdivianum*, a black currant native to Chile, is an extreme example of the relationship between fruit size and ACY within *Ribes* (**Table 3**). Even at 907 berries/100 g, ACY of *R. valdivianum* was comparable to that of *R. nigrum* cultivars 5 to 8 times as large. Only one gooseberry, cv. Captivator (*R. uva-crispa* L.) was evaluated (**Table 3**).

Anthocyanin Levels and Flesh Color. A few samples we examined contained dark skin and dark flesh: the ORUS 6-10 series of *Ribes* × *nidigrolaria* Bauer (**Table 3**), and the *V. membranaceum* Douglas ex Torrey selections (**Table 1**). Surprisingly, these were among the lowest in ACY, as compared to that of fruits with nonpigmented flesh. We note that the ACY extraction method used in this study removes virtually all the skin pigments, whereas after commercial pressing for blueberry juice, substantial ACY remains in the skin-rich press-cake (*29*). Thus for certain food processing applications, dark-fleshed *Vaccinium* or *Ribes* berries may still represent a valued source of ACY.

Maturity Effects. Summit, a Southern highbush blueberry cultivar, is represented twice (**Table 1**), as "Summit" and "Summit II". Ripe fruit was picked from the same field very early (July 10) and very late (August 24) in the harvest season for this cultivar. For ACY, TPH, ORAC, and FRAP, increases of 163%, 175%, 182%, and 129% occurred during the growing season. Both maturity (*11*) and postharvest storage (*21*, *30*) have been reported to increase *Vaccinium* ACY levels. The Summit ACY/TPH ratios remained nearly constant over time, 0.345 and 0.323, perhaps due to a concomitant rise in ACY and TPH. A significant 250% decrease in berry size was observed over time, which altered the skin to whole fruit ratio between the two samples. In *Vaccinium*, changes in berry size and thus skin area per berry should be considered in studies of maturity effects on ACY or antioxidant capacity.

Implications for Fruit Selection and Breeding. *Vaccinium*. The upper range of ACY (**Table 1**) for seedling highbush blueberries such as CVAC 5.001 and CVAC 23.001 indicates that cultivars with increased ACY, TPH, and antioxidant capacity may still be selected or developed from wild *V. corymbosum* L. material. Four rabbiteye selections had among the highest antioxidant capacities of all *Vaccinium* samples examined ($n = 31$) reflecting very high levels of both ACY and TPH (**Figure 1** *Vaccinium*). Bluegem, the lone *V. ashei* cultivar tested, had an ORAC value more than twice that of any highbush (*V. corymbosum* L.) cultivar tested ($n = 7$). Magee (*30*) reported a similar 2-fold level of ACY in two rabbiteye cultivars compared to three southern highbush cultivars, whereas Prior et al. (*11*) observed equivalent ORAC means for several *V. ashei* vs *V. corymbosum* cultivars. The two *Vaccinium ovatum* ("evergreen huckleberry") selections we tested were also quite high in ACY, TPH, and antioxidant capacity.

Rubus. Of the 32 blackberries tested, Marion cv. had the highest ACY levels and was among the highest in ORAC and FRAP values (**Table 2**). Yet, the full range of TPH and FRAP diversity is displayed among hybrid blackberry cultivars, recent crosses, and advanced selections (**Table 2**, **Figure 1** *Rubus*). There is a significant gap reported (**Table 2**, **Figure 1** *Rubus*) between *R. ursinus* hybrid blackberry and black raspberry (*R. occidentalis*) values; Earlysweet, Jewel and Munger black raspberries cultivars have phytochemical and antioxidant values well above those of all other *Rubus* in this study. Deighton et al. (*12*) found the blackberry *R. caucasicus* Focke with the highest antioxidant capacity of 18 selections from 12 *Rubus* species, and posited *R. caucasicus* as a donor to increase antioxidant capacity in blackberry hybrids. *R. caucasicus* fruit was unavailable for this study, but six *R. caucasicus* × Chester Thornless or Cherokee hybrids were examined (**Table 2**). One of these, ORUS1719A (*R. caucasicus* × Chester Thornless) was

high in TPH and antioxidant capacity as compared to other blackberry hybrids, confirming the potential value of *R. caucasicus* crosses, as predicted by Deighton et al. (*12*).

Ribes. Compared to highbush blueberries, blackcurrant (*R. nigrum* L.) cultivars displayed a lower correlation ($r = 0.41$) between size and ACY levels (**Table 4**). In addition, the correlation ($r = 0.30$) between size and TPH in blackcurrants ($n = 32$) was not significant. Larger fruited *R. nigrum* cultivars might be selected without concomitant lowering of ACY or TPH.

CONCLUSION

Our survey of small fruit germplasm for ACY, TPH, and antioxidant capacity shows distinctives in each of three genera examined, and confirms each as an excellent source of dietary phytochemicals.

ABBREVIATIONS USED

ACY, total anthocyanin content; TPH, total phenolic content; ORAC, oxygen radical absorbing capacity; FRAP, ferric reducing antioxidant power; Trolox, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; TE, Trolox equivalents.

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LITERATURE CITED

(1) Hertog, M. G. L.; Hollman, P. C. H.; Katan, M. B. Content of potentially anti-carcinogenic flavonoids of 28 vegetables and 9 fruits commonly consumed in The Netherlands. *J. Agric. Food Chem.* **1993**, *40*, 2379−2383.

(2) Hertog, M. G. L.; Kromhout, D.; Aravanis, C.; Blackburn, H.; Buzina, R.; Fidanza, F.; Ciampaoli, S.; Jansen, A.; Menotti, A.; Nedeljkovic, S.; Pekkarinen, M.; Simic, B. S.; Toshima, H.; Feskens, E. J. M.; Hollman, P. C. H.; Katan, M. B. Flavonoid intake and long-term risk of coronary heart disease and cancer in the seven countries study. *Arch. Int. Med.* **1995**, *155*, 381−386.

(3) Ames, B. M.; Shigena, M. K.; Hagen, T. M. Oxidants, antioxidants and the degenerative diseases of aging. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 7915−7922.

(4) Rice-Evans, C. A.; Miller, N. J.; Paganga, G. Structure−antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biol. Med.* **1996**, *20*, 933−956.

(5) Cao, G.; Sofic, E.; Prior, R. L. Antioxidant and prooxidant behavior of flavonoids: Structure−activity relationships. *Free Radical Biol. Med.* **1997**, *22*, 749−760.

(6) Wang, H.; Cao, G.; Prior, R. L. Oxygen radical absorbing capacity of anthocyanins. *J. Agric. Food Chem.* **1997**, *45*, 304−309.

(7) Mazza, G.; Miniati, E. *Anthocyanins in Fruits, Vegetables and Grains*; CRC Press: Boca Raton, FL, 1993.

(8) Wang, H.; Cao, G.; Prior, R. L. Total antioxidant capacity of fruits. *J. Agric. Food Chem.* **1996**, *44*, 701−705.

(9) Kähkönen, M. J.; Hopia, A. I.; Vuorela, H. J.; Rauha, J.; Pihlaja, K.; Kujala, T. S.; Heinonen, M. Antioxidant activity of plant extracts containing phenolic compounds. *J. Agric. Food Chem.* **1999**, *47*, 3954−3962.

(10) Fukomoto, L. R.; Mazza, G. Assessing antioxidant and prooxidant activities of phenolic compounds. *J. Agric. Food Chem.* **2000**, *48*, 3597−3604.

(11) Prior, R. L.; Cao, G.; Martin, A.; Sofic, E.; McEwan, J.; O'Brien, C.; Lischner, N.; Ehlenfeldt, M.; Kalt, W.; Krewer, G.; Mainland, C. M. Antioxidant capacity as influenced by total phenolic and anthocyanin content, maturity and variety of *Vaccinium* species. *J. Agric. Food Chem.* **1998**, *46*, 2686−2693.

(12) Deighton, N.; Brennan, R.; Finn, C.; Davies, H. V. Antioxidant properties of domesticated and wild *Rubus* species. *J. Sci. Food Agric.* **2000**, *80*, 1307−1313.

(13) Connor, A. M.; Luby, J. J.; Tong, C. B. S.; Finn, C. E.; Hancock, J. F. Genotypic and environmental variation in antioxidant activity, total phenolic content and anthocyanin content among blueberry cultivars. *J. Am. Soc. Hort. Sci.* **2002**, *127*, in press.

(14) Heiberg, N.; Mage, F.; Haffner, K. Chemical composition of 10 blackcurrant (*Ribes nigrum* L.) cultivars. *Acta Agric. Scand.* **1992**, *42*, 251−254.

(15) Rodriguez-Saona, L. E.; Wrolstad, R. E. Unit F1.1.1-11: Anthocyanins. Extraction, Isolation and Purification of Anthocyanins. In *Current Protocols in Food Analytical Chemistry*; Wrolstad, R. E., Ed.; John Wiley & Sons: New York, 2001.

(16) Giusti, M. M.; Wrolstad, R. E. Unit F1.2.1-13. Anthocyanins. Characterization and Measurement with UV−Visible Spectroscopy. In *Current Protocols in Food Analytical Chemistry*; Wrolstad, R. E., Ed.; John Wiley & Sons: New York, 2001.

(17) Singleton, V. L.; Rossi, J. A. Colorimetry of total phenolics with phosphomolybdic−phosphotungstic acid reagents. *Am. J. Enol. Vitic.* **1965**, *16*, 144−158.

(18) Cao, G.; Alessio, H. M.; Culter, R. G. Oxygen-radical absorbance capacity assay for antioxidants. *Free Radical Biol. Med.* **1993**, *14*, 303−311.

(19) Benzie, I. F. F.; Strain, J. J. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Anal. Biochem.* **1996**, *239*, 70−76.

(20) Ehlenfeldt, M. K.; Prior, R. L. Oxygen radical absorbance capacity (ORAC) and phenolic and anthocyanin concentrations in fruit and leaf tissues of highbush blueberry. *J. Agric. Food Chem.* **2001**, *49*, 2222−2227.

(21) Kalt, W.; Forney, C. F.; Martin, A.; Prior, R. L. Antioxidant capacity, vitamin C, phenolics, and anthocyanins after fresh storage of small fruits. *J. Agric. Food Chem.* **1999**, *47*, 4638−4644.

(22) Finn, C.; Strik, B. C.; Lawrence, R. J. Marion trailing blackberry. *Fruit Var. J.* **1997**, *51*, 130−132.

(23) Fan-Chiang, H.-J. Anthocyanin pigment, nonvolatile acid and sugar composition of blackberries. M.S. Thesis, Oregon State University, Corvallis, OR, December 14, 1999.

(24) Wang, S. Y.; Lin, H. Antioxidant activity in fruits and leaves of blackberry, raspberry, and strawberry varies with cultivar and developmental stage. *J. Agric. Food Chem.* **2000**, *48*, 140−146.

(25) ORAC Values. In *Oregon Raspberry and Blackberry Commission, Nutraceuticals*. http://www.oregon-berries.com; accessed July 27, 2001.

(26) Banaszczyk, J.; Pluta, S. Quality characteristics of fruits of new black currant selections released at Skierniewice. *J. Fruit Ornam. Plant Res.* **1997**, *5*, 13−17.

(27) Brennan, R. Currants and gooseberries. In *Fruit Breeding Volume II. Vine and Small Fruit Crops*; Janick, J., Moore, J. N., Eds.; John Wiley & Sons: New York, 1996; pp 191−295.

(28) Coville, F. V. Improving the wild blueberry. In *Yearbook of Agriculture, 1937*; U.S. Department of Agriculture, U.S. Government Printing Office: Washington, DC, 1937; pp 559−574.

(29) Skrede, G.; Wrolstad, R. E.; Durst, R. W. Changes in anthocyanins and polyphenolics during juice processing of highbush blueberries (*Vaccinium corymbosum* L.) *J. Food Sci.* **2000**, *65*, 357−364.

(30) Magee, J. B. Storage quality evaluation of southern highbush blueberry cultivars Jubilee, Magnolia and Pearl River. *Fruit Varieties J.* **1999**, *53*, 10−15.

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