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Phenolic Compounds and Antioxidant Capacity of Georgia-Grown Blueberries and Blackberries

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Blueberries and blackberries grown at various locations in Georgia were collected and analyzed for flavonoids, total anthocyanins, total polyphenols, and Trolox-equivalent antioxidant capacity (TEAC). Each sample was analyzed for phenolic acids (gallic acid, *p*-hydroxybenzoic acid, caffeic acid, ferulic acid, and ellagic acid) and flavonoids (catechin, epicatechin, myricetin, quercetin, and kaempferol). A high-performance liquid chromatographic (HPLC) method with photodiode array detection was used for analysis. Compounds were analyzed as aglycons after acid hydrolysis with 1.2 M HCl. Identification of each compound was based on retention time and UV spectra by comparison with pure commercial standards. Phenolic acids ranged from 0.19 to 258.90 mg/100 g fresh weight (FW), and flavonoids ranged from 2.50 to 387.48 mg/100 g FW. Total polyphenols ranged from 261.95 to 929.62 mg/100 g FW, and total anthocyanins ranged from 12.70 to 197.34 mg/100 g FW. TEAC values varied from 8.11 to a maximum of 38.29 μM/g FW. A linear relationship was observed between TEAC values and total polyphenols or total anthocyanins. The data indicate that blueberries and blackberries are rich sources of antioxidants.

KEYWORDS: Anthocyanins; antioxidant capacity; blackberries; blueberries; flavonoids; HPLC diode-array; phenolic acids; total polyphenols

INTRODUCTION

Phenolics are naturally occurring secondary metabolites from plants. They are present in fruits, vegetables, leaves, nuts, seeds, flowers, and barks. These compounds are an integral part of the human diet and are also taken intentionally as medicinal preparations. Since ancient times, plant preparations have been used by man to deal with common health problems. However, the importance of these compounds as health-promoting and disease-preventing substances has recently been realized through scientific investigations. Phenolic compounds are considered as nonnutrient biologically active compounds (*1*). The functionality of these compounds is expressed through their action as an inhibitor or an activator for a large variety of mammalian enzyme systems, and as metal chelators and scavenger of free oxygen radicals (*2*−*4*). Oxygen free radicals are involved in many pathological conditions such as atherosclerosis, cancer, and chronic inflammation (*5*). Phenolics interfere with the pathways that regulate cell division and proliferation, platelet aggregation, detoxification, and inflammatory and immune response (*6*). Among these phenolic substances, flavonoids, and in particular anthocyanins, are of interest because of their high occurrence in foods, especially in fruits, vegetables, and green leafy vegetables including green tea. Flavonoids are known to reduce coronary heart disease (*7*), and they have anticancer (*8*, *9*) and antioxidant properties (*10*).

In view of growing interest in these compounds, there is a need to identify and quantify these important compounds in fruits and vegetables. Some of the compounds are present in many fruits but others are specific for a particular kind of fruit or vegetable. Furthermore, within the same fruit type, the growing season, variety, environmental and climatic conditions, plant disease, soil type, geographic locations, and even maturity, seem to influence the concentration of phenolic compounds.

Because blueberries are grown in large scale in Georgia and they are currently being promoted as a rich source of antioxidants, the present work focused on further characterization of berries grown in Georgia as possible sources of phenolics for functional foods application. We analyzed different varieties of blueberries and blackberries for total anthocyanins, total polyphenols, and Trolox-equivalent antioxidant capacity. The phenolics analyzed were gallic acid, *p*-hydroxybenzoic acid, caffeic acid, *p*-coumaric acid, ferulic acid, ellagic acid, (+)-catechin, (−)-epicatechin, myricetin, quercetin, and kaempferol.

MATERIALS AND METHODS

Chemicals. Pure standards were purchased from Sigma (St. Louis, MO) and Fluka (Milwaukee, WI). Standards were dissolved in methanol as follows: gallic acid, 2.1 mg; *p*-hydroxybenzoic acid, 2.1 mg; (+)-

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Table 1. Solvent Gradient Elution Program

time (min)	A 1% formic acid in 70:30 water/methanol (%)	B methanol (%)	C 1% formic acid in water (%)	flow (mL/min)
0.00	0.0	0.0	100	1.3
5.00	0.0	0.0	100	1.3
5.01	50.0	0.0	50.0	1.3
10.00	85.0	15.0	0.0	1.0
10.01	50.0	5.0	45.0	1.0
20.00	85.0	15.0	0.0	1.0
25.00	85.0	15.0	0.0	1.0
60.00	45.0	55.0	0.0	1.0
60.01	0.0	100.0	0.0	1.0
65.00	0.0	100.0	0.0	1.0
65.01	50.0	0.0	50.0	1.0
75.00	50.0	0.0	50.0	1.0

catechin, 2.5 mg; caffeic acid, 2 mg; (−)-epicatechin, 2.9 mg; *p*-coumaric acid, 2.3 mg; ferulic acid, 2.4 mg; ellagic acid, 2.4 mg; myricetin, 2.3 mg; quercetin, 2.1 mg; and kaempferol 2.2 mg, all per 10 mL. Working solutions were prepared each day by appropriate dilution with methanol. 2,2′-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), Trolox (6-hydroxy-2,5,7,8-tetramethychroman-2-carboxylic acid), and ascorbic acid were purchased from Fluka (Milwaukee, WI). Methanol and water (HPLC grade), formic acid, and hydrochloric acid (analytical grade) were purchased from Fisher Scientific (Norcross, GA). Ascorbic acid was procured from BASF Corporation (Parsippany, NJ).

Sample Collection. Blueberry and blackberry samples were collected from Chula, Alapaha, and Attapulgus (Georgia) in June 2000. Varieties of berries collected were rabbiteye blueberries (*Vaccinium ashei* Reade) cultivars: Austin, Brightblue, Brightwell, Climax (Alapaha), Climax (irrigated, Attapulgus), Climax (nonirrigated, Attapulgus, drought-strickened), FL 80-11, Premier, FL 81-156, T 460, Tifblue, and Woodard; Southern highbush blueberries (*Vaccinium corymbosum L.* Hybrids) cultivars: FL 86-19, TH 161, TH 440, TH 442, and Sharpblue; and blackberries (*Rubus L.*) cultivars: Choctaw and Kiowa. All cultivars were grown with irrigation or under conditions of adequate rainfall, except the nonirrigated Climax which were drought strickened. Samples were frozen, transported to the University of Georgia, and stored at −80 °C for further use.

Extraction and Hydrolysis. A 10-g portion of frozen whole fruit sample was ground to paste with mortar and pestle in the presence of 100 mg of ascorbic acid, 500 mg of washed sand, and 10 mL of 6 M HCl. Volume was made to 50 mL with methanol (final concentration of 1.2 M HCl). The flask was wrapped with aluminum foil and flushed with nitrogen for 5 min. The deoxygenated sample was refluxed at 95 °C for 2 h to hydrolyze the flavonoid glycosides to aglycons. The hydrolyzed sample was cooled in dark and filtered through a 0.2-micron syringe nylon filter. A 20-μL aliquot of filtered sample was injected into the HPLC for analysis.

HPLC Analysis. HPLC was performed with a Hewlett-Packard (Avondale, PA), model 1090 liquid chromatograph with quaternary pumps and a diode array UV-visible detector (*11*−*13*) coupled to a HP ChemStation. A Phenomenex (Torrance, CA) Prodigy 5-μ, ODS-2, RP C_{18} column (250 × 4.6 mm) protected by guard column was the stationary phase. Gradient of mobile phase (A) water/methanol (70:30 vol/vol) with 1% formic acid, (B) methanol, and (C) 1% formic acid in water with a flow rate of 1−1.3 mL/min was used as shown in **Table 1**. UV spectra were recorded from 220 to 450 nm at a rate of 1.00 spectrum/1.28 s and a resolution of 2 nm with a bandwidth of 4 nm and reference wavelength in off mode.

Blueberries have been analyzed by HPLC with photodiode array detection (*11*−*13*) using a C_{18} reverse-phase column in acidic pH. However, our attempt to follow those methods resulted in an unsatisfactory performance due to baseline drift. To improve the separation of these compounds in berries we modified the method of Justesen et al. (*14*) as described in **Table 1**. Separation of the following compounds occurred in the order listed: gallic acid, catechin, *p*-hydroxybenzoic acid, epicatechin, caffeic acid, *p*-coumaric acid, ferulic acid, ellagic acid, myricetin, quercetin, and kaempferol. Satisfactory separation was achieved and the separation of epicatechin and caffeic acid was much closer than that of any other compounds. The HPLC chromatogram of separated standard compounds and a blueberry sample is shown in **Figure 1**. However, the presence of many other unknown compounds in the real sample made the chromatogram too crowded; but the unwanted peaks could be selectively suppressed by scanning at specific predetermined wavelengths of 260, 280, 320, and 360 nm. Use of narrower bandwidth of 4 nm in each signal improved the peak sharpness.

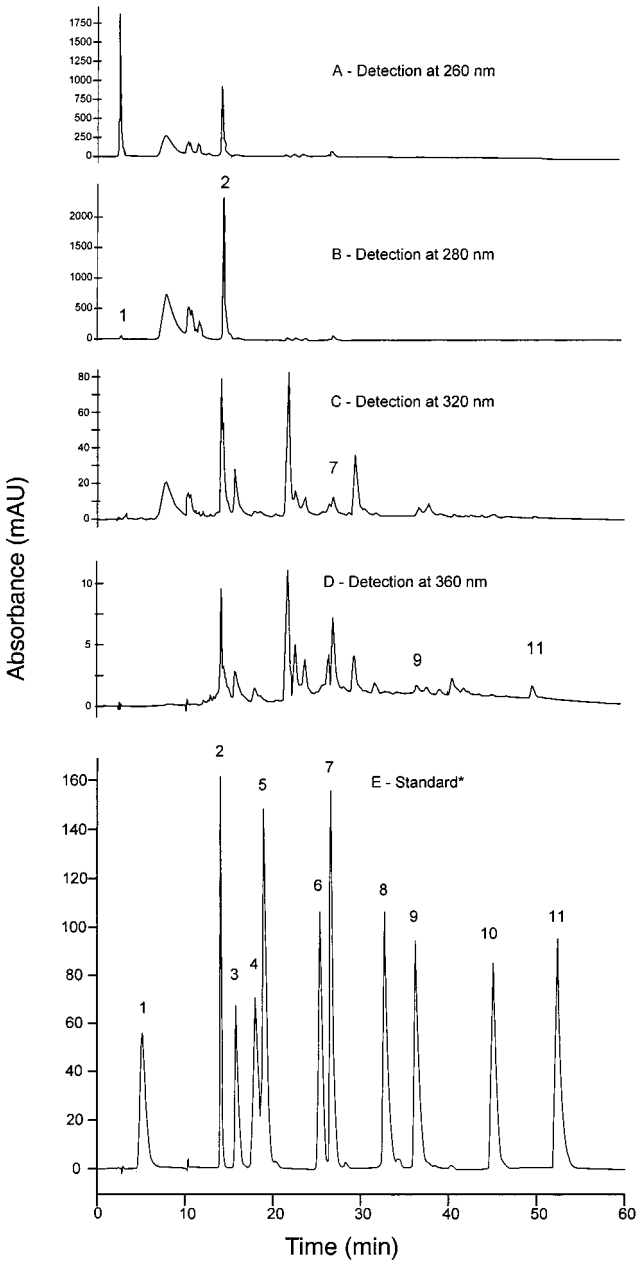

Figure 1. HPLC chromatogram of rabbiteye blueberry sample FL 80-11 detected at 260 nm (A), 280 nm (B), 320 nm (C), and 360 nm (D). The chromatogram of standard is presented as E. *The standards were detected at 280 nm from 0 to 19 min, 320 nm from 19 to 30 min, 260 nm from 30 to 35 min, and 360 nm from 35 to 60 min. Compounds: (1) gallic acid, (2) (+)-catechin, (3) *p*-hydroxybenzoic acid, (4) (–)-epicatechin, (5) caffeic acid, (6) *p*-coumaric acid, (7) ferulic acid, (8) ellagic acid, (9) myricetin, (10) quercetin, and (11) kaempferol.

Quantitation. Quantification was performed based on external standards of known concentrations. Peak areas were used to quantify

the compounds in the sample. Calibration curves of the standards ranging from 20 to 240 ng/mL were used with good linearity and R^2 values exceeding 0.99 (peak areas vs concentration).

Analysis of Total Anthocyanins, Total Polyphenols, and Antioxidant Capacity. *Extraction.* The method of Prior et al. (*15*) was adopted with minor modification for the extraction of phenolics. Briefly, 1 g of frozen sample was pasted with mortar and pestle in 10 mL of 4% acetic acid in acetonitrile, and the final volume was made up to 25 mL with the same solution. Contents were shaken at 200 rpm for 1 h at 30 °C in a Gyratory water bath shaker. The extract was filtered with 0.2-micron syringe nylon filter before analysis.

Estimation of Anthocyanins. Total anthocyanin content of berries was estimated on a UV−visible spectrophotometer (Shimadzu UV-1601, Norcross, GA) by the pH-differential method using two buffer systems − potassium chloride buffer, pH 1.0 (0.025 M) and sodium acetate buffer, pH 4.5 (0.4 M). A diluted sample of 0.2 mL (to give optical density in the range of 0.1−1.2 at 510 nm) was mixed with 1.8 mL of corresponding buffer and read against a blank at 510 and 700 nm. Absorbance was calculated as

$$A = (A_{510\text{nm}} - A_{700\text{nm}})_{\text{pH1.0}} - (A_{510\text{nm}} - A_{700\text{nm}})_{\text{pH4.5}}$$

Monomeric anthocyanin pigment concentration in the extract was calculated as cyanidin-3-glucoside (*16*).

$$\text{Monomeric anthocyanin pigment (mg/L)} = A \times \text{MW} \times \text{DF} \times 1000/(\epsilon \times 1)$$

where A = absorbance, MW = molecular weight (449.2); DF = dilution factor, ϵ = molar absorptivity (26,900). The final concentration of anthocyanins (mg/100 g) was calculated based on total volume of extract and weight of sample.

Estimation of Total Polyphenols. Total polyphenols were estimated colorimetrically using the Folin−Ciocalteau method (*17*). Extracted samples were filtered through a 0.2-μm nylon syringe filter. A sample aliquot of 200 μL was added to 800 μL of water, 5 mL of 0.2 N Folin−Ciocalteau reagent, and 4 mL of saturated sodium carbonate solution (75 g/L) and mixed in a cyclomixer. The absorbance was measured at 765 nm with a Shimadzu UV−Visible spectrophotometer after incubation for 2 h at room temperature. Quantification was based on the standard curve generated with 100, 200, 300, and 400 mg/L of gallic acid.

Assay of Antioxidant Capacity. Antioxidant capacity was performed on the Shimadzu UV−Visible spectrophotometer in a kinetic mode based on the method of Re et al. (*18*). Briefly, $ABTS^{\bullet+}$ radical cation was produced by reacting 7 mM of 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) and 2.45 mM potassium persulfate after incubation at room temperature in dark for 16 h. The $ABTS^{\bullet+}$ solution was diluted with ethanol to an absorbance of 0.70 ± 0.1 at 734 nm. Filtered sample was diluted with ethanol so as to give 20−80% inhibition of the blank absorbance with 20 μL of sample. A 980 μL aliquot of $ABTS^{\bullet+}$ solution (absorbance of 0.70 ± 0.1) was read at 734 nm for a minute; after exactly 1 min, 20 μL of the sample was added and mixed thoroughly. Absorbance was continuously taken at every 6 s up to 7 min. Trolox (6-hydroxy-2,5,7,8-tetramethychroman-2-carboxylic acid, a vitamin E analogue) standards of final concentration 0−15 μM in ethanol were prepared and assayed under the same condition. The Trolox-equivalent antioxidant capacity (TEAC) of the sample was calculated based on the inhibition exerted by standard Trolox solution at 6 min.

RESULTS

Phenolic Acids and Flavonoids. The contents of individual phenolic acids and flavonoids are reported in **Table 2**. The highest concentration of gallic acid was found in rabbiteye blueberry, Tifblue at 258.90 mg/100 g fresh weight [FW], compared to southern highbush blueberries and blackberries. Other varieties within rabbiteye blueberries have comparable gallic acid content. Climax (irrigated, late June) and Climax (nonirrigated, late June) had no detectable amounts of gallic acid. *p*-Hydroxybenzoic acid was found only in rabbiteye blueberry, Climax-irrigated, at 103.67 mg/100 g FW. Southern highbush blueberries and blackberries had no detectable amounts of *p*-hydroxybenzoic acid. Caffeic acid was up to 6.32 mg/100 g FW in rabbiteye blueberries, which is slightly higher than southern highbush blueberries and blackberries containing up to 3.33 and 3.64 mg/100 g FW, respectively. The lowest concentration (1.38 mg/100 g FW) of caffeic acid was found in Choctaw cultivar of blackberry. *p*-Coumaric acid was found in all three varieties. Rabbiteye blueberry, Climax (nonirrigated) had highest *p*-coumaric acid of 15.78 mg/100 g among the three varieties. Blackberries had the lowest content of *p*-coumaric acid, 0.40 mg/100 FW. The majority of the cultivars within rabbiteye blueberries contain *p*-coumaric acid, except Austin, Climax-irrigated, FL-80-11, Fl-81-156, and Tifblue. Southern highbush blueberry cultivars contain *p*-coumaric acid in the range of 2.40−7.15 mg/100 g FW. The highest amount of ferulic acid was found in rabbiteye blueberries, Tifblue at 16.97 mg/100 g, and moderate amounts of up to 4.16 and 3.51 mg/100 g FW were found in southern highbush blueberries and blackberries, respectively. Rabbiteye blueberry cultivars Climax-irrigated and Climax-nonirrigated, and southern highbush blueberry cultivars TH 442 and Sharpblue, did not have any detectable amounts of ferulic acid. We did not detect ellagic acid in many cultivars of rabbiteye blueberries. However, high concentrations of ellagic acid were found in blackberries, Choctaw (33.81 mg/100 g FW) and Kiowa (30.01 mg/100 g FW). Southern highbush blueberries contained 0.75−6.65 mg/100 g FW ellagic acid, whereas rabbiteye blueberries contained 0.19−33.81 mg/100 g FW ellagic acid.

Catechin was the major flavonoid, with concentrations of up to 387.48 mg/100 g FW, followed by epicatechin at up to 129.51 mg/100 g FW. Flavonoids myricetin, quercetin, and kaempferol contents were less than 14.60 mg/100 g FW among all three varieties. The highest concentration of catechin was found in rabbiteye blueberries, Austin (387.48 mg/100 g FW), followed by blackberries, Choctaw (312.86 mg/100 g FW). Southern highbush blueberries contained up to 29.28 mg catechin/100 g FW. The presence of high concentrations of catechins suggests the possible occurrence of more polar dimeric and oligomeric proanthocyanidins with important biological activities. Epicatechin was found only in rabbiteye blueberries and ranged from 34 to 129.51 mg/100 g FW, with the highest concentration foundin Briteblue (129.51 mg/100 g FW); moderate concentrations were found in Climax (Alapaha), Climax-irrigated (Attapulgus), T460, and Woodard. Southern highbush blueberries and blackberries did not have any detectable amount of epicatechin. The majority of the cultivars contained myricetin in the range of 6.68−9.99 mg/100 g FW. Kiowa (blackberry) had the highest myricetin concentration of 9.99 mg, and the lowest concentration was found in Climax (6.68 mg/100 g FW). Southern highbush blueberries had up to 6.98 mg/100 g FW. Myricetin content was found to be higher than that previously reported by Häkkinen et al. (*12*, *13*), which was 23−26 mg/kg FW. Southern highbush blueberry FL 86-19 had the highest concentration of quercetin, 14.60 mg/100 g FW, followed by Climax (Attapulgus) at 9.97 mg/100 g FW in rabbiteye blueberries. These values are slightly lower than previous reports of 17−24 mg/kg FW (*12*), but they are in good agreement with Häkkinen et al. (*13*), who reported 10.5−16.0 mg/100 g FW. No quercetin was detected in blackberries or the following cultivars of rabbiteye blueberries: Austin, Climax-irrigated, FL 80-11, FL 81-156, and Tifblue. Kaempferol contents of rabbiteye blueberries and southern highbush blueberries were up to 3.72 and 3.17 mg/

Table 2. Individual Phenolic Acids and Flavonoids in Blueberries and Blackberries (values are averages of triplicate analyses)

	phenolic acids (mg/100 g freshweight)						flavonoids (mg/100 g freshweight)				
cultivar and sample location	gallic acid	*p*-hydroxy benzoic acid	caffeic acid	*p*-coumaric acid	ferulicacid	ellagic acid	catechin	epicatechin	myricetin	quercetin	kaempferol
Rabbiteye blueberries (*Vaccinium ashei* Reade)											
Austin[a]	1.53 ± 0.31	nd[d]	nd	nd	3.57 ± 0.59	0.22 ± 0.05	387.48 ± 13.90	nd	nd	nd	2.60 ± 0.07
Briteblue[a]	2.83 ± 0.97	nd	nd	7.91 ± 1.65	4.51 ± 0.59	nd	28.04 ± 16.75	129.51 ± 1.91	6.69 ± 0.02	5.82 ± 0.09	2.59 ± 0.04
Brightwell[a]	4.03 ± 1.24	nd	nd	4.37 ± 0.94	3.02 ± 0.64	6.02 ± 0.71	15.51 ± 4.27	nd	7.05 ± 0.45	6.81 ± 0.68	2.68 ± 0.17
Climax (early June)[a]	4.04 ± 0.47	nd	2.40 ± 0.35	3.78 ± 0.92	5.14 ± 1.06	1.12 ± 0.15	17.51 ± 6.57	57.68 ± 4.37	6.68 ± 0.06	6.20 ± 0.23	2.50± 0.12
Climax (irrigated, late June)	nd	103.67 ± 9.95	nd	nd	nd	nd	17.43 ± 7.33	nd	nd	nd	3.07 ± 0.06
Climax (nonirrigated, late June)[b]	nd	nd	6.32 ± 0.10	15.78 ± 3.09	nd	nd	34.75 ± 1.51	34.23 ± 0.30	nd	9.97 ± 0.88	3.21 ± 0.18
FL 80-11[a]	1.57 ± 0.18	nd	nd	nd	5.09 ± 0.03	nd	277.83 ± 18.23	nd	6.73 ± 0.34	nd	2.70 ± 0.06
Premier[a]	4.23 ± 0.90	nd	nd	7.48 ± 0.13	4.34 ± 0.04	nd	17.26 ± 0.07	nd	7.07 ± 0.07	6.10 ± 0.14	3.72 ± 0.04
FL 81-156[c]	1.55 ± 0.06	nd	nd	nd	3.22 ± 0.46	0.19 ± 0.00	246.66 ± 55.67	nd	8.62 ± 0.09	nd	nd
T 460[a]	3.42 ± 0.48	nd	nd	5.13 ± 0.90	3.17 ± 0.04	nd	14.53 ± 4.50	37.89 ± 4.61	6.69 ± 0.1	7.59 ± 0.61	2.51 ± 0.04
Tifblue[b]	258.90± 69.21	nd	nd	nd	16.97 ± 0.06	nd	107.00 ± 22.26	nd	nd	nd	nd
Woodard[a]	4.01 ± 0.38	nd	4.07± 0.76	10.16 ± 1.51	4.10 ± 0.07	nd	17.69 ± 0.22	48.66 ± 1.23	6.81 ± 0.08	5.88 ± 0.22	2.69 ± 0.08
Southern highbush blueberries (*Vaccinium corymbosum L.* Hybrids)											
FL 86-19[c]	4.55 ± 1.04	nd	nd	4.75 ± 0.83	3.45 ± 0.33	nd	11.66 ± 0.74	nd	6.72 ± 0.12	14.60±0.38	2.52 ± 0.03
TH 161[a]	2.72 ± 0.64	nd	nd	7.15 ± 1.50	4.16 ± 0.64	4.45 ± 0.17	21.43 ± 2.84	nd	6.91 ± 0.34	9.85 ± 1.56	2.59 ± 0.20
TH 440[a]	1.95 ± 0.06	nd	nd	4.62 ± 0.39	3.63 ± 0.59	6.65 ± 0.69	25.25 ± 2.13	nd	nd	12.03 ± 0.28	2.88 ± 0.10
TH 442[a]	4.76 ± 0.89	nd	3.33 ± 0.13	6.27 ± 0.36	nd	0.75 ± 0.10	29.28 ± 1.75	nd	6.98 ± 0.37	10.28 ± 0.65	3.17 ± 0.62
Sharpblue	2.83 ± 0.83	nd	3.00 ± 1.88	2.40 ± 0.62	nd	2.68 ± 0.32	9.87 ± 0.00	nd	nd	9.70 ± 4.23	3.13 ± 0.01
Blackberries (*Rubus L.*) cultivars											
Choctaw[c]	6.42 ± 0.32	nd	1.38 ± 0.83	2.08 ± 0.29	3.51 ± 0.59	33.81 ± 2.62	312.86 ± 8.71	nd	nd	nd	nd
Kiowa[c]	4.12 ± 0.35	nd	3.64 ± 0.26	0.40 ± 0.01	2.99 ± 0.22	30.01 ± 1.23	265.75 ± 11.46	nd	9.99 ± 1.05	nd	nd

[a] Alapaha, GA. [b] Attapulgus, GA. [c] Chula, GA. [d] nd = Not detected.

Table 3. Total Anthocyanins, Total Polyphenolics, and TEAC Values of Blueberries and Blackberries (values are averages of triplicates)

cultivar and location	total anthocyanins[d] (mg/100 g FW)	total polyphenolics (mg/100 g FW)	TEAC[e] μM/g FW
	Rabbiteye blueberries (*Vaccinium ashei* Reade)		
Austin[a]	178.15 ± 11.62	669.01 ± 6.57	29.52 ± 3.42
Brightblue[a]	16.37 ± 0.39	929.62 ± 20.40	26.74 ± 1.96
Brightwell[a]	87.38 ± 8.10	386.86 ± 10.64	29.81± 1.72
Climax[a] (early June)	105.21 ± 5.16	288.00 ± 9.16	22.65 ± 3.12
Climax (irrigated, late June)[b]	197.34 ± 5.69	641.07 ± 21.33	30.06 ± 2.67
Climax (nonirrigated, late June)[b]	99.33 ± 2.81	270.02 ± 15.42	19.73 ± 0.98
FL 80-11[a]	171.92 ± 4.36	911.78 ± 8.70	24.99 ± 2.60
Premier[a]	157.31 ± 1.82	522.13 ± 25.25	38.29 ± 2.89
FL 81-156[b]	111.47 ± 3.68	603.36 ± 21.19	24.87 ± 3.45
T 460[a]	12.70 ± 0.33	437.37 ± 20.27	21.19 ± 2.32
Tifblue[b]	108.62 ± 1.90	391.57 ± 10.17	29.66 ± 3.10
Woodard[a]	116.85 ± 1.05	622.89 ± 13.74	33.76 ± 3.81
average	113.55 ± 58.10	556.14 ± 216.87	27.60 ± 5.33
	Southern highbush blueberries (*Vaccinium corymbosum L.* Hybrids)		
FL 86-19[c]	35.47 ± 1.37	261.95 ± 62.93	8.11 ± 1.81
TH 161[a]	87.63 ± 9.36	287.87 ± 56.97	8.62 ± 1.39
TH 440[a]	53.49 ± 5.48	327.93 ± 60.65	10.37 ± 1.38
TH 442[a]	114.06 ± 15.55	585.34 ± 3.57	26.45 ± 3.60
Sharpblue	129.93 ± 4.10	533.32 ± 47.55	20.60 ± 2.89
average	84.12 ± 39.72	399.28 ± 149.12	14.83 ± 8.24
	Blackberries (*Rubus L.*) cultivars		
Choctaw[c]	110.52 ± 3.04	555.21 ± 68.36	18.04 ± 4.16
Kiowa[c]	122.66 ± 4.73	417.84 ± 25.80	2.65 ± 1.22
average	116.59 ± 8.58	486.53 ± 97.13	20.35 ± 3.25

[a] Alapaha, GA. [b] Attapulgus, GA. [c] Chula, GA. [d] Total anthocyanins were expressed as cyanidin-3-glucoside equivalents. [e] TEAC: Trolox-equivalent antioxidant capacity at 6 min.

100 g FW, respectively. These values are slightly higher than previous reports of 0−0.6 mg/100 g FW (*12*). Blackberries do not have any detectable amount of kaempferol.

Overall, our results are in good agreement with those reported in the literature (*19*−*22*) except for gallic acid. Unlike these authors, our findings show high concentrations of gallic acid in many of the cultivars. The concentrations of caffeic acid, ellagic acid, and myricetin in blueberries are in good agreement with the previous findings (*23*). Nevertheless, the concentrations of kaempferol, *p*-coumaric acid, and ferulic acid were slightly higher than the reported values of 0.6, 0.7, and 0.8 mg, respectively (*23*). The presence of quercetin and kaempferol in highbush blueberries is in good agreement with previous findings by Kader et al. (*23*). The large standard deviation in some of the results underscores the difficulty in obtaining evenly matured fruit samples for analysis.

Total Anthocyanins. Anthocyanins content of individual cultivars are shown in **Table 3**. The average total anthocyanin contents among rabbiteye blueberries, southern highbush blueberries, and blackberries are 113.55, 84.12, and 116.59 mg/100 g FW, respectively. Blackberries had the highest concentration and southern highbush blueberries had the least. Among rabbiteye blueberry varieties, Climax (irrigated-late June harvest) had the highest concentration of anthocyanins at 197.34 mg/100 g FW. On a very dry site, irrigation appeared to double the anthocyanin content of Climax. Sharpblue southern highbush blueberries and Kiowa in blackberries had the highest concentrations of 129.93 and 122.66 mg anthocyanins/100 g FW, respectively. These values are in good agreement with, and within the range of, those in previous reports (*15*).

Total Polyphenols. All flavonoids, anthocyanins, and nonflavonoid phenolic compounds are estimated in this parameter. Because only a few of the whole spectra of compounds could be identified and quantified, total polyphenols (TPP) estimates the whole amount of phenolics present in samples. **Table 3** presents the TPP of all samples. Rabbiteye blueberries had the highest average concentration of TPP (556.14 mg) and southern highbush blueberries had the lowest (399.28 mg/100 g FW). Blackberries had an average 486.53 mg TPP/100 g FW. Briteblue from rabbiteye blueberries had the highest amount of TPP at 929.62 mg/100 g FW, followed by FL-80-11 at 911.78/100 g FW, among all cultivars. Climax (nonirrigated) was found to contain the least amount (270.02 mg TPP/100 g FW) among rabbiteye blueberries. Overall, the lowest concentration of TPP was found in the southern highbush FL86-19 variety at 261.95 mg/100 g FW.

Antioxidant Capacity. Assessing the capacity of a compound to scavenge $ABTS^{\bullet+}$ radicals in terms of Trolox equivalent is known as Trolox-equivalent antioxidant capacity (TEAC) as first reported by Miller et al. (*26*). Various phytochemical components, including the flavonoids, phenylpropanoids, and phenolic acids are known to be responsible for antioxidant capacity in fruits and vegetables (*27*). Among the cultivars assayed, the values were found to be in the range of 8.11 to 38.29 μM TEAC/g of FW (**Table 3**). The average TEAC values for rabbiteye blueberries, southern highbush blueberries, and blackberries were 27.60, 14.83, and 20.35 μM TEAC/g FW, respectively. The antioxidant capacity may be related to the content of phenolic compounds in these samples. Higher content of TPP reflected higher TEAC values, and reduction in TPP decreased the TEAC value (*26*). The Premier cultivar from rabbiteye blueberries gave the highest TEAC value of 38.29 μM TEAC/g FW. Nonirrigated drought-strickened Climax gave the lowest antioxidant capacity of 19.73 μM TEAC/g FW among the rabbiteye blueberries. TH 442 had the highest TEAC value of 26.45, and FL-86-19 had the lowest (8.11 TEAC) value in southern highbush blueberries. Blackberry cultivars, Choctaw

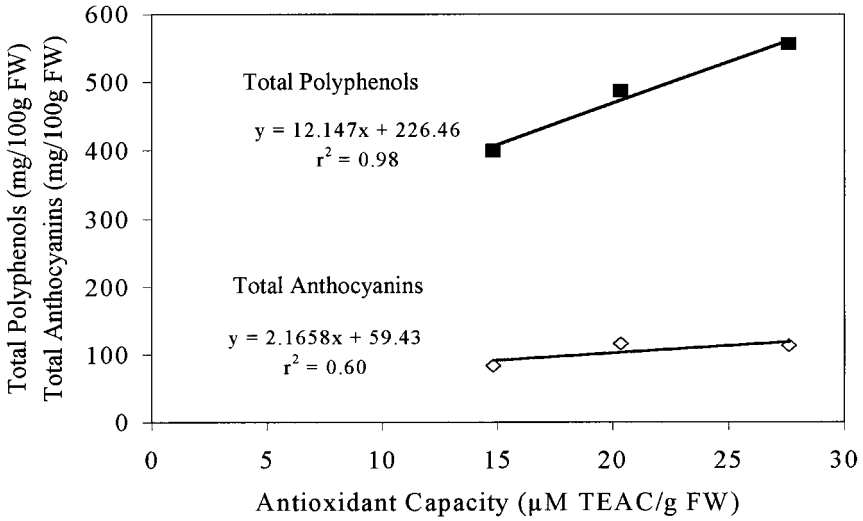

Figure 2. Correlation between total polyphenols (*y*-axis), $r^2 = 0.98$, and total anthocyanins (*y*-axis), $r^2 = 0.60$, to TEAC value (*x*-axis). Average values for rabbiteye blueberries, southern highbush blueberries, and blackberries were used for the plots.

and Kiowa, had moderate TEAC values of 18.04 and 22.65 μM TEAC/g FW, respectively, in comparison with those of other cultivars.

Catechin was present in all types of berries analyzed. The majority of the berries contained gallic acid, ferulic acid, myricetin, quercetin, and kaempferol. Anthocyanins were present from 12 to 197 mg/100 g FW and total polyphenols varied from 261 to 929 mg/100 g FW. TEAC values of berries analyzed were in the range of 8 to 38 μM/g FW. These variations may be due to the variety, type of the cultivars, maturity, and soil conditions in Georgia.

The correlation between TEAC and total polyphenols or total anthocyanins contents of different blueberry and blackberry samples are presented in **Figure 2**. The average values of TEAC showed positive correlation with average values of total anthocyanins and total polyphenols. A linear relationship was observed between TEAC and total polyphenols or total anthocyanins. The correlation coefficient, r^2, is 0.98 for total polyphenols and 0.60 for total anthocyanins. These values indicate that the antioxidant capacity is strongly related to total polyphenols and moderately related to total anthocyanins. Similar correlation was reported with oxygen radical absorbance capacity (ORAC) for other cultivars of southern highbush and lowbush blueberries with total polyphenols $r^2 = 0.85$, and $r^2 = 0.77$ for total anthocyanins (*15*). However, the correlation of TEAC values to total anthocyanins or total polyphenols for individual varieties deviated much. The correlation coefficient between TEAC values and total anthocyanins was 0.20; and between TEAC and total polyphenols was 0.05, for rabbiteye blueberries. Southern highbush blueberry TEAC values showed strong correlation with total anthocyanins, $r^2 = 0.65$ and total polyphenols, $r^2 = 0.98$. Blackberry TEAC values were correlated with total anthocyanins, $r^2 = 1$; and total polyphenols, $r^2 = 1$. These differences can be explained by the wide range of values in certain varieties (**Table 3**) and/or by the lack of enough cultivars in the case of blackberries.

Our findings are in general agreement with the work of Prior et al. (*15*) regarding total anthocyanins, total polyphenols, and antioxidant capacity of rabbiteye blueberries. The average content of total anthocyanins, total polyphenols, and antioxidant capacity of rabbiteye blueberries were higher than than those of southern highbush. Blackberry averages were similar to those of rabbiteye blueberries. Georgia grown blueberries and blackberries are a good source of antioxidants that can be used in foods and nutritional supplement formulations.

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