Antioxidant Capacity As Influenced by Total Phenolic and Anthocyanin Content, Maturity, and Variety of Vaccinium Species

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Antioxidant Capacity As Influenced by Total Phenolic and Anthocyanin Content, Maturity, and Variety of *Vaccinium* Species

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Different cultivars of four *Vaccinium* species [*Vaccinium corymbosum* L (Highbush), *Vaccinium ashei* Reade (Rabbiteye), *Vaccinium angustifolium* (Lowbush), and *Vaccinium myrtillus* L (Bilberry)] were analyzed for total phenolics, total anthocyanins, and antioxidant capacity (oxygen radical absorbance capacity, ORAC). The total antioxidant capacity of different berries studied ranged from a low of 13.9 to 45.9 μmol Trolox equivalents (TE)/g of fresh berry (63.2−282.3 μmol TE/g of dry matter) in different species and cultivars of *Vaccinium*. Brightwell and Tifblue cultivars of rabbiteye blueberries were harvested at 2 times, 49 days apart. Increased maturity at harvest increased the ORAC, the anthocyanin, and the total phenolic content. The growing location (Oregon vs Michigan vs New Jersey) did not affect ORAC, anthocyanin or total phenolic content of the cv. Jersey of highbush blueberries. A linear relationship existed between ORAC and anthocyanin (r_{xy} = 0.77) or total phenolic (r_{xy} = 0.92) content. In general, blueberries are one of the richest sources of antioxidant phytonutrients of the fresh fruits and vegetables we have studied.

Keywords: *Vitamin C; ascorbate; blueberry; bilberry; highbush; lowbush; rabbiteye; ORAC; HPLC*

INTRODUCTION

Fruits and vegetables contain many different phytonutrients, many of which have antioxidant properties. Research has shown that fruits and vegetables contain other antioxidant nutrients, in addition to the well-known vitamins C and E, or carotenoids, that significantly contribute to their total antioxidant capacity (Cao et al., 1996; Wang et al., 1996). For example, flavonoids (including compounds such as flavones, isoflavones, flavonones, anthocyanins, and catechins) that are components of fruits and vegetables have strong antioxidant capacity (Cao et al., 1997; Wang et al., 1997). There is convincing epidemiologic evidence showing that fruits and vegetables are in general beneficial to health and contribute to the prevention of degenerative processes (Ames et al., 1993). Thus, it is important to characterize the beneficial phytonutrients present in these foods and the mechanisms responsible for these effects.

The protection provided against diseases by fruits and vegetables has been attributed to the various antioxidants contained in these foods (Ames et al., 1993; Gey et al., 1991; Steinberg et al., 1989). At present, there is overwhelming evidence to indicate that free radicals cause oxidative damage to lipids, proteins, and nucleic acids. Free radicals may lie at the heart of the etiology or natural history of a number of diseases, including cancer, heart, vascular, and neurodegenerative diseases (Halliwell, 1994; Yu, 1994). Therefore, antioxidants, which can neutralize free radicals, may be of central importance in the prevention of these disease states. What is not clear from the available literature is what phytochemicals are responsible for or associated with the protection against carcinogenicity, cardiovascular, and neurodegenerative changes associated with aging and if specific fruits or vegetables might be more effective than others in preventing these disorders.

There have been few attempts at quantifying the total antioxidant capacity in foods. Studies from our laboratory represent the first attempt to accurately measure the total antioxidant capacity of fruits and vegetables (Cao et al., 1996; Wang et al., 1996). The oxygen radical absorbance capacity (ORAC) procedure, which has been automated and utilized extensively in our laboratory, lends itself well to identifying foods with high antioxidant capacity and to evaluating in vivo responses to dietary antioxidant manipulation (Cao et al., 1993, 1995, 1996; Wang et al., 1996). Results from these studies using a peroxyl radical generator ($ORAC_{ROO\cdot}$) indicated that the antioxidant capacities of common fruits, vegetables, and teas had a considerable range. On the basis of wet weight (edible portion), kale, strawberry, and

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spinach had relatively high antioxidant capacities of 17.7, 15.4, and 12.6 μmol Trolox equivalents (TE)/g of fresh weight, respectively (Cao et al., 1996; Wang et al., 1996). Interestingly, additional analyses indicated that the major source of antioxidant capacity of most of these fruits is not from their inherent vitamin C content (Wang et al., 1996).

Blueberries have been of specific interest in our laboratory because of their high antioxidant capacity (in some cases as high as 40−50 μmol TE/g). However, in our early analyses of fruits purchased in the local supermarket, we found considerable variation in ORAC. Because of the wide range of anthocyanin concentrations reported (Mazza and Miniati, 1993), we suspected that the antioxidant capacity might vary accordingly. Thus, we undertook the studies reported in this paper to evaluate different cultivars of four *Vaccinium* species. The purpose of this study was to compare total phenolics, total anthocyanins and antioxidant capacity in appropriate berry samples from selected cultivars of the *Vaccinium* (*V.*) species [*V. corymbosum* L (Highbush), *V. ashei* Reade (Rabbiteye), *V. angustifolium* (Lowbush), and *V. myrtillus* L (Bilberry)]. With the cv. Jersey of *V. corymbosum* L. we obtained samples from three locations within the United States (New Jersey, Michigan, Oregon) to evaluate effects of differing climatic or growing conditions on the antioxidant capacity. Bilberry was included in the sampling because of the long history in folk medicinal uses of the bilberry pharmaceutical products, which has existed for most of this century (Morazzoni and Bombardelli, 1996).

MATERIALS AND METHODS

Chemicals. *R*-Phycoerythrin (*R*-PE), ascorbic acid, gallic acid, and acetonitrile (HPLC grade) were purchased from Sigma Chemical Co. (St. Louis, MO). 6-Hydroxy-2,5,7,8-tetramethyl-2-carboxylic acid (Trolox) was obtained from Aldrich (Milwaukee, WI). 2,2′-Azobis(2-amidinopropane) dihydrochloride (AAPH) was obtained from Wako Chemicals USA, Inc. (Richmond, VA). Methanol (HPLC grade) was from Fisher Scientific (Boston, MA). HPLC grade water was obtained from J. T. Baker Inc. (Phillipsburg, NJ).

***Vaccinium* Species and Cultivars.** Four *Vaccinium* species with a total of 23 cultivars were examined in this study. The four species included *V. corymbosum* L (Highbush), *V. ashei* Reade (Rabbiteye), *V. angustifolium* (Lowbush), and *V. myrtillus* L (Bilberry). Thirteen cultivars are commercially available, including Bluecrop, Jersey, Croatan, Duke, Rancocas, Rubel, and O'Neal of highbush blueberries; Climax, Brightwell, Tifblue, and Little Giant from rabbiteye blueberries; Lowbush blueberries from Maine; and bilberry. Cultivars which are not commercially available include the following: Reveille, Blue Ridge, Cape Fear, Pender, and Bladen of the highbush blueberries; Cumberland, Blomidin, and Fundy lowbush blueberries; and lowbush blueberries sampled from Nova Scotia and Prince Edward Island.

Sampling Procedures. The commercially available varieties of *Vaccinium* species were selected with input from industry sources. Highbush blueberries were harvested fresh from six to eight bushes (about 2 kg), sorted to remove green, overripe, or damaged berries and mixed together, and approximately 500 g were sampled and shipped fresh in cooled containers with 24 h delivery from the point of harvest to the USDA Human Nutrition Research Center on Aging (HNRCA) in Boston. All fresh samples came from the 1997 crop of blueberries. Fresh samples received at the USDA-HNRCA were stored at −70 °C until analyzed which was usually no longer than 2 months. Extractions and analyses were performed on six cultivar/site samples at a time. Berries were not selected for size but reflected the typical and average for the cultivar. Harvesting at a uniform stage of maturity was attempted.

The varieties of highbush blueberries not commercially available were obtained from North Carolina. They were harvested about midway through the normal harvest period. The lowbush blueberries obtained from Nova Scotia and Prince Edward Island were a composite of approximately equal volumes of 20 different clones. The fruit was considered at "commercial maturity" (i.e. any fruit that appeared underripe or not completely blue were removed). With the variety of genotypes and their ripening dates, there were undoubtedly differences in maturity within the lowbush blueberry clones. The lowbush clones are planted and maintained at research facilities in Nova Scotia but provide an opportunity to look at genotypic effects within this species. Bilberry, which also represents a mixture of wild clones, was imported from Germany.

Determination of ORAC, Total Anthocyanins, and Total Phenoloics. Blueberries were extracted with acetonitrile/acetic acid for the analysis of ORAC, total anthocyanins, and total phenolics. A 50 g sample of each blueberry source was added to 50 mL of acetonitrile containing 4% acetic acid and homogenized in a blender for 2 min. After the recovery of the homogenate, 25 mL of acetonitrile containing 4% acetic acid was used to wash the blender and pooled with the first homogenate. The pooled homogenate was left at room temperature with shaking every 3 min for at least 30 min and then centrifuged at 13000*g* for 15 min at 4 °C. The pellet following centrifugation was washed with 50 mL of acetonitrile containing 4% acetic acid and centrifuged, and the resulting supernatants were combined with the initial extract. Triplicate extractions were prepared from each blueberry source. Blueberries were also extracted with water followed by an acetone extraction of the pellet. However, total recoveries of total anthocyanins and phenolics was generally lower with H_2O/acetone extraction than with acetonitrile. Thus, only the acetonitrile data are presented.

Automated $ORAC_{ROO\cdot}$ Assay. The automated ORAC assay was carried out on a COBAS FARA II spectrofluorometric centrifugal analyzer (Roche Diagnostic System Inc., Branchburg, NJ; emission filter = 565 nm). The procedure was based on a previous report of Cao and co-workers (1993), as modified for the COBAS FARA II (Cao et al., 1995). Briefly, in the final assay mixture (0.4 mL total volume), *R*-phycoerythrin (16.7 nM; Sigma) was used as a target of free radical attack, with AAPH (4 mM) as a peroxyl radical generator. Trolox (1.0 μmol/L), a water soluble analogue of vitamin E, was used as a control standard. The analyzer was programmed to record the fluorescence of *R*-PE every 2 min after addition of AAPH. All fluorescent measurements are expressed relative to the initial reading. Final results were calculated using the differences of areas under the *R*-PE decay curves between the blank and a sample and expressed as micromole Trolox equivalents (TE) per gram of fresh weight.

Total Anthocyanins Assay. The total anthocyanins were estimated by a pH differential method (Cheng and Breen, 1991). Absorbance was measured in a Beckman spectrophotometer at 510 nm and at 700 nm in buffers at pH 1.0 and 4.5, using $A = [(A_{510} - A_{700})_{pH1.0} - (A_{510} - A_{700})_{pH4.5}]$ with a molar extinction coefficient of cyanidin-3-glucoside of 29 600. Results were expressed as milligrams of cyanidin-3-glucoside equivalent per 100 g of fresh weight.

Total Phenolics Assay. Total soluble phenolics in the acetonitrile extracts were determined with Folin-Ciocalteu reagent by the method of Slinkard and Singleton (1977) using gallic acid as a standard.

Determination of Ascorbate. For ascorbate analysis, blueberries were homogenized in a blender with cold 5% (w/v) metaphosphoric acid in phosphate buffered saline (pH 1.6; 1:9 (w/w)) containing 1 mmol/L of the metal ion chelator diethylenetriaminepentaacetic acid. The mixutre was homogenized for 2 min and centrifuged at 12 000 rpm at 4 °C. The supernatant was stored at −70 °C until analyses of ascorbate were performed. Ascorbate was analyzed by paired-ion,

Table 1. Antioxidant Activity, Anthocyanin, Phenolic Contents of Acetonitrile Extract, and Ascorbate Concentrations Based upon the Fresh Weight of Berries from Different Commercially Available Varieties of *Vaccinium* Species

cultivar, state, and source	$ORAC_{ROO}$[a] (μmol TE/g)	anthocyanin[b] (mg/100 g)	phenolics[c] (mg/100 g)	*A/P* [d] (mg/mg)	ascorbate (mg/100 g)
		Vaccinium corymbosum L. (Northern Highbush)			
Bluecrop (MI)[e]	17.0 ± 1.0 (70.5)	93.1 ± 1.6	189.8 ± 10.9	0.491	8.1 ± 0.27
Jersey (OR)[f]	18.1 ± 0.2 (76.1)	101.2 ± 1.5	181.1 ± 10.4	0.559	11.1 ± 0.13
Jersey (MI)[e]	20.8 ± 0.6 (63.2)	100.1 ± 2.3	206.2 ± 4.1	0.485	8.3 ± 0.17
Jersey (NJ)[g]	21.4 ± 0.4 (91.9)	116.6 ± 1.1	221.3 ± 4.3	0.527	12.1 ± 0.64
Croatan (NC)[h]	20.0 ± 0.6 (134.2)	118.8 ± 6.3	275.3 ± 7.9	0.432	7.2 ± 0.23
Duke (NJ)[g]	25.1 ± 0.9 (121.8)	127.4 ± 2.1	305.9 ± 3.4	0.416	7.3 ± 0.03
Rancocas (BC)[k]	32.4 ± 1.4 (117.3)	140.9 ± 4.9	317.4 ± 7.3	0.444	13.0 ± 0.11
Rubel (MI)[e]	37.1 ± 0.5 (182.8)	235.4 ± 6.1	390.5 ± 6.5	0.603	14.6 ± 0.55
means (N. Highbush)	*24.0 ± 0.7 (107.2)*	*129.2 ± 3.2*	*260.9 ± 6.9*	*0.494 ± 0.02*	*10.2 ± 0.27*
		Vaccinium corymbosum L. (Southern Highbush)			
O'Neal (NC)[h]	16.8 ± 1.9 (105.0)	92.6 ± 4.6	227.3 ± 6.9	0.407	4.9 ± 0.12
		Vaccinium ashei Reade (Rabbiteye)			
Climax (GA)[i]	13.9 ± 4.1 (86.3)	90.8 ± 5.2	230.8 ± 7.3	0.393	8.5 ± 0.0
Brightwell (GA)[i]	15.3 ± 2.8 (85.0)	61.8 ± 1.8	271.4 ± 12.7	0.228	8.1 ± 0.1
Tifblue (GA)[i]	23.0 ± 2.6 (129.9)	87.4 ± 5.6	361.1 ± 16.6	0.242	8.6 ± 0.1
Brightwell (GA)[i,n]	34.3 ± 2.9 (130.9)	161.7 ± 3.5	457.5 ± 14.1	0.353	6.2 ± 0.4
Tifblue (GA)[i,n]	37.8 ± 3.6 (206.5)	154.2 ± 2.6	409.3 ± 22.5	0.377	10.5 ± 0.6
Little Giant (MI)[e,m]	25.5 ± 1.2 (178.3)	187.2 ± 6.2	307.8 ± 14.6	0.608	8.3 ± 0.08
means (Rabbiteye)	*25.0 ± 2.7 (136.2)*	*123.9 ± 4.2*	*339.7 ± 14.6*	*0.370 ± 0.06*	*8.4 ± 0.21*
		Vaccinium angustifolium (Lowbush)			
Lowbush (ME)[j]	25.9 ± 0.4 (82.7)	95.4 ± 2.6	299.0 ± 18.9	0.319	16.4 ± 0.1
overall means	*24.0 ± 2.0 (116.4)*	*122.7 ± 11.0*	*290.7 ± 20.5*	*0.430 ± 0.02*	*9.6 ± 0.8*

[a] Expressed as micromole Trolox equivalents per gram of fresh fruit. Data in parentheses expressed per gram of dry matter. See Table 2 for harvest dates. [b] Concentration based upon cyanidin-3-glucoside as standard expressed per gram of fresh weight. [c] Concentration based upon gallic acid as standard expressed per gram of fresh weight. [d] Anthocyanin/phenolics. [e] Source: MBG Marketing, Grand Junction, MI. [f] Source: USDA-ARS, Hort Crops Research Laboratory, Corvalis, OR. [g] Source: USDA-ARS, Blueberry & Cranberry Research Center, Chatsworth, NJ. [h] Source: NC State University, Castle Hayne, NC. [i] Source: University of Georgia, Tifton, GA. [j] Source: Tru Blu CoOp, New Lisbon, NJ. [k] Source: Berryhill Foods, Abbotsford, British Columbia, Canada. [l] Source: Wilhelm Dierking Beemobst, OT Nienhagen, Gilton, Germany. [m] 50% *V. constablaei*. [n] Very late harvest (07/28/97) after most harvesting for fresh market sales was completed.

reversed-phase HPLC coupled with electrochemical detection. The procedure was based on the method of Behrens and Madere (1987) and has been previously described (Martin and Frei, 1997). Briefly, an aliquot of the acidic supernatant was chromatographed on a LC8 column (150 mm × 4.6 mm i.d., 3 μm particle size; Supelco, Bellefonte, PA) using 99% deionized water and 1% methanol containing 40 mmol/L sodium acetate and 1.5 mmol/L dodecyltriethylammonium phosphate (Q12 ion pair cocktail; Regis, Morton Grove, IL) as the mobile phase. Vitamin C was detected at an applied potential of +0.6 V by a LC 4B amperometric electrochemical detector (Bioanalytical Systems, West Lafayette, IN).

Determination of Fruit Weight and Size, Total Soluble Solids, and Total Titratable Acid (TTA). The average berry weight was determined by weighing approximately 50 g of berries and counting the number of berries. The average berry diameter was determined using calipers as the average of two measurements; one from the stem to a point opposite the stem and the second measurement perpendicular to the stem measurement. Total soluble solids (TSS), expressed as °Brix, were determined using a refractometer on juice obtained from squeezing the liquid from the blueberries through four layers of gauze. The total titratable acid (TTA) was determined on 50 g of berries following homogenization of the berries with an equal weight of water for 5 min. The homogenate was titrated to pH 8.1 with 0.1 N NaOH, and TTA was calculated and expressed as milliequivalents per gram of fresh weight.

Statistical Analysis. Correlation and regression analyses and principal component analysis were performed using Systat (1992). The component loadings included total phenolics, ORAC, total anthocyanins, the anthocyanin to total phenolic ratio (*A*/*P*), and ascorbate.

RESULTS

ORAC, Total Anthocyanins, Total Phenolics, and Vitamin C. Total antioxidant capacity, measured as ORAC, ranged from a low of 13.9 to 45.9 μmol TE/g of fresh berries in the acetonitrile extracts of the different cultivars of blueberries (Tables 1 and 2). The overall mean of all commercially available cultivars was 24.0 ± 2.0. The highbush varieties of Rancocas, Rubel (Table 1), and Bladen (Table 2) and the late harvest on the rabbiteye cv. Tifblue and Brightwell (Table 1) had ORAC values (i.e. 32.4, 37.1, 42.3, 37.8, and 34.3, respectively) that approached that observed for the Bilberry (44.6). Bilberry and the lowbush blueberries from Nova Scotia had the highest antioxidant capacity (44.6 ± 2.3 and 45.9 ± 2.2, respectively) as well as in total phenolics (525 ± 5.0 and 495 ± 3.5, respectively) (Table 2). There appear to be two clusters of ORAC values in the lowbush blueberries. The first included lowbush-PEI, lowbush-NS, and Fundy lowbush blueberries (Table 2) which were relatively high in ORAC (mean: 41.8), anthocyanins, and total phenolics. The second cluster included lowbush from ME (Table 1), cv. Cumberland, and cv. Blomidin (Table 2) lowbush blueberries which were lower in ORAC (mean: 27.5). At this point, it is not clear as to the source (genetics, location, maturity, etc.) of this variation. Anthocyanins in the lowbush blueberries were not as high as the bilberry compared to the relative ORAC values as reflected in the ratio of anthocyanins to ORAC (0.37 vs 0.57).

The relationship between ORAC and the total anthocyanin or total phenolic content in all these different blueberry samples is presented in Figures 1 and 2. A significant linear relationship was observed between ORAC and the total anthocyanin or total phenolic content. The correlation coefficient was much higher between ORAC and the total phenolics (r_{xy} = 0.85)

Table 2. Antioxidant Activity (ORAC), Anthocyanin, and Total Phenolic Contents of Acetonitrile Extract and Ascorbate Concentrations Based upon Fresh Weight of Berries from Different Varieties of *Vaccinium* Species Not Readily Available or Identifiable Commercially and from Bilberry (*Vaccinium myrtillus* L.)

cultivar, state, and source	$ORAC_{ROO\cdot}$[a] (μmol/g)	anthocyanin[b] (mg/100 g)	phenolics[c] (mg/100 g)	*A/P* [d] (mg/mg)	ascorbate (mg/100 g)
		Vaccinium corymbosum L. (Southern Highbush)			
Reveille (NC)[e]	17.8 ± 1.0 (144.8)	62.6 ± 3.8	233 ± 1.5	0.269	4.9 ± 0.1
Blue Ridge (NC)[e]	25.7 ± 2.3 (153.9)	110.8 ± 3.5	347 ± 10.9	0.319	9.5 ± 0.8
Cape Fear (NC)[e]	26.3 ± 3.4 (156.5)	157.3 ± 5.2	331 ± 10.3	0.476	NA
Pender (NC)[e]	30.5 ± 2.9 (180.5)	157.4 ± 3.7	349 ± 7.1	0.451	NA
Bladen (NC)[e]	42.3 ± 0.3 (231.1)	130.9 ± 5.5	473 ± 10.7	0.277	NA
mean ± SEM	*28.5 ± 4.0 (173.4)*	*123.8 ± 17.6*	*347 ± 38.2*	*0.358 ± 0.04*	*7.2 ± 1.5*
		Vaccinium angustifolium (Lowbush)			
Cumberland (NS)[l]	27.8 ± 2.6 (167.4)	103.6 ± 0.9	295 ± 13.2	0.351	8.0 ± 0.2
Blomidin (NS)[l]	28.8 ± 2.1 (223.8)	91.1 ± 0.7	313 ± 6.4	0.291	3.6 ± 0.2
Lowbush (PEI)[l]	37.4 ± 0.9 (277.2)	179.6 ± 3.4	453 ± 18.5	0.396	1.7 ± 0.2
Fundy (NS)[l]	42.0 ± 2.0 (209.3)	191.5 ± 2.5	433 ± 45.5	0.442	4.3 ± 0.1
Lowbush (NS)[l]	45.9 ± 2.2 (271.2)	175.0 ± 1.6	495 ± 3.5	0.354	9.7 ± 0.1
mean ± SEM	*36.4 ± 3.6 (229.8)*	*148.2 ± 21.0*	*398 ± 39.6*	*0.367 ± 0.03*	*5.5 ± 1.5*
		Vaccinium myrtillus L.			
Bilberry (GER)[l]	44.6 ± 2.3 (282.3)	299.6 ± 12.9	525.0 ± 5.0	0.571	1.3 ± 0.1

[a] Expressed as micromole Trolox equivalents per gram of fresh fruit. Data in parentheses expressed per gram of dry matter. Southern highbush samples were harvested on 6/4/97 and bilberry on 7/2/97. [b] Concentration based upon cyanidin-3-glucoside as standard. [c] Concentration based upon gallic acid as standard. [d] Anthocyanin/phenolics.

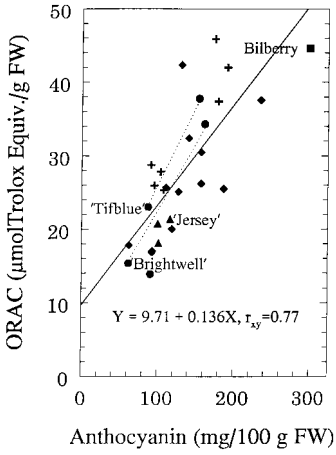

Figure 1. Relationship between anthocyanin concentrations (*X*) (mg/100 g of fresh weight) to ORAC (*Y*) (μmol Trolox equiv/g of fresh berry) in different cultivars of *Vaccinium* species ($Y = 9.71 + 0.136X$; $r_{xy} = 0.77$; $n = 28$). ♦, *Vaccinium corymbosum L.* (highbush); ▲, cv. Jersey (Highbush); +, *Vaccinium angustifolium* Ait., Lowbush; ■, *Vaccinium myrtillus* L. (bilberry); ●, Rabbiteye. Data for the two points for the early and late harvest dates for cv. Tifblue and cv. Brightwell are connected by a line.

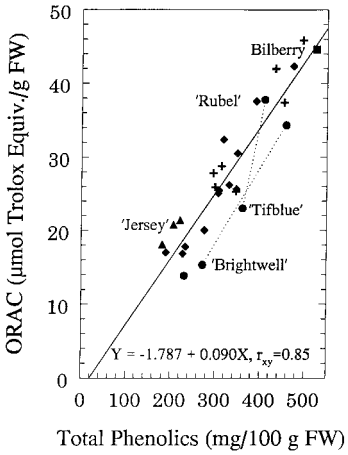

Figure 2. Relationship between total phenolic concentrations (*X*) (mg/100 g of fresh weight) to ORAC (*Y*) (μmol Trolox equiv/g of fresh berry) in different cultivars of *Vaccinium* species ($Y = -1.787 + 0.090X$, $r_{xy} = 0.845$; $n = 28$). ♦, *Vaccinium corymbosum* L. (Highbush); ▲, cv. Jersey (highbush); +, *Vaccinium angustifolium* Ait. Lowbush; ■, *Vaccinium myrtillus* L. (bilberry); ●, Rabbiteye. Data for the two points for the early and late harvest dates for cv. Tifblue and cv. Brightwell are connected by a line.

compared to ORAC and anthocyanins ($r_{xy} = 0.77$) (Figures 1 and 2).

Data for ORAC, the total anthocyanins, the total phenolics, the total anthocyanins/total phenolics ratio, and ascorbate were used as components in the principal component analysis using three factors (Table 3). Those species or cultivars falling in the upper right-hand quadrant of Figure 3 would be high in anthocyanins and total antioxidant capacity. Factor 1 is determined primarily by the quantities of ORAC, total anthocyanins, and total phenolics, while factor 2 is determined by the total anthocyanins and total anthocyanins/total phenolics ratio. Factor 3 is dominated by ascorbate concentrations. A plot of the calculated values for factor 1 and factor 2 is shown in Figure 3. Ascorbate has an ORAC of 5.6 mmol TE/g. With a maximum ascorbate concentration of 15 mg/100 g of blueberry, the ORAC from ascorbate would be 0.84 μmol TE, which is less than 5% of the total ORAC. Thus, because ascorbate was not a significant contributor to the measured antioxidant activity, factor 3 was not evaluated further.

Ascorbate concentrations (1.3−16.4 mg/100 g) showed a significant variability between cultivars and species. Although most of the samples had an ascorbate concentration between 9 and 16 mg/100 g, no consistent pattern emerged relative to ORAC or anthocyanins or to total phenolics. We observed during the course of this study that if the skin of the blueberry was broken, ascorbate may be oxidized and the concentration may be significantly reduced. We tried to control for this phenomenon and not use samples that were obviously damaged. However, this may account for the low ascorbate value observed in the bilberry (1.3 g/100 g), as there was minor damage to the berries in the shipment from Germany. Using an ORAC value for ascorbate of 5.6 mmol TE/g, it was calculated that the antioxidant capacity contributed by ascorbate to the total antioxidant capacity, measured as ORAC, was

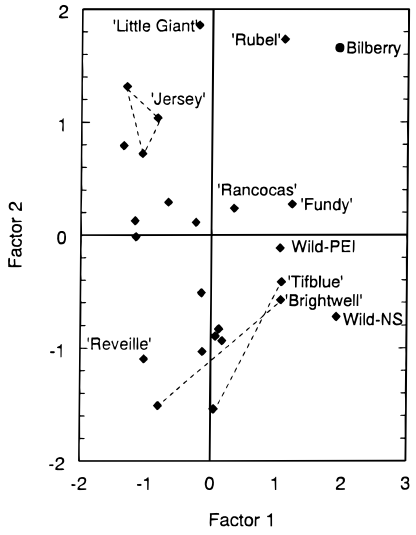

Figure 3. Principal component analysis of *Vaccinium* sp. The coefficients for standardized factor scores were as follows for the following components: (1) total phenolics, (2) ORAC, (3) anthocyanin, (4) anthocyanin/phenolics, and (5) ascorbate (see Table 3 for weighting for each of the three factors; only factors 1 and 2 are plotted in this graph).

Table 3. Principal Component Analysis of Different Species and Cultivars of *Vaccinium*

item	for given factor 1	2	3
rotated loading matrix			
total phenolics	0.976	−0.080	0.149
ORAC	0.967	0.123	0.033
anthocyanins	0.792	0.580	0.124
anthocyanin/phenolic	0.045	0.995	−0.060
ascorbate	−0.116	0.034	−0.992
% of total variation explained	50.6	27.0	20.5
coefficients of standardized factor scores			
total phenolics	0.441	−0.236	−0.028
ORAC	0.417	−0.080	−0.128
anthocyanins	0.234	0.337	0.042
anthocyanin/phenolic	−0.157	0.801	0.024
ascorbate	0.119	−0.045	−1.013

2.3% for the highbush and rabbiteye berries. Ascorbate in lowbush berries contributed only 1.5% while, in the bilberry sample, the contribution of ascorbate to ORAC was only 0.2%. Thus, it is clear that ascorbate does not make a major contribution to the antioxidant capacity of any of the blueberries sampled. In calculations with other fruits, ascorbate has generally contributed less than 10% of the total antioxidant capacity (Wang et al., 1996).

Maturity Effects. Maturity at harvest had a marked effect on ORAC, the total anthocyanins, and the total phenolics of the berries, for the cv. Brightwell and Tifblue of rabbiteye blueberries (Table 1) which were the only two cultivars evaluated. Berries harvested immediately after turning blue had lower ORAC and total anthocyanins than berries well-matured that were harvested 49 days later. ORAC and total anthocyanins increased 224 and 261%, respectively, in the cv. Brightwell while in cv. Tifblue they increased 164 and 176%, respectively, with increasing maturity. Total phenolics increased by 169 and 113% in the Brightwell and cv. Tifblue cultivars, respectively, with increased maturity.

Location Effects. Location (Oregon vs Michigan vs New Jersey) did not affect the ORAC value (range 18.1−21.4 μmol TE/g of fresh weight), the total anthocyanin (range 101.2−116.6 mg/100 g of fresh weight), or the total phenolic (range 181.1−221.3 mg/100 g of fresh weight) content of the cv. Jersey of highbush blueberries (Table 1).

Fruit Weight and Size, Total Soluble Solids, and Total Titratable Acid. Data for harvest date, dry matter, TSS, TTA, diameter, weight/berry and calculated surface area per berry are presented in Tables 4 and 5. There was not a consistent difference in these parameters with species of blueberry from highbush and rabbiteye blueberries. However, the range in TTA, weight, and surface area was large (Tables 3 and 4). Bilberry had the highest TTA and a low TSS, thus giving the lowest ratio of TSS/TTA of any of the cultivars. The smallest berries were seen with the bilberry, lowbush-ME, cv. Little Giant and cv. Rubel. The calculated regression equation for ORAC (μmol/g) (Y) and either weight/berry or average berry diameter (X) was

$$Y = 37.5 - 12.0X \quad (X = \text{weight (g)}; r_{xy} = 0.62; p = 0.005; n = 19)$$

$$Y = 58.0 - 29.1X \quad (X = \text{diameter (cm)}; r_{xy} = 0.62; p = 0.004; n = 19)$$

The ratio of the surface area to volume was also calculated, and both the total anthocyanins and ORAC increased as the surface area-to-volume ratio increased (Figures 4 and 5).

DISCUSSION

Acetonitrile/acetic acid extract was used in this study for the determination of ORAC, total anthocyanins, and total phenolics in different *Vaccinium* berries. Acetonitrile/acetic acid extraction increased recovery of anthocyanins, total phenolics, and ORAC in these *Vaccinium* berries by an average of 59.8 ± 4.8 mg/100 g (45.5%), 39.0 ± 12.3 mg/100 g (11.8%), and 16.6 ± 1.0 μmol/g (16.8%), respectively (data not shown), compared to the water/acetone extraction used previously with other fruits and vegetables (Cao et al., 1996; Wang et al., 1996). However, in general, the rank of these different *Vaccinium* species and cultivars determined using their acetonitrile/acetic acid extractions was similar to that determined using their water/acetone extractions, in terms of their ORAC, total anthocyanin, or phenolic content.

Previous reports from our laboratories presented data on the total antioxidant capacity in other fruits and vegetables (Cao et al., 1996; Wang et al., 1996). The results presented in this paper represent the first published data on the total antioxidant capacity in blueberries. Data presented here indicate that, on a fresh weight basis, blueberries have the highest antioxidant capacity, as estimated using the average ORAC of different species and cultivars, of all the fresh fruits and vegetables tested to date (Cao et al., 1996; Wang et al., 1996). However, considerable variability seemed to exist among the initial analyses that were performed on blueberry samples obtained from the commercial supermarket, suggesting that variation exists in the antioxidant capacity of different varieties of the *Vaccinium* species. We have previously analyzed the antioxidant capacity of anthocyanins (Wang et al., 1997) and other flavonoids (Cao et al., 1997) and found them to have 2−6 times the activity found in common anti-

Table 4. Harvest Dates, Total Solids, and Total Acid Content of Berries from Different Commercially Available Varieties of *Vaccinium* Species

cultivar, state, and source	harvest date	DM[a] (%)	TSS[b] (°Brix)	TTA[c] (mequiv/g of DM)	TSS/TTA	diam (cm)	wt[d] (g/berry)	SA[e] (cm^2)
				Vaccinium corymbosum L. (Northern Highbush)				
Bluecrop (MI)[l]	08/18/97	24.1	12.0	0.25	47.7	1.39	1.77	6.08
Jersey (OR)[f]	07/22/97	23.8	16.5	0.24	69.8	1.39	1.76	6.10
Jersey (MI)[l]	08/18/97	32.9	13.5	0.22	61.1	1.22	1.22	4.67
Jersey (NJ)[g]	07/17/97	23.3	16.5	0.60	27.6	1.17	1.12	4.31
Croatan (NC)[k]	06/04/97	14.9	NA	NA	NA	NA	NA	NA
Duke (NJ)[g]	06/26/97	20.6	10.0	0.73	13.7	1.16	1.10	4.21
Rancocas (BC)[h]	07/14/97	27.6	19.0	0.22	86.9	1.12	1.04	3.96
Rubel (MI)[l]	08/26/97	20.3	15.8	0.41	38.4	0.98	0.63	3.04
means (N. Highbush)		*23.4 ± 1.9*	*14.8 ± 1.2*	*0.38 ± 0.08*	*49.3 ± 9.6*	*1.20 ± 0.06*	*1.23 ± 0.15*	*4.62 ± 0.42*
				Vaccinium corymbosum L. (Southern Highbush)				
O'Neal (NC)[n]	06/04/97	16.0	15.4	0.82	18.8	1.34	1.54	5.64
				Vaccinium ashei Reade (Rabbiteye)				
Climax (GA)[i]	06/09/97	16.1	12.0	0.65	18.5	1.24	1.23	4.81
Brightwell (GA)[i]	06/09/97	18.0	12.5	0.51	24.8	1.24	1.25	4.82
Tifblue (GA)[i]	06/09/97	17.7	11.5	0.84	13.6	1.29	1.33	5.21
Brightwell (GA)[i,m]	07/28/97	26.2	16.0	0.26	61.5	1.23	1.29	4.71
Tifblue (GA)[i,m]	07/28/97	18.3	17.0	0.35	48.6	1.23	1.27	4.76
Little Giant (MI)[l]	08/18/97	14.3	10.0	0.85	11.8	0.96	0.49	2.89
means (Rabbiteye)		*18.4 ± 0.8*	*13.2 ± 1.12*	*0.58 ± 0.10*	*29.8 ± 8.36*	*1.20 ± 0.05*	*1.14 ± 0.13*	*4.53 ± 0.34*
				Vaccinium angustifolium (Lowbush)				
Lowbush (ME)[j]	09/03/97	31.3	16.5	0.20	80.8	0.73	0.27	1.67
overall means		20.9 ± 1.4	14.3 ± 0.2	0.47 ± 0.06	41.6 ± 6.6	1.17 ± 0.05	1.15 ± 0.11	4.45 ± 0.31

[a] Dry matter, %. [b] Total soluble solids, °Brix. [c] Total titratable acid, milliequivalents per g of dry matter. [d] Weight, grams per berry. [e] Calculated surface area, cm^2. [f] Source: USDA-ARS, Hort Crops Research Laboratory, Corvalis, OR. [g] Source: USDA-ARS, Blueberry & Cranberry Research Center, Chatsworth, NJ. [h] Source: Berryhill Foods, Abbotsford, British Columbia, Canada. [i] Source: University of Georgia, Tifton, GA. [j] Source: Tru Blu CoOp, New Lisbon, NJ. [k] Source: North Carolina State University, Castle Hayne, NC. [l] Source: MBG Marketing, Grand Junction, MI. [m] Very late harvest, after most harvest for fresh-market sales was completed.

Table 5. Harvest Dates, Total Solids, and Acid Content of Berries from Different Varieties of *Vaccinium corymbosum* L. Not Readily Available Commercially and from Bilberry (*Vaccinium myrtillus* L.)

cultivar, state, and source	DM[a] (%)	TSS[b] (°Brix)	TTA[c] (mequiv/g of DM)	TSS/TTA	diam (cm)	wt[d] (g/berry)	SA[e] (cm^2)
			Vaccinium corymbosum L. (Southern Highbush)				
Reveille (NC)[f]	19.2	14.5	0.57	25.3	1.10	0.86	3.83
Cape Fear (NC)[f]	16.8	14.5	0.79	18.4	1.3	1.45	5.33
Pender (NC)[f]	16.9	13.0	0.93	13.9	1.10	0.88	3.79
Bladen (NC)[f]	18.3	13.5	0.66	20.6	1.00	0.66	3.14
means (S. Highbush)	*17.6 ± 0.50*	*13.9 ± 0.38*	*0.74 ± 0.08*	*19.6 ± 2.4*	*1.12 ± 0.06*	*0.96 ± 0.17*	*4.02 ± 0.46*
			Vaccinium myrtillus L.				
Bilberry[m]	15.8	10.0	1.52	6.60	0.84	0.40	2.21

[a] Dry matter, %. [b] Total soluble solids, °Brix. [c] Total titratable acid, milliequivalents per gram dry matter. [d] Weight, grams per berry. [e] Calculated surface area, cm^2. [f] Source: North Carolina State University, Castle Hayne, NC. Samples harvested on 6/4/97. [g] Samples harvested on 7/2/97.

oxidants such as ascorbate, glutathione, etc. Thus, in these studies, we also determined the anthocyanin and total phenolic concentrations in the different blueberry samples. Previous reports of anthocyanin content in *Vaccinium* species have also indicated a large variation. Highbush blueberries (*V. corymbosum L.*) have been reported to have an anthocyanin content of 25−495 mg/100 g (Mazza and Miniati, 1993). By using HPLC techniques, Gao and Mazza (1994) found that two highbush blueberry cultivars contained about 100 mg of anthocyanins/100 g and most lowbush blueberry (*V. angustifolium* Ait.) cultivars contained 150−200 mg of anthocyanins/100 g. Kalt and McDonald (1996) also reported that lowbush blueberries had about 138 mg of anthocyanins/100 g. Highbush blueberry and lowbush blueberry are the primary species of blueberries used by the food industry in the United States. Rabbiteye blueberries (*V. ashei* Reade), grown in the southern U. S., have been reported to have an anthocyanin content of 210 (Tifblue) to 272 (Bluegem) mg/100 g (Ballinger et al., 1979; Gao and Mazza, 1994). Bilberry (*V. myrtillus* L.), native to parts of Europe and northern regions of Asia, has been reported to have the highest anthocyanin content (300−698 mg of anthocyanin/100 g) (Mazza and Miniati, 1993). We observed anthocyanin concentrations in the range of 62 mg/100 g for cv. Reveille blueberries to 300 mg/100 g for bilberries (*V. myrtillus* L.; Table 1). Our results in general seem to be a little lower than some of the other reports; however, the particular anthocyanin compound used as a standard and its associated molar absorption coefficient can influence the absolute amounts calculated. It is likely that bilberry from different sources will have similar variability as observed with blueberry, which must be recognized in any comparisons in the present data. Unfortunately, we were not in a position to obtain multiple samplings of bilberry.

The 3-glucoside(s) and 3-galactoside(s) of delphinidin, malvidin, petunidin, cyanidin, and peonidin are the primary anthocyanins that have been identified in blueberries (Mazza and Miniati, 1993; Gao and Mazza, 1994). The anthocyanin content of the different blueberry samples was linearly related to the ORAC measurement ($r_{xy} = 0.77$; $p < 0.01$) (Figure 1); however, the

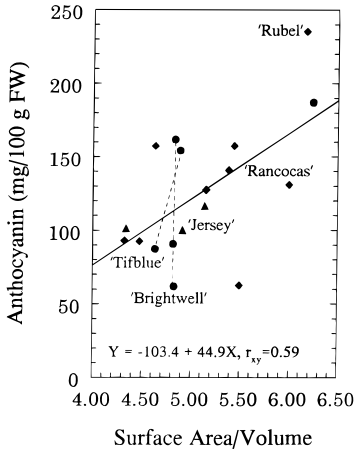

Figure 4. Relationship between anthocyanin concentrations (X) (mg/100 g of fresh weight) to calculated blueberry surface area/volume ratio (Y) in different cultivars of *Vaccinium* species. ($Y = -103.4 + 44.9X$; $r_{xy} = 0.59$; $n = 18$) ♦, *Vaccinium corymbosum L.* (highbush); ▲, cv. Jersey (Highbush); ●, Rabbiteye. Data for the two points for the early and late harvest dates for cv. Tifblue and cv. Brightwell are connected by a line.

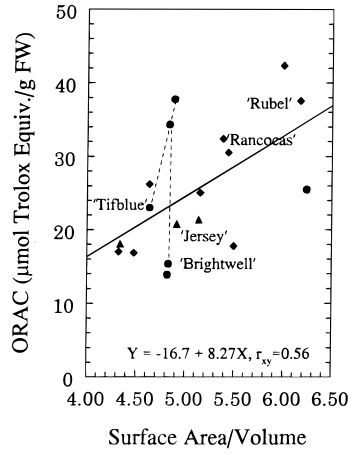

Figure 5. Relationship between ORAC concentrations (X) (μmol Trolox equiv/g of fresh weight) to calculated blueberry surface area/volume ratio (Y) in different cultivars of *Vaccinium* species. ($Y = -16.7 + 8.27X$; $r_{xy} = 0.56$; $n = 18$) ♦, *Vaccinium corymbosum L.* (highbush); ▲, cv. Jersey (Highbush); ●, Rabbiteye. Data for the two points for the early and late harvest dates for cv. Tifblue and cv. Brightwell are connected by a line.

agreement as indicated by the correlation coefficient was not as high as between total phenolics and ORAC ($r_{xy} = 0.85$; $p < 0.01$) (Figure 2), although both were significant.

Because the anthocyanins seem to be concentrated in the skin of the blueberry (the bilberry may be an exception), it is expected that the total anthocyanins would increase in proportion to the calculated surface area/volume ratio of the blueberries. In general, this relationship held across cultivars within the *V. corymbosum* and *V. ashei* Reade species. However, changes in anthocyanin content at different stages of maturity can cause this relationship to break down as indicated by the cv. Tifblue and cv. Brightwell cultivars. Anthocyanins increased markedly with maturity, but the surface area/volume estimate did not change.

The phytochemicals responsible for the antioxidant capacity most likely can be accounted for by the phenolic acids, anthocyanins, and other flavonoid compounds (Cao et al., 1997). We are in the process of identification of the compounds represented in our HPLC chromatograms, the results of which will be published at a later time.

The polyphenolic components present within blueberries may have multiple health benefits which at this point are difficult to understand. The potential beneficial effects of the high antioxidant capacity and protection of cells from free radical attack seem clear (Yu, 1994; Halliwell, 1994), but other possible effects which might be independent of antioxidant effects remain open to question. Anthocyanins in blueberries may have potential health benefits that are independent of or in addition to their antioxidant effects. Several studies have been undertaken with a highly purified extract of *V. myrtillus* L., designated Myrtocyan, which contains 36% anthocyanosides (Morazzoni and Bombardelli, 1996). Cyanidin 3-glucoside, a major component of Myrtocyan, was shown to be the most active compound tested against carbon tetrachloride induced lipoperoxidation (Morazzoni and Bombardelli, 1996) and was the anthocyanin with the highest ORAC that we tested (Wang et al., 1997). In addition to the antioxidant activity, Myrtocyan has been shown to (1) prevent or control interstitial fluid formation and contribute to controlling the blood flow redistribution in the microvascular network, (2) modulate capillary resistance and permeability, improving visual function by promoting dark adaptation after dazzling, (3) promote wound-healing, and (4) have antiulcer and antiatherosclerotic activity (Morazzoni and Bombardelli, 1996; Murray, 1997). However, studies have not been done to determine whether consumption of anthocyanins from other *Vaccinium* species might have similar health benefits. On the basis of our measurements of antioxidant capacity, other *Vaccinium* species might be good sources of anthocyanins and other antioxidants.

Studies are continuing in our laboratory of the implications of consuming foods containing increased quantities of ORAC. The antioxidant rich phytochemicals in strawberries have been shown in rat models to reduce or retard the central nervous system deficits seen in aging (Bickford et al., 1997) and to protect against the oxidative stress caused by 100% oxygen exposure (Sofic et al., 1997). Since the antioxidant capacity of blueberries is higher than for strawberries, a benefit of consuming antioxidants from blueberries would also be expected. Furthermore, consumption of a more concentrated source of antioxidants will have the greatest impact on in vivo antioxidant capacity. We have estimated that normal intake in humans of antioxidants as measured by ORAC within the U.S. is in the range of 1.2−1.7 mmol ORAC/day (Prior et al., unpublished data). Increases in serum ORAC are observed with intakes of 3−4 mmol Trolox equiv/day, and some individuals have been observed to have ORAC intakes as high as 6 mmol/day (Cao et al., unpublished data). Consumption of $^1/_2$ cup of blueberries/day (72.5 g) would increase ORAC intake by 1−3.2 mmol, depending upon the blueberry variety and maturity. Thus, the ORAC of the blueberry source can have marked effects on the total daily ORAC intake.

ABBREVIATIONS USED

ORAC, oxygen radical absorbance capacity; AAPH, 2,2′-azobis(2-amidinopropane) dihydrochloride; Trolox,

6-hydroxy-2,5,7,8-tetramethyl-2-carboxylic acid; ODS, octadecylsiloxane; TE, trolox equivalents.

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