

Original article

Antioxidant capacities vary substantially among cultivars of rabbiteye blueberry (*Vaccinium ashei* Reade)

Shiow Y. Wang,^{1*} Hangjun Chen^{1,2} & Mark K. Ehlenfeldt³

1 Genetic Improvement of Fruits and Vegetables Laboratory, Beltsville Agricultural Research Center, Agricultural Research Service, U. S. Department of Agriculture, Beltsville, MD 20705-2350, USA

2 Food Science Institute, Zhejiang Academy of Agricultural Sciences, Hangzhou, 310021, Zhejiang, China

3 Genetic Improvement of Fruits and Vegetables Laboratory, U. S. Department of Agriculture, Agricultural Research Service, Located at the Marucci Center for Blueberry and Cranberry Research and Extension, Rutgers University, Chatsworth, NJ 08019, USA

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Summary Fruits from forty-two blueberry cultivars, including thirty-six rabbiteye (*Vaccinium ashei* Reade), three *V. ashei* hybrid derivatives and three northern highbush (*V. corymbosum* L.) standards, were evaluated for their antioxidant activities against peroxy free radicals (ROO·), hydroxyl radicals (OH·), hydrogen peroxide (H₂O₂), superoxide radicals (O₂⁻) and singlet oxygen (¹O₂) radicals. The differences in scavenging capacities for these radicals among forty-two selected blueberry cultivars were significant. Oxygen radical absorbance capacity values ranged from 33.8 to 118.7 μmol Trolox equivalents (TE) g fresh wt⁻¹, 196.1 to 518.8 μmol TE g dry wt⁻¹ and 7.1 to 22.2 μmol cm⁻²-surface area. Extracts from fruit of pure rabbiteye had higher levels of scavenging capacities of oxygen species O₂⁻, ¹O₂ and H₂O₂ compared to *V. ashei* hybrid derivatives and northern highbush blueberry standards. The rabbiteye cultivars 'Early May' and 'Centurion' had the highest scavenging capacity for the reactive oxygen species, not only for ROO· and ·OH, but also for O₂⁻, ¹O₂ and a strong oxidant, H₂O₂. In contrast, 'Pink Lemonade' (pink-fruited) had the lowest ability to inhibit free radical activity of ROO·, ·OH, ¹O₂, and H₂O₂. 'Snowflake' had the lowest scavenging capacity for O₂⁻. Blueberry cultivars with high antioxidant activity and radical scavenging capacity have potential to improve human health and can possibly be used as parents for future blueberry breeding programs to develop new blueberry cultivars with higher antioxidant activity.

Keywords Antioxidant capacity, reactive oxygen species, *Vaccinium* species.

Introduction

Blueberries contain high levels of antioxidant compounds and high antioxidant capacity (Prior *et al.*, 1998; Wang & Jiao, 2000; Ehlenfeldt & Prior, 2001; Connor *et al.*, 2002a,b). Antioxidants are compounds that can delay or inhibit the oxidation of lipids, nucleic acids and/or other molecules by inhibiting the initiation or propagation of oxidising chain reactions. Eating fruits and vegetables has shown to reduce blood pressure, enhances the immune system, detoxifies contaminants and pollutants and reduces inflammation (Löf *et al.*, 2011). Berry fruits have been shown to possess radical scavenging and antioxidant capacity. Consumption of berry fruits has been associated with lower incidence and mortality rates of cancer in several human cohort and

case-control studies (Soerjomataram *et al.*, 2010; Jing & Giusti, 2011). A high intake of berry fruits has also been reported to prevent urinary tract infections, enhance the immune function and reduce blood pressure and cardiovascular diseases (Löf *et al.*, 2011).

Berry fruits not only possess peroxy radical (ROO·) scavenging capacity but also have antioxidant activities against other biological free radicals such as superoxide radicals (O₂⁻), hydroxyl radicals (·OH), singlet oxygen (¹O₂) and a strong oxidant, hydrogen peroxide (H₂O₂) (Wang & Jiao, 2000). Antioxidant compounds differ in their ability to scavenge different reactive oxygen species, and genotypic variation in antioxidant compounds among berry fruits has been shown to have markedly different effects on antioxidant capacity (Prior *et al.*, 1998; Wang & Jiao, 2000; Ehlenfeldt & Prior, 2001; Connor *et al.*, 2002a,b). The purpose of this study was to evaluate and select cultivars with high free radical scavenging capacities of peroxy radical (ROO·), super-

*Correspondent: Fax: +1 301 504 5107;
e-mail: shiow.wang@ars.usda.gov

oxide radicals ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), hydroxyl radicals ($\cdot OH$) and singlet oxygen (1O_2) radicals in blueberries. The forty-two tested cultivars included thirty-six rabbiteye cultivars along with three *V. ashei* hybrid derivatives and three northern highbush blueberry standards.

Materials and methods

Fruit sample handling and preparation

Blueberry fruits (*Vaccinium* species) used in this study were grown at USDA-ARS plots at the Marucci Center for Blueberry and Cranberry Research and Extension Center, Chatsworth, NJ, USA. Fully mature (100% blue) blueberries were hand-harvested at bush maturity from 45% to 100% with the most typical value being approximately 60%. Approximately 500–900 g of fruits were harvested per genotype from test plots, and forty-two different genotypes were sampled and used for this study. This included thirty-six rabbiteye (*Vaccinium ashei* Reade) blueberry cultivars along with three *V. ashei* hybrid derivatives and three northern highbush blueberry standards as shown in Tables 1–3. Berries were initially frozen in a $-70^\circ C$ freezer, then transported to Beltsville with freezer packs in a cooler and ultimately stored at $-80^\circ C$ until they were used for analysis.

For dry weight measurement, fruits were dried in the oven at $70^\circ C$ for 72 h. The unit of antioxidant activity from fresh weight (fw) then converted to dry wt (dw) basis. The surface areas were calculated using the formula $4\pi(d/2)^2$ with $\pi = 3.1416$ and d = average equatorial diameter of the berries of a given cultivar.

Triplicate composite 5 g samples cut from twenty fruits each per cultivar were extracted three times with 50% acetone using a Polytron (Brinkmann Instruments, Inc., Westbury, NY, USA). The homogenised samples from the acetone extracts were then centrifuged at 14 000 g for 20 min at $4^\circ C$. The supernatants were combined with final volumes 25 mL, then transferred to vials, stored at $-80^\circ C$ and later used for measuring oxygen radical scavenging capacity.

Antioxidant properties of blueberry fruit extracts

Peroxyl radical ($ROO\cdot$) assay

The $ROO\cdot$ assay was carried out using a high-throughput instrument platform consisting of a robotic eight-channel liquid handling system (Precision 2000; Bio-Tek Instrument, Winooski, VT, USA) and a microplate fluorescence reader (FL800; Bio-Tek Instrument). Final oxygen radical absorbance capacity (ORAC) values were calculated using the regression equation between Trolox concentration and the net area under the curve (AUC) (Huang *et al.*, 2002) and were expressed as μmol

Trolox equivalents (TE) per gram of fresh weight (g fw), μmol TE per gram of dry weight (g dw) and μmol TE per square centimetre-surface area (cm^2 -SA).

Hydroxyl radical scavenging capacity ($\cdot OH$; HOSC) assay

The HOSC assay was conducted with acetone solutions according to a previously published protocol (Moore *et al.*, 2006) with some modifications. Reaction mixtures consisted of 170 μL of 9.28×10^{-8} M fluorescein prepared in 75 mM sodium phosphate buffer, 30 μL of standard or sample or blank, 40 μL of 0.1990 M H_2O_2 and 60 μL of 3.43 mM $FeCl_3$. Trolox prepared in 50% acetone at concentrations of 20, 40, 60, 80 and 100 μM was used to prepare the standard curve. The HOSC values were determined by calculating the net AUC of the standards and samples. Final HOSC values were calculated using the regression equation between Trolox concentration and the net AUC and were expressed as μmol TE per gram fw, μmol TE per gram dw and μmol TE per square centimetre-SA.

Hydrogen peroxide (H_2O_2) assay

The assay for hydrogen peroxide in fruit extracts of blueberry was carried out following procedures previously described by Patterson *et al.* (1984). The antioxidant capacity of fruit extract against H_2O_2 value was expressed as mg ascorbate equivalents (asc-eq) per gram fw, mg asc-eq per gram dw and mg asc-eq per square centimetre-SA.

Superoxide radical ($O_2^{\cdot-}$) assay

The assay for superoxide radical ($O_2^{\cdot-}$) was determined using the methods of Richmond *et al.* (1981). The $O_2^{\cdot-}$ was generated by the xanthine oxidase system. The antioxidant capacity of fruit extract against $O_2^{\cdot-}$ value was expressed as mg asc-eq) per gram fw, mg asc-eq per gram dw and mg asc-eq per square centimetre-SA.

Singlet oxygen (1O_2) assay

The assay for singlet oxygen (1O_2) in blueberry extracts was according to Chakraborty & Tripathy (1992) with minor modifications in which *N, N*, dimethyl-*p*-nitrosoaniline was used as a selective scavenger of 1O_2 and histidine as a trap for 1O_2 acceptor. The bleaching of *N, N*, dimethyl-*p*-nitrosoaniline as induced by the reaction of 1O_2 with histidine was monitored spectrophotometrically at 440 nm. The extent of 1O_2 production was determined by measuring the decrease in absorbance of *N, N*, dimethyl-*p*-nitrosoaniline at 440 nm. The scavenging capacity of ascorbate at various concentrations (1–10 μg) on singlet oxygen (1O_2) was measured and used for determining the 1O_2 scavenging capacity of berry extracts. The antioxidant capacity of berry extracts against 1O_2 was expressed as asc-eq per gram fw, mg asc-eq per gram dw and mg asc-eq per square centimetre-SA.

Table 1 Cultivar variations of scavenging capacities for peroxy radicals (ROO·) and hydroxyl radicals (·OH) from fruit extracts of forty-two blueberry cultivars (thirty-six *Vaccinium ashei*, three *V. ashei* derivative hybrids and three northern highbush standards)

Blueberry cultivar	Peroxy radicals (ROO·; ORAC)			Hydroxy radicals (·OH)		
	μmol g fw ⁻¹	μmol g dw ⁻¹	μmol cm ⁻² -SA	μmol g fw ⁻¹	μmol g dw ⁻¹	μmol cm ⁻² -SA
<i>V. ashei</i> Reade (rabbiteye)						
Alapaha	49.8 de	263.8 e	11.2 ef	60.3 fgh	319.6 i	13.6 fg
Aliceblue	82.4 pqr	438.8 x	18.7 s	73.1 kl	383.6 mn	16.6 kl
Austin	45.2 c	251.2 d	10.8 de	47.7 e	256.9 e	11.4 e
Baldwin	63.6 fg	362.9 m-p	15.8 k-o	68.0 ij	386.9 no	16.9 lm
Beckyblue	33.8 a	196.1 a	7.1 a	35.3 bc	196.2 b	9.5 d
Bluebelle	46.9 cd	297.1 g	12.6 g	43.5 de	274.9 f	11.7 e
Bluegem	92.3 s	454.0 y	20.1 tu	113.9 tu	563.4 y	24.9 s
Bonita	62.4 f	340.9 ij	15.2 i-l	64.9 hi	348.1 k	15.8 jk
Brightwell	60.4 f	335.3 hi	13.8 h	59.4 fg	327.2 j	13.6 fgh
Briteblue	76.8 mno	417.7 w	17.4 pqr	87.7 op	475.3 v	19.9 o
Callaway	69.8 ijk	357.7 lmn	14.7 h-k	84.1 no	421.9 r	17.7 mn
Centurion	81.3 pq	503.4 z	20.4 u	115.4 tu	687.2 z	28.9 t
Chaucer	61.2 f	293.9 g	15.7 k-n	68.4 ijk	327.1 j	17.5 lmn
Choice	76.0 lmn	386.1 st	18.5 rs	72.6 jkl	378.6 lm	17.7 mn
Clara	90.6 s	387.8 tu	17.0 opq	118.0 u	504.6 x	22.1 qr
Climax	86.0 r	372.1 pqr	19.0 st	98.1 r	436.0 t	21.7 q
Coastal	49.4 cde	280.8 f	11.5 efg	55.6 f	297.7 h	12.9 f
Delite	39.2 b	241.6 cd	9.6 c	40.1 cd	245.2 d	9.8 d
Early May	118.7 t	518.8 a	22.2 v	164.5 v	745.4 a	33.6 u
Ethel	73.6 klm	365.3 nop	16.1 l-o	64.5 hi	319.7 i	14.1 ghi
Garden Blue	67.6 ghi	364.1 nop	15.6 k-n	63.8 ghi	344.1 k	14.7 hij
Homebell	75.6 lmn	349.8 jkl	18.0 qrs	92.1 pq	432.3 t	21.9 qr
Ira	70.2 ijk	364.1 nop	16.9 opq	75.9 lm	391.6 op	18.3 n
Menditoo	68.7 ij	326.1 h	15.5 k-n	90.4 p	423.9 rs	20.5 op
Montgomery	61.4 f	377.7 qrs	14.0 hi	65.7 i	405.9 q	14.9 ij
Myers	83.1 qr	368.5 opq	17.3 pqr	95.4 qr	419.4 r	19.9 o
Owen	94.1 s	397.0 uv	18.3 rs	112.1 st	469.4 uv	21.9 q
Powderblue	64.2 fgh	353.2 klm	15.9 l-o	79.4 mn	394.7 p	19.7 o
Premier	67.3 ghi	358.3 lmn	16.5 nop	81.2 n	429.1 st	19.9 o
Satilla	60.2 f	344.7 ijk	14.3 hij	76.0 lm	423.3 rs	18.0 mn
Southland	68.3 hi	381.4 rst	16.4 m-p	91.2 pq	504.3 x	21.9 qr
Suwanee	61.6 f	297.0 g	12.4 fg	76.4 lm	375.6 l	15.4 j
Tifblue	76.7 l-o	414.3 w	19.2 st	92.0 pq	495.1 w	23.0 r
Walker	49.1 cde	263.0 e	12.2 fg	57.0 f	296.2 h	14.1 ghi
Windy	68.0 hi	383.6 st	15.2 j-m	75.7 lm	422.3 rs	17.0 lm
Woodard	80.8 opq	414.8 w	14.7 h-k	108.2 s	562.7 y	19.6 o
Mean	68.8	356.2	15.5	79.7	407.9	18.1
<i>V. ashei</i> hybrid derivatives						
Pearl River	38.4 b	225.9 b	8.6 bc	39.2 cd	229.6 c	8.8 cd
Snowflake	52.8 e	404.5 v	9.4 c	64.1 ghi	489.7 w	9.8 d
Pink Lemonade	37.3 ab	233.7 bc	7.1 a	14.8 a	93.6 a	3.0 a
Mean	42.8	288.0	8.4	39.4	271.0	7.2
<i>V. corymbosum</i> L. (northern highbush)						
Bluecrop	46.0 cd	250.5 d	11.5 efg	31.5 b	194.6 b	7.9 bc
Duke	45.7 cd	303.1 g	9.7 cd	43.8 de	285.0 g	9.3 d
Elliott	78.5 nop	372.5 pqr	16.0 l-o	97.3 r	464.3 u	19.8 o
Mean	56.7	308.7	12.4	57.6	314.6	12.3

fw, fresh weight; dw, dry weight; SA, surface area.

Means within same column followed by different letters were significantly different at $P \leq 0.05$.

Table 2 Cultivar variations in scavenging capacities for hydrogen peroxide (H₂O₂) and superoxide radicals (O₂⁻) from fruit extracts of forty-two blueberry cultivars (thirty-six *Vaccinium ashei*, three *V. ashei* derivative hybrids and three northern highbush standards)

Blueberry cultivar	Hydrogen peroxidase (H ₂ O ₂)			Superoxide radicals (O ₂ ⁻)		
	μmol g fw ⁻¹	μmol g dw ⁻¹	μmol cm ⁻² -surface area	μmol g fw ⁻¹	μmol g dw ⁻¹	μmol cm ⁻² -SA
<i>V. ashei</i> Reade (rabbiteye)						
Alapaha	1.8 bc	9.5 bc	40.2 b-e	3.3 bcd	17.3 bc	0.73 cd
Aliceblue	3.4 op	17.9 rs	76.2 qrs	4.0 j-o	21.4 j-o	0.91 i-p
Austin	2.7 h-l	14.8 j-q	63.9 k-p	3.8 e-n	20.9 g-n	0.90 h-p
Baldwin	3.1 mno	17.9 rs	77.9 rs	3.9 f-n	22.1 mno	0.96 n-q
Beckyblue	1.2 a	7.0 a	33.3 bc	3.1 b	17.5 bcd	0.84 d-m
Bluebelle	2.0 b-e	12.4 d-i	53.2 g-j	3.4 b-f	21.2 h-o	0.91 i-p
Bluegem	3.9 q	19.0 st	84.1 st	4.2 no	21.8 h-o	1.01 q
Bonita	2.3 e-i	12.7 e-j	57.1 h-l	3.7 d-m	20.2 e-m	0.90 h-p
Brightwell	2.5 g-k	14.1 h-n	58.3 h-m	3.6 b-j	19.7 c-m	0.82 c-j
Briteblue	3.0 l-o	16.4 o-r	68.3 n-r	3.8 e-n	20.9 g-n	0.87 f-n
Callaway	2.7 i-l	13.4 f-l	56.2 h-k	3.6 c-k	18.0 b-e	0.76 cde
Centurion	4.0 q	23.4 u	96.9 u	4.3 no	25.3 p	1.05 q
Chaucer	2.2 d-g	10.8 b-e	57.1 h-l	3.5 b-g	16.7 b	0.88 g-p
Choice	3.2 mno	16.0 n-r	76.8 qrs	3.9 g-o	19.7 c-m	0.95 m-q
Clara	3.8 pq	16.0 n-r	70.3 o-r	4.1 k-o	17.3 bc	0.76 c-f
Climax	3.3 no	14.3 i-p	72.0 pqr	4.2 mno	18.3 b-f	0.92 j-p
Coastal	2.6 g-k	13.5 f-m	60.0 i-n	3.9 f-o	20.3 e-n	0.90 h-p
Delite	2.1 b-f	12.7 e-j	50.3 fgh	3.3 bcd	20.2 e-m	0.80 c-h
Early May	4.5 r	20.6 t	92.9 tu	4.3 p	26.2 p	1.04 q
Ethel	2.9 k-n	14.6 j-q	63.9 k-p	3.8 e-n	19.0 b-k	0.83 d-l
Garden Blue	2.9 j-n	15.2 k-q	66.1 l-p	3.8 e-n	19.9 d-m	0.87 f-n
Homebell	3.2 no	15.0 k-q	76.3 qrs	4.1 l-o	19.3 c-k	0.98 opq
Ira	2.6 g-k	13.2 f-k	62.1 j-o	3.7 d-l	18.8 b-i	0.88 g-p
Menditoo	2.7 i-m	12.8 e-j	61.8 j-o	3.7 d-m	17.4 bc	0.84 d-m
Montgomery	2.2 d-h	13.9 g-n	51.1 f-i	3.5 b-i	21.9 l-o	0.81 c-i
Myers	3.2 no	14.0 h-n	67.4 m-q	4.0 h-o	17.4 bc	0.84 d-m
Owen	4.0 q	16.8 qr	77.5 rs	4.1 l-o	17.3 rs	0.80 c-h
Powderblue	2.9 j-n	14.2 i-o	70.7 o-r	3.8 e-n	18.7 b-h	0.93 k-p
Premier	2.6 g-k	13.6 f-m	63.0 k-p	4.0 i-o	21.3 i-o	0.99 pq
Satilla	2.4 e-i	13.8 g-n	56.7 h-l	3.7 d-m	21.5 k-o	0.88 g-p
Southland	3.0 k-o	16.5 pqr	71.0 o-r	4.1 k-o	22.8 no	0.98 opq
Suwanee	2.4 f-j	11.9 d-h	49.2 e-h	3.9 g-o	18.9 b-j	0.78 c-g
Tifblue	2.9 j-n	15.4 k-q	71.3 o-r	3.9 f-n	20.8 f-n	0.96 n-q
Walker	1.7 b	8.8 ab	41.5 c-f	3.5 b-h	18.5 b-g	0.87 g-o
Windy	2.8 i-m	15.5 l-q	61.8 j-o	3.8 e-n	21.2 h-o	0.85 e-m
Woodard	3.1 mno	15.7 m-r	72.7 pqr	4.0 i-o	20.2 e-m	0.94 l-p
Mean	2.8	14.5	64.7	3.8	20.0	0.89
<i>V. ashei</i> hybrid derivatives						
Pearl River	1.8 bc	10.4 bcd	39.2 bcd	3.2 bc	18.8 b-i	0.71 bc
Snowflake	2.2 c-g	16.7 qr	24.8 ab	2.1 a	11.4 a	0.46 a
Pink Lemonade	1.1 a	6.6 a	21.1 a	3.1 b	19.6 c-l	0.62 b
Mean	1.7	11.2	28.4	2.8	16.6	0.60
<i>V. corymbosum</i> L. (northern highbush)						
Bluecrop	1.8 bcd	11.4 c-f	45.8 d-g	3.4 b-e	21.0 h-n	0.84 d-m
Duke	1.8 bc	11.7 c-g	37.2 bc	3.5 b-i	23.6 op	0.75 cde
Elliott	3.2 no	15.4 k-q	66.1 l-p	4.0 j-o	19.2 c-k	0.82 d-k
Mean	2.3	12.9	49.7	3.6	21.3	0.80

fw, fresh weight; dw, dry weight; SA, surface area.

Means within same column followed by different letters were significantly different at ≤0.05.

Table 3 Cultivar variations in scavenging capacities for singlet oxygen ($^1\text{O}_2$) radicals from fruit extracts of forty-two blueberry cultivars (thirty-six *Vaccinium ashei*, three *V. ashei* derivative hybrids and three northern highbush standards)

Blueberry cultivar	Singlet oxygen ($^1\text{O}_2$)		
	$\mu\text{mol g fw}^{-1}$	$\mu\text{mol g dw}^{-1}$	$\mu\text{mol cm}^{-2}\text{-SA}$
<i>V. ashei</i> Reade (rabbiteye)			
Alapaha	0.25 b-f	1.35 a-d	5.71 cde
Aliceblue	0.32 k-p	1.68 k-n	7.16 j-o
Austin	0.27 b-i	1.48 b-k	6.39 e-k
Baldwin	0.30 h-p	1.70 mn	7.38 l-p
Beckyblue	0.25 a-d	1.39 a-g	6.64 g-m
Bluebelle	0.28 d-k	1.74 n	7.50 m-p
Bluegem	0.33 p	1.60 h-n	7.09 i-o
Bonita	0.29 e-p	1.56 e-n	6.99 h-o
Brightwell	0.28 d-l	1.56 f-n	6.46 e-k
Briteblue	0.31 j-p	1.69 lmn	7.02 h-o
Callaway	0.30 h-p	1.48 b-k	6.21 d-i
Centurion	0.34 p	1.96 o	8.12 p
Chaucer	0.26 b-h	1.27 a	6.71 g-m
Choice	0.30 h-p	1.52 c-m	6.27 d-i
Clara	0.32 l-p	1.36 a-e	5.97 c-g
Climax	0.32 m-p	1.41 a-h	7.09 h-o
Coastal	0.27 c-j	1.43 a-i	6.35 e-j
Delite	0.23 ab	1.45 a-j	5.74 c-f
Early May	0.38 q	1.71 mn	7.70 op
Ethel	0.31 j-p	1.54 c-m	6.73 g-n
Garden Blue	0.28 d-m	1.49 b-l	6.51 e-l
Homebell	0.30 i-p	1.41 a-h	7.17 j-o
Ira	0.28 d-k	1.43 a-i	6.72 g-m
Menditoo	0.29 e-o	1.34 a-d	6.50 e-l
Montgomery	0.27 c-j	1.69 lmn	6.20 d-h
Myers	0.31 j-p	1.34 abc	6.44 e-k
Owen	0.32 nop	1.36 a-e	6.28 d-j
Powderblue	0.29 g-p	1.47 a-j	7.28 k-p
Premier	0.30 h-p	1.57 f-n	7.27 k-p
Satilla	0.28 d-j	1.59 g-n	6.53 e-l
Southland	0.29 f-p	1.62 i-n	6.96 h-o
Suwanee	0.28 e-n	1.39 a-f	5.73 cde
Tifblue	0.31 j-p	1.64 j-n	7.62 nop
Walker	0.27 d-j	1.43 a-i	6.78 g-n
Windy	0.28 d-j	1.56 e-n	6.22 d-i
Woodard	0.30 i-p	1.52 c-m	7.05 h-o
Mean	0.29	1.52	6.74
<i>V. ashei</i> hybrid derivatives			
Pearl River	0.24 abc	1.40 a-g	5.28 c
Snowflake	0.26 b-g	1.37 a	2.94 a
Pink	0.21 a	1.31 ab	2.17 a
Mean	0.23	1.36	3.46
<i>V. corymbosum</i> L. (northern highbush)			
Bluecrop	0.25 b-e	1.57 f-n	6.30 d-j
Duke	0.26 b-g	1.71 mn	5.43 cd
Elliott	0.32 op	1.55 d-n	6.62 f-m
Mean	0.28	1.61	6.12

fw, fresh weight; dw, dry weight; SA, surface area.

Means within same column followed by different letters were significantly different at $P \leq 0.05$.

Statistical analysis

Data were subjected to analysis of variance using SPSS (2008). Values for antioxidant capacity of $\text{ROO}\cdot$, $\text{O}_2^{\cdot-}$, $\text{OH}\cdot$, $^1\text{O}_2$ and H_2O_2 were evaluated by the Duncan's test. Differences at $P \leq 0.05$ were considered significant. Correlation coefficients (r) among scavenging activities of $\text{ROO}\cdot$ radicals vs. antioxidant activities of $\text{O}_2^{\cdot-}$, H_2O_2 , $\text{OH}\cdot$ and $^1\text{O}_2$ were also calculated using SPSS (2008) and were reported as r values.

Results

Antioxidant activity

The differences in scavenging capacity of $\text{ROO}\cdot$, $\text{O}_2^{\cdot-}$, H_2O_2 , $\text{OH}\cdot$ and $^1\text{O}_2$ radicals among forty-two selected blueberry cultivars were significant. ORAC values ranged from 33.8 to 118.7 $\mu\text{mol TE g fw}^{-1}$, 196.1 to 518.8 $\mu\text{mol TE g dw}^{-1}$ and 7.1 to 22.2 $\mu\text{mol cm}^{-2}\text{-SA}$. High radical scavenging capacity for $\text{ROO}\cdot$ (ORAC) was found in rabbiteye cultivars 'Early May', 'Owen', 'Bluegem' and 'Clara', while 'Beckyblue' had the lowest. This was approximately a 3.5-fold difference between the highest and lowest values (Table 1). If expressed on the basis gram dw or square centimetre-SA, the same trend was found between cultivars for the lowest and the highest, but the differences from cultivar to cultivar for ORAC values were less, and a 2.6-fold difference on dry wt basis and a 3.1-fold difference on square centimetre-SA basis were found between lowest and highest values (Table 1). 'Early May', 'Bluegem', 'Centurion' and 'Aliceblue' all had high ORAC values when expressed as fresh wt, dry wt or square centimetre-SA basis.

Among *V. ashei* hybrid derivatives, 'Snowflake' had higher ORAC values than 'Pink Lemonade' and 'Pearl River', but the mean ORAC value of *V. ashei* hybrid derivatives was lower than the mean of thirty-six rabbiteye cultivars on the basis of fw, dw or square centimetre-SA. Among the northern highbush cultivars, 'Elliott' had higher ORAC values compared to other two northern highbush cultivars 'Bluecrop' and 'Duke' and the mean of thirty-six rabbiteye cultivars (Table 1).

Blueberries had high scavenging capacity against $\cdot\text{OH}$ radicals. A wide variation was found among blueberry cultivars in their ability to react and quench $\cdot\text{OH}$ radicals (Table 1). The scavenging capacities ranged from 14.8 to 164.5 $\mu\text{mol TE g fw}^{-1}$, 93.6 to 745.4 $\mu\text{mol TE g dw}^{-1}$ and 3.0 to 33.6 $\mu\text{mol TE cm}^{-2}\text{-SA}$, reflecting 11.1-fold (expressed as fw), 8.0-fold (expressed as dw) or 11.4-fold (expressed as SA) differences among cultivars (Table 1). The rabbiteye cultivars 'Early May' and 'Centurion' had the highest scavenging activities against $\cdot\text{OH}$ radicals, whereas *V. ashei* hybrid derivative 'Pink Lemonade' (50% rabbiteye: 17% *V. darrowii* and 33% *V. corymbosum*) and the northern highbush 'Bluecrop'

had the lowest $\cdot\text{OH}$ scavenging efficiency. Other cultivars with high $\cdot\text{OH}$ scavenging capacity were rabbiteyes 'Aliceblue', 'Baldwin', 'Bluegem' and 'Owen' (Table 1).

Among the three *V. ashei* hybrid derivatives used in this study, 'Snowflake' (75% rabbiteye and 25% *V. constablaei*) had higher scavenging capacity for $\cdot\text{OH}$ radicals than both 'Pearl River' (50% rabbiteye and 50% southern highbush) and 'Pink Lemonade' as expressed on fw, dw or SA basis. However, the $\cdot\text{OH}$ radical quenching capacity of 'Snowflake' was lower than the mean values of thirty-six rabbiteyes (Table 1). 'Bluecrop', 'Duke' and 'Elliott' are northern highbush blueberries (*V. corymbosum* L.). 'Elliott' had the highest $\cdot\text{OH}$ scavenging capacity compared to 'Bluecrop' and 'Duke', and its antioxidant activity for scavenging $\cdot\text{OH}$ radicals also was higher than the mean values of thirty-six rabbiteyes. Among the three blueberry types (rabbiteye, *V. ashei* hybrid derivatives and northern highbush blueberry) used in this study, berry extracts from rabbiteye had the highest mean of antioxidant activities, whereas *V. ashei* hybrid derivatives had the lowest.

The scavenging capacity for H_2O_2 as expressed as asc-eq ranged from 1.1 to 4.5 mg asc-eq g fw⁻¹, 6.6 to 23.4 mg asc-eq g dw⁻¹ and 21.1 to 96.9 mg asc-eq cm⁻²-SA. Among different blueberry cultivars, rabbiteye 'Early May' and 'Centurion' had the highest antioxidant capacity for H_2O_2 , whereas 'Beckyblue' and 'Pink Lemonade' had the lowest values (Table 2).

The scavenging capacity of these blueberry cultivars against $\text{O}_2^{\cdot-}$ ranged from 2.1 to 4.3 mg asc-eq g fw⁻¹, 11.4 to 26.2 mg asc-eq g dw⁻¹ and 0.5 to 1.1 mg asc-eq cm⁻²-SA (Table 2), and the scavenging capacity for $^1\text{O}_2$ ranged from 0.2 to 0.4 mg asc-eq g fw⁻¹, 1.3 to 2.0 mg asc-eq g dw⁻¹ and 2.2 to 8.1 mg asc-eq cm⁻²-SA (Table 3). The rabbiteye cultivars 'Early May' and 'Centurion' had the best scavenging capacity for the reactive oxygen species not only for $\text{ROO}\cdot$ and $\cdot\text{OH}$, but also for H_2O_2 , $\text{O}_2^{\cdot-}$ and $^1\text{O}_2$ (Tables 1–3). Meanwhile, 'Pink Lemonade' had the lowest ability to scavenging free radical activity for H_2O_2 and $^1\text{O}_2$, and 'Snowflake' had the lowest scavenging capacity for $\text{O}_2^{\cdot-}$. Among different blueberry types, extracts from rabbiteye berries had the highest scavenging activities against H_2O_2 , $\text{O}_2^{\cdot-}$ and $^1\text{O}_2$ compared with *V. ashei* hybrid derivatives and northern highbush blueberries.

The correlation coefficients (*r*) among the antioxidant activities of $\text{ROO}\cdot$, $\cdot\text{OH}$, H_2O_2 , $\text{O}_2^{\cdot-}$ and $^1\text{O}_2$ from berry extracts of forty-two cultivars (thirty-six rabbiteye, three *V. ashei* hybrid derivatives and three northern highbush standards), when expressed as fresh weight, ranged from 0.88 to 0.94. There was a positive correlation between $\text{ROO}\cdot$ scavenging activities and $\cdot\text{OH}$, H_2O_2 , $\text{O}_2^{\cdot-}$ and $^1\text{O}_2$ with *r* values 0.93, 0.93, 0.94 and 0.94, respectively. The correlation values between $\cdot\text{OH}$ radical scavenging and H_2O_2 , $\text{O}_2^{\cdot-}$ and $^1\text{O}_2$ were 0.90, 0.88 and 0.91, respectively. On dry matter basis or on SA basis, the

same patterns of correlations among scavenging activities for $\text{ROO}\cdot$, $\cdot\text{OH}$, H_2O_2 , $\text{O}_2^{\cdot-}$ and $^1\text{O}_2$ were also found (data not shown).

Discussion

Blueberries represent one of the most important sources of bioactive compounds with respect to antioxidant activities. Rabbiteye blueberries, grown in the southern United States, have been reported to have high anthocyanin contents (Gao & Mazza, 1994). Several genetic and environmental factors have been reported to affect the antioxidant activity in blueberry fruits (Wang & Jiao, 2000; Ehlenfeldt & Prior, 2001; Connor *et al.*, 2002a,b). The present study has shown that different cultivars of blueberries have varying degrees of scavenging capacity for different active oxygen species including $\text{ROO}\cdot$, $\cdot\text{OH}$, H_2O_2 , $\text{O}_2^{\cdot-}$ and $^1\text{O}_2$ (Tables 1–3). This is probably due to the genetic variation in flavonoid content and composition in the berries. Chlorogenic acid, resveratrol, myricetin 3-arabinoside, quercetin 3-galactoside, quercetin 3-glucoside, delphinidin 3-galactoside, delphinidin 3-glucoside, cyanidin 3-galactoside, cyanidin 3-glucoside, delphinidin 3-arabinoside, petunidin 3-galactoside, petunidin 3-glucoside, petunidin 3-arabinoside, malvidin 3-galactoside, malvidin 3-glucoside and malvidin 3-arabinoside were found to be varied substantially among the forty-two blueberry cultivars examined in this study, and the contribution of individual phenolics to total antioxidant capacity was generally dependent on their content and structure in the berries (Data available upon request). Previous study reported that the antioxidant activity of myricetin was higher than that of quercetin for ORAC values (Wang *et al.*, 1997). Kaempferol, with a structure related to that of quercetin, had just 27% of quercetin's antioxidant activity. Quercetin and kaempferol are potent quenchers of $\text{ROO}\cdot$ and $^1\text{O}_2$ (Larson, 1988). The antioxidant activities of different individual anthocyanins were also different and have been ranked as follows: delphinidin > cyanidin > malvidin > pelargonidin (Satué-Gracia *et al.*, 1997).

The rabbiteye blueberry cultivars 'Early May', 'Centurion', 'Bluegem', 'Clara', 'Owen', 'Climax', 'Aliceblue', 'Myers' and 'Baldwin' had higher antioxidant capacity compared to three *V. ashei* hybrid derivatives and three northern blueberries to suppress free radicals. 'Pink Lemonade', a *V. ashei* hybrid derivative and a pigmentation mutant (Ehlenfeldt & Finn, 2007), had the lowest antioxidant capacity. This may be due to its low content of anthocyanin and phenolics. Substantial differences in antioxidant capacities ($\text{ROO}\cdot$, $\text{O}_2^{\cdot-}$, H_2O_2 , $\cdot\text{OH}$ and $^1\text{O}_2$ radicals) among different blueberry cultivars were found in this study. Antioxidant capacity was calculated and expressed on fw vs. dw basis or SA basis. If expressed on fw, the antioxidant activity for

ROO \cdot scavenging activity followed the order of 'Early May' > 'Owen' = 'Bluegem' = 'Clara' > 'Climax' > 'Aliceblue' > 'Myers' > 'Centurion' > 'Woodard'. If expressed on dw, the ranks were 'Early May' > 'Bluegem' > 'Aliceblue' > 'Briteblue' > 'Woodard' = 'Tifblue' > 'Snowflake' > 'Owen' > 'Clara' = 'Choice' = 'Windy', whereas when expressed on square centimetre-SA, the ranks were 'Early May' > 'Centurion' > 'Bluegem' > 'Tifblue' > 'Climax' = 'Aliceblue' = 'Choice' = 'Owen'. The difference in ranking order with different expressions may be due to the variation in dry matter content among fruits of different cultivars as well as variation in fruit size. Smaller fruit in general had higher surface area per unit of weight; this resulted in higher flavonoid content in smaller berry fruit than larger fruit and in turn had higher antioxidant activity (Ehlenfeldt & Prior, 2001). Anthocyanins and other phenolic compounds are confined principally to the fruit skin that is a significant contributor to the high antioxidant activity in blueberry (Prior *et al.*, 1998; Kalt *et al.*, 1999); thus, variation in antioxidant activity among cultivars may simply reflect smaller berry size of those cultivars with higher activity (Ehlenfeldt & Prior, 2001; Connor *et al.*, 2002a,b). Ehlenfeldt & Prior (2001) suggested that antioxidant activity in blueberries should be standardised to surface area for breeders to compare antioxidant activity in blueberry genotypes or cultivars, but blueberries are not perfectly spherical and that skin thickness can also vary among cultivars. In general, flavonoid content was higher in the outer pericarp fruit tissue compared to pulp of fruit.

'Clara', 'Owen' and 'Woodard' are small fruits (0.84–0.86 g per berry), whereas 'Aliceblue', 'Bluegem', 'Centurion', 'Climax', 'Early May' and 'Myer' are medium-sized fruits (1.18–1.59 g per berry-fresh wt). 'Bluecrop', a northern highbush cultivar, (2.46 \pm 0.57 g per berry) has larger fruit than the average of blueberries tested. It is the leading commercial variety in North America and has a largest surface area (1032.6 mm² per berry) per fruit among the forty-two cultivars. If we calculated the whole berry on fresh wt or total SA basis, the antioxidant activity of 'Bluecrop' for ROO \cdot radical scavenging capacity was 113.1 μ mol TE per berry-fw or 118.8 μ mol TE per berry-SA. In comparison, the antioxidant activity for ROO \cdot radical scavenging capacity of the smallest berries, 'Woodard', was 69.5 μ mol TE per berry-fw or 74.9 μ mol TE per berry-SA. These findings suggest that selection for high antioxidant activity in blueberries will not necessarily result in concomitant selection for small berry size or large surface area, because berries are not perfectly spherical and that skin thicknesses also vary among cultivars as mentioned earlier, but it is useful in standardising values so no bias occurs in relative ranking of antioxidant activity based on size.

Sellapan *et al.* (2002) found the average content of antioxidant capacity of rabbiteye blueberries was higher than those of southern highbush. Howard *et al.* (2003) reported ORAC values of eighteen blueberry cultivars and showed a 2.9-fold difference among cultivars. Other studies also reported that ORAC values among blueberry genotypes varied 1.8-fold (Kalt *et al.*, 1999), 2.5-fold (Prior *et al.*, 1998), 3.3-fold (Sellapan *et al.*, 2002), 4.7-fold (Connor *et al.*, 2002a,b), 5.2-fold (Moyer *et al.*, 2002) or 6.8-fold (Ehlenfeldt & Prior, 2001). These studies indicate that ample genetic variation exists for exploitation by plant breeders. Moyer *et al.* (2002) evaluated thirty genotypes of nine species of *Vaccinium* and found *V. ashei* had the highest antioxidant capacity with ORAC. The antioxidant activity (ORAC values) of some blueberry cultivars (such as 'Bluecrop', 'Duke', 'Climax' and 'Tifblue') measured in this study is substantially different from those previously reported by others (Prior *et al.*, 1998; Ehlenfeldt & Prior, 2001; Connor *et al.*, 2002a,b). This may reflect differences in environmental conditions such as variations in ultraviolet radiation, temperature, water stress, mineral nutrient availability, or differences in extraction and assay methods. In general, the ORAC values were 1.5–2.0 times higher by using fluorescein as fluorescent probe than those obtained using β -phycoerythrin.

Blueberries not only possess antioxidant activities against ROO \cdot radicals but also have the capacity to scavenge H₂O₂, \cdot OH, O₂⁻ and ¹O₂. Imbalances in the production and metabolism of ROS can cause oxidative stress and lead to cell death (Mahalingam & Fedoroff, 2003). Cells contain several mechanisms to inactivate these reactive oxygen species and repair or replace damaged cellular molecules to maintain cellular homeostasis (Yu, 1994). A multiplicity of antioxidants in blueberries is beneficial because specific antioxidant molecules can be particularly effective for neutralising specific reactive oxygen species. Hydroxyl radicals are short-lived but are the most damaging radicals within the body. Hydrogen peroxide is naturally produced in organisms as a by-product of oxygen metabolism. Hydrogen peroxide is unique in that it can be converted to the highly damaging hydroxyl radical or be catalysed and excreted harmlessly as water. The oxidizing capacity of hydrogen peroxide is considered a highly reactive oxygen species, and although short-lived, it can damage DNA and lipids, ultimately leading to the damage of cellular membranes (Pryor, 1986; Ames *et al.*, 1993). Peroxyl radicals (ROO \cdot) are formed in biological systems and are important oxidants found in cells whose ability to react with the DNA and cause damage is well established. Superoxide anions are formed when oxygen acquires an additional electron, leaving the molecule with only one unpaired electron. Within the mitochondria, O₂⁻ is formed continuously, with the rate of formation dependent on the amount of oxygen

flowing through the mitochondria at any given time (Pryor, 1986). Singlet oxygen is another reactive oxygen species, can transfer the energy to a new molecule and act as a catalyst for free radical formation. The molecule can also interact with other molecules leading to the formation of a new free radical. Singlet oxygen has electrons in an excited state that react destructively with biomolecules containing double bonds and is linked to oxidation of LDL cholesterol (Pryor, 1986). Anything that boosts the immune system is protective against chronic diseases, but antioxidants have an additional anti-chronic disease effect through protection against DNA damage (Yu, 1994; Mahalingam & Fedoroff, 2003; Jing & Giusti, 2011).

Conclusions

Our results show that fruits of *V. ashei* are good sources of antioxidants and providing high scavenging activities against the ROO \cdot , O $_2^{\cdot-}$, H $_2$ O $_2$, \cdot OH and 1 O $_2$. A considerable variation exists in antioxidant activity among blueberry cultivars, which clearly shows the potential value of certain cultivars for use in breeding programs to develop new blueberry cultivars with higher antioxidant capacity. Even through years of breeding may be required for the selection of a good genotype, cultivars with high antioxidant trait could be used as good parentage for crosses to develop new varieties with high antioxidants. With few exceptions, usually if the parents are superior to a trait, the progeny will also be superior to that trait (Finn, 2009). Heritability for antioxidant activity and total phenolic and anthocyanin content in blueberry progenies have been reported (Connor *et al.*, 2002a,b). Moreover, development of new blueberry fruit with high antioxidant capacity may also stimulate additional interest in the nutraceutical and functional food aspects resulting in greater blueberry consumption.

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