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Antioxidant capacity and α-glucosidase inhibitory activity in peel and flesh of blueberry (*Vaccinium* spp.) cultivars

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ABSTRACT

This study was designed to evaluate cultivar variations in phenolic content, anthocyanin content, and antioxidant activity of peel and flesh; and to determine their potential inhibitory effects on α-glucosidase in 33 blueberry (*Vaccinium* species) cultivars, including 29 rabbiteye (*Vaccinium ashei* Reade) blueberries, two *V. ashei* hybrid derivatives, and two northern highbush (*Vaccinium corymbosum* L.). The relation of phenolic, anthocyanin, and antioxidant activity to α-glucosidase inhibition in blueberries also was investigated. It was found that peel tissue possessed higher levels of total anthocyanins (TA), total phenolics (TP), antioxidant capacity, and α-glucosidase inhibitory activity than flesh tissue in all blueberries tested. The percentage contributions of peel to whole berry on scavenging capacity against peroxyl free radicals (ROO·), hydroxyl radicals (·OH), hydrogen peroxide (H_2O_2), and singlet oxygen ($^1O_2$) radicals, were higher than those of flesh, even though the fruit contained much higher amounts of flesh than peel in terms of dry weight. Cultivars with high levels of phenolic compounds, antioxidant capacities, and α-glucosidase inhibitory activities could be selected for use in blueberry breeding programs to develop new lines with improved health benefits.

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1. Introduction

Blueberries are an excellent source for antioxidants that include phenolics acids, tannins, flavonols, and anthocyanins (Connor, Luby, & Tong, 2002; Ehlenfeldt & Prior, 2001; Prior et al., 1998; Wang & Jiao, 2000). Antioxidants help neutralise free radicals which are unstable molecules linked to the development of a number of diseases including cancer, cardiovascular disease, and other age-related conditions. Antioxidant compounds absorbed from our diet are thought to play a role in preventing these and other diseases that result from oxidative damage. Berries of *Vaccinium* species have been shown to possess high radical scavenging capacity (Moyer, Hummer, Finn, Frei, & Wrolstad, 2002; Taruscio, Barney, & Exon, 2004; Viljanen, Kylli, Kivikari, & Heinonen, 2004; Zheng & Wang, 2003). Therefore, diets rich in phytonutrients such as blueberries may reduce the risk of many types of chronic disease (Ames, Shigena, & Hagen, 1993; Ascherio et al., 1992; Heinonen, Meyer, & Frankel, 1998).

It has been shown that blueberry (cv. Berkley) extracts are efficient inhibitors of α-glucosidase (McDougall et al., 2005; McDougall & Stewart, 2005). α-Glucosidases are enzymes that catalyse the absorption of digested glucose from dietary polysaccharides in the small intestine. Inhibition of α-glucosidase is considered one of the effective measures for regulating type II diabetes by controlling glucose uptake (Puls, Keup, Krause, Thomas, & Hoffmeister, 1977). Type II diabetes is a major health concern worldwide. In the United States, an estimated 25.8 million people suffer from diabetes, and another 79 million are considered pre-diabetic (American Diabetes Association, 2011). Diabetes is a chronic condition that develops when the body becomes resistant to insulin or when the pancreas stops producing enough insulin; untreated type II diabetes can be life-threatening. It has been reported that acylated anthocyanins inhibit α-glucosidase and reduce glucose uptake in rats (Matsui et al., 2001a, 2001b). α-Glucosidase inhibition by small-fruit extracts is related to their anthocyanin content. Therefore, phenolic phytochemicals potentially provide a natural source of α-glucosidase inhibitors. Accumulated evidence has also suggested that diabetic patients are under oxidative stress, with an imbalance between free-radical-generating and radical-scavenging capacities (Maritim, Sanders, & Watkins, 2003). Augmentation of radical scavenging capacity by a diet rich in berries may help reduce this oxidative stress.

It is well known that wide differences in antioxidant activities exist among cultivars and between species. However, little is

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known about the varietal variations in the inhibition of α-glucosidase activities. There are also no reports on the differences in phenolic content and α-glucosidase activities between peel and flesh of the blueberries. The objective of this research therefore, was to study the effect of cultivar on phenolic content, anthocyanin content, antioxidant activity of peel and flesh, and to determine their potential inhibitory effect of α-glucosidase in 33 blueberry cultivars. The relation of phenolic, anthocyanin, and antioxidant activity to α-glucosidase inhibition in blueberries was also investigated.

2. Materials and methods

2.1. Chemicals

2′,2′-Azobis (2-amidinopropane) dihydrochloride (AAPH) was obtained from Wako Chemicals USA Inc., (Richmond VA). Ascorbate, fluorescein disodium, $FeCl_3$, histidine, 2-deoxy-D-ribose, hydrogen peroxide (30% w/w), manganese dioxide, hypoxanthine, sodium hydrochloride, *p*-nitrophenyl-α-d-glucopyranoside (*p*NPG), *N,N*-dimethyl-*p*-nitrosoaniline, xanthine, xanthine oxide, and yeast α-glucosidase (EC 3.2.1.20), were obtained from Sigma Chemical Co. (St. Louis, MO), EDTA (ethylenediaminetetracetic acid, disodium salt, dihydrate-Na_2 EDTA·$2H_2O$), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), and titanium (IV) chloride, and thiobarbituric acid were obtained from Aldrich Chemical Co. (Milwaukee, WI). All other chemicals and solvents were of the highest commercial grade and used without further purification.

2.2. Fruit sample handling and preparation

Blueberry fruit (*Vaccinium* species) used in this study were grown at USDA-ARS plots at the Marucci Center for Blueberry and Cranberry Research and Extension 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 fruit was harvested per genotype from test plots of two plants, and 33 different genotypes were sampled and used for this study. This included 29 rabbiteye (*Vaccinium ashei* Reade) blueberry cultivars, along with two *V. ashei* hybrid derivatives and two northern highbush blueberry standards as shown in Tables 1–5. The fruit were sorted to eliminate damaged, shrivelled, and unripe fruit. Fruit was then selected for uniform size and colour. Berries were initially frozen in a −70 °C freezer, then transported to Beltsville with freezer packs in a cooler, and ultimately stored at −80 °C until they were used for analysis.

Table 1
Total anthocyanins (TA), total phenolics (TP), and scavenging capacity of peroxyl radicals (ROO·; ORAC) from flesh and peel extracts of 33 blueberry cultivars (29 *V. ashei*, two *V. ashei* derivative hybrids, and two northern highbush standards) as express on dry weight (dw).

Blueberry cultivar	TA (mg/100 g dw)		TP (mg/100 g dw)		ORAC (μmol TE/g dw)	
	Flesh	Peel	Flesh	Peel	Flesh	Peel
Vaccinium ashei Reade (rabbiteye)						
Aliceblue	339.8 jk[a]	4892.2 q	394.2 h	3193.1 s	328.3 n	678.3 p
Austin	363.0 i	4613.0 w	358.4 j	2841.8 vw	422.0 j	634.2 q
Beckyblue	93.8 u	4708.8 t	188.9 p	2681.7 x	164.6 t	533.7 t
Bluebelle	117.2 t	4839.8 r	219.4 o	2545.7 y	175.8 st	611.5 r
Bluegem	314.8 mn	4664.6 uv	368.7 i	3591.3 p	422.2 j	876.7 k
Briteblue	408.7 g	6241.1 f	410.9 gh	5230.3 f	443.1 hi	1483.4 e
Callaway	557.9 b	7373.7 c	520.1 c	5929.7 b	607.4 b	1683.5 b
Centurion	385.0 h	5794.1 k	288.8 m	5711.6 c	356.0 l	1527.5 cd
Chaucer	196.1 qr	5217.8 o	151.1 q	3151.8 t	143.3 u	804.9 lm
Choice	635.6 a	8028.2 b	692.1 a	5355.8 e	701.9 a	1517.6 cd
Clara	429.1 f	6098.1 g	355.6 ijk	5174.8 g	345.0 lm	1187.5 g
Coastal	433.9 f	5994.4 h	459.3 de	4752.6 k	521.1 d	1007.7 i
Delite	384.3 h	5839.3 j	473.4 d	3663.7 o	502.3 e	798.7 m
Early May	319.0 mn	5972.8 h	449.6 ef	5021.5 i	489.4 f	1539.7 c
Ethel	392.6 h	5699.7 l	444.7 f	3582.9 p	467.7 g	824.3 l
Garden Blue	306.1 n	4435.8 y	250.2 n	3418.9 r	331.3 n	666.9 p
Homebell	334.0 kl	4609.9 w	420.2 g	2827.1 w	394.2 k	505.1 u
Ira	203.1 q	4636.8 vw	221.8 o	2509.2 z	308.1 o	559.1 s
Menditoo	451.8 e	4850.0 r	296.7 m	4198.8 l	454.1 h	1014.1 i
Myers	480.4 d	5548.6 n	565.9 b	4927.9 j	442.2 i	1110.5 h
Owen	388.2 h	5661.3 m	474.7 d	4090.8 n	413.8 j	1512.1 d
Premier	175.2 rs	5013.1 p	189.0 p	3436.8 r	167.5 t	707.2 o
Satilla	320.8 lm	7018.5 d	356.9 ijk	5646.2 d	334.5 mn	1480.4 e
Southland	528.6 c	8804.7 a	511.2 c	5128.2 h	600.4 b	1683.9 b
Suwanee	468.2 d	8814.9 a	474.2 d	6987.3 a	569.7 c	2243.7 a
Tifblue	237.1 p	4687.2 tu	347.0 k	4100.6 n	352.7 l	865.7 k
Walker	347.7 j	4558.2 x	363.8 ij	2865.1 v	447.1 hi	622.1 qr
Windy	203.0 q	4010.8 z	333.8 l	2820.4 w	355.5 l	514.4 tu
Woodard	345.7 jk	6965.8 e	404.1 h	3650.9 o	448.0 hi	1345.1 f
Mean	350.4	5710.1	378.8	4104.7	403.8	1053.1
Vaccinium ashei hybrid derivatives						
Pearl River	178.8 s	5879.8 i	195.0 p	2962.1 u	182.9 s	800.6 m
Pink Lemonade	384.5 h	693.0 aa	255.2 n	984.4 aa	231.0 q	401.4 v
Mean	281.7	3286.4	225.1	1973.3	207.0	601.0
Vaccinium corymbosum L. (northern highbush)						
Bluecrop	255.8 o	4750.4 s	296.9 m	4142.3 m	287.5 p	958.9 j
Duke	248.1 op	5196.8 o	251.5 n	3551.5 q	199.7 r	768.7 n
Mean	252.0	4973.6	274.2	3846.9	243.6	863.8

Values within the same column followed by different letters were significantly different at $p < 0.05$.
[a] Data were expressed as means of three assays for each cultivars.

Table 2
Scavenging capacity of hydrogen peroxidase (H_2O_2), singlet oxygen ($^1O_2$), and hydroxyl radicals ($^{\cdot}OH$) from flesh and peel extracts of 33 blueberry cultivars (29 *V. ashei*, two *V. ashei* derivative hybrids, and two northern highbush standards) as express on dry weight (dw).

Blueberry cultivars	H_2O_2 (mg ascorbate/10 g dw)		$^1O_2$ (mg ascorbate/10 g dw)		$^{\cdot}OH$ (μmol TE/g dw)	
	Flesh	Peel	Flesh	Peel	Flesh	Peel
Vaccinium ashei Reade (rabbiteye)						
Aliceblue	11.1 d-h[a]	52.9 g-l	10.9 c-i	67.3 ij	399.0 gh	1535.5 n
Austin	12.3 c-f	45.5 m-p	8.6 ghi	62.5 kl	356.2 kl	1276.2 q
Beckyblue	5.9 p	49.4 j-p	5.8 no	55.5 mn	230.7 p	955.2 t
Bluebelle	7.1 l-p	44.7 op	5.1 opq	67.3 ij	232.2 p	923.8 u
Bluegem	10.8 e-h	53.6 f-l	8.3 f-l	78.9 gh	363.9 k	1748.9 k
Briteblue	13.6 c	66.8 a-e	8.5 hi	117.8 cd	379.5 ij	1911.6 g
Callaway	18.6 b	78.0 a	16.6 a	162.9 a	419.4f	2135.6 c
Centurion	13.4 c	72.3 a-d	15.2 b	116.3 d	417.1 f	2206.3 b
Chaucer	7.6 k-p	51.8 g-m	4.3 pq	60.6 klm	108.3 s	1518.1 o
Choice	21.8 a	69.0 a-d	17.4 a	127.7 b	663.0 a	2077.3 e
Clara	10.1 ghi	63.2 c-g	9.9 efg	108.3 de	354.7 kl	1924.8 g
Coastal	13.1 cd	57.7 e-k	11.0 de	76.7 h	433.1 e	1830.4 i
Delite	9.5 h-k	50.8 klm	10.3 d-h	77.4 h	400.2 g	1751.4 k
Early May	10.5 f-i	61.8 d-h	10.7 def	99.9 f	319.0 m	2076.5 e
Ethel	13.1 cd	51.5 j-m	13.1 c	84.0 g	414.3 fg	1641.2 m
Garden Blue	9.1 h-l	48.2 j-p	6.1 mno	63.1 jkl	250.4 o	1523.6 no
Homebell	11.6 c-g	50.8 klm	12.6 cd	74.8 hi	381.3 ij	1260.2 q
Ira	8.5 i-n	45.9 nop	7.0 i-o	57.1 m	228.8 p	903.5 v
Menditoo	13.9 c	60.0 e-i	8.5 hi	65.0 jk	382.5 ij	1826.5 i
Myers	11.7 c-g	65.1 b-f	10.6 d-g	104.0 ef	540.2 b	1867.8 h
Owen	8.6 i-m	51.0 j-o	8.0 g-m	57.3 m	387.1 hi	1775.7 j
Premier	6.4 op	49.0 j-p	6.7 i-o	56.6 lmn	208.4 q	1623.5 m
Satilla	10.0 g-j	72.9 abc	11.1 de	127.2 b	275.2 n	1982.0 f
Southland	13.7 c	71.6 a-d	13.3 cd	132.6 b	494.9 c	2094.3 d
Suwanee	12.7 cde	73.7 abc	8.1 e-p	154.0 a	482.0 d	2429.8 a
Tifblue	11.0 e-h	57.6 f-l	4.9 n-q	61.3 klm	315.1 m	1705.4 l
Walker	10.4 f-i	43.1 p	3.6 q	41.9 o	375.0 j	1005.3 s
Windy	9.8 g-h	47.6 m-p	4.8 n-q	55.2 mn	348.3 l	1142.1 r
Woodard	10.9 e-h	65.1 b-f	7.5 il	84.1 g	385.3 hij	1742.1 k
Mean	11.3	57.6	9.3	86.1	363.6	1668.8
Vaccinium ashei hybrid derivatives						
Pearl River	6.5 nop	46.7 m-p	6.2 l-o	51.3 n	207.8 q	1372.0 p
Pink Lemonade	8.0 j-o	17.0 q	1.4 r	3.8 p	136.1 r	453.4 w
Mean	7.3	31.8	3.8	27.6	171.9	912.7
Vaccinium corymbosum L. (northern highbush)						
Bluecrop	9.3 h-k	56.4 f-j	8.9 fk	81.4 gh	310.6 m	1821.6 i
Duke	6.6 m-p	52.8 h-m	7.2 i-n	64.7 jk	260.9 no	1700.2 l
Mean	8.0	54.6	8.1	73.0	285.7	1760.9

Values within the same column followed by different letters were significantly different at $p < 0.05$.
[a] Data were expressed as means of three assays for each cultivars.

Each blueberry fruit was separated into peel and flesh. For dry weight measurement, peel and flesh were dried in the oven at 70 °C for 72 h. The relative contributions of peel and flesh for a given cultivar were obtained by first multiplying the amounts of TA, TP, or antioxidant activities (per dry weight unit), with the actual dry weight of the peel or flesh from each fruit. These values from peel or flesh were then divided by the total values of whole fruit and then multiplied 100 to get the percentage of contributions from peel or flesh.

Triplicate composite 4 g of peel and 12 g of flesh cut from 25 to 35 fruit each per cultivar were extracted four times with 50% acetone using a Polytron (Brinkmann Instruments, Inc., Westbury, NY). The homogenised samples from the acetone extracts were then centrifuged at 14,000*g* for 20 min at 4 °C. The supernatants were combined and final volume of 50 ml was transferred to vials, stored at −80 °C until analysis for total anthocyanins, total phenolics, antioxidant activity, and α-glucosidase inhibitory activity assay.

2.3. Total phenolic content (TP) and anthocyanin (TA) assay

Total soluble phenolics in the fruit extract were determined with Folin–Ciocalteu reagent. Since the Folin–Ciocalteu assay could be affected by several interfering substances, such as sugars, aromatic amine, sulphur dioxide, ascorbic acid, organic acids, Fe (II), as well as some non-phenolic organic substances (Box, 1983; Peterson, 1979), a solid-phase extraction (SPE) procedure was used to remove such water soluble compounds from fruit extract samples. Five millilitres from the above acetone–formic acid extracts were concentrated to 1 ml using a Buchler Evapomix (Fort Lee, NJ) in a water bath at 30 °C. The concentrated samples were dissolved in 4 ml of acidified water (3% formic acid) then passed through a C_{18} Sep–Pak cartridge (Waters), which was previously activated with methanol followed by water and 3% aqueous formic acid. The interfering substances such as sugars, ascorbic acid, organic acids, and non-phenolic organic substances that react with Folin–Ciocalteu were all passed through C_{18} Sep–Pak column. Anthocyanins and other phenolics were retained by the column and then recovered with 5.0 ml of acidified methanol containing 3% formic acid. Total soluble phenolics were then determined with Folin–Ciocalteu reagent by the method of Slinkard and Singleton (1977), using gallic acid as standard. Results were expressed as milligrams gallic acid equivalent (GAE), in the blueberry extract, per 100 g dry weight (dw).

Total anthocyanin contents in blueberry extract were determined by using the pH differential method (Cheng & Breen, 1991). Absorbance was measured in a Shimadzu Spectrophotometer (Shimadzu UV-160) (Shimadzu Scientific Instruments, Inc.,

Table 3
Peel to flesh ratios (by dry weight) for total anthocyanins (TA), total phenolics (TP), scavenging capacity for peroxyl radicals (ROO·), hydroxyl radicals (·OH), hydrogen peroxidase (H_2O_2), and singlet oxygen ($^1O_2$) from peel and flesh extracts of 33 blueberry cultivars (29 *V. ashei*, two *V. ashei* derivative hybrids, and two northern highbush standards).

Blueberry cultivar	Ratio peel/flesh (on dry weight basis)					
	TA	TP	ORAC	·OH	H_2O_2	$^1O_2$
Vaccinium ashei Reade (rabbiteye)						
Aliceblue	14.4	8.1	2.1	3.8	4.8	6.2
Austin	12.7	7.9	1.5	3.6	3.7	7.2
Beckyblue	50.2	14.2	3.2	4.1	8.4	9.6
Bluebelle	41.3	11.6	3.5	4.0	6.3	13.3
Bluegem	14.8	9.7	2.1	4.8	5.0	9.6
Briteblue	15.3	12.7	3.3	5.0	4.9	13.9
Callaway	13.2	11.4	2.8	5.1	4.2	9.8
Centurion	15.1	19.8	4.3	5.3	5.4	7.7
Chaucer	26.6	20.9	5.6	14.0	6.8	14.3
Choice	12.6	7.7	2.2	3.1	3.2	7.3
Clara	14.2	14.6	3.4	5.4	6.3	11.0
Coastal	13.8	10.3	1.9	4.2	4.4	7.0
Delite	15.2	7.7	1.6	4.4	5.4	7.5
Early May	18.7	11.2	3.2	6.5	5.9	9.4
Ethel	14.5	8.1	1.8	4.0	3.9	6.4
Garden Blue	14.5	13.7	2.0	6.1	5.3	10.3
Homebell	13.8	6.7	1.3	3.3	4.4	5.9
Ira	22.8	11.3	1.8	3.9	5.4	8.2
Menditoo	10.7	14.2	2.2	4.8	4.3	7.7
Myers	11.6	8.7	2.5	3.5	5.6	9.9
Owen	14.6	8.6	3.7	4.6	5.9	7.2
Premier	28.6	18.2	4.2	7.8	7.7	8.4
Satilla	21.9	15.8	4.4	7.2	7.3	11.4
Southland	16.7	10.0	2.8	4.2	5.2	10.0
Suwanee	18.8	14.7	3.9	5.0	5.8	19.0
Tifblue	19.8	11.8	2.5	5.4	5.3	12.5
Walker	13.1	7.9	1.4	2.7	4.1	11.7
Windy	19.8	8.4	1.4	3.3	4.9	11.5
Woodard	20.1	9.0	3.0	4.5	6.0	11.2
Mean	18.6	11.6	2.7	5.0	5.4	9.8
Vaccinium ashei hybrid derivatives						
Pearl River	32.9	15.2	4.4	6.6	7.2	8.2
Pink Lemonade	1.8	3.9	1.7	3.3	2.1	2.8
Mean	17.3	9.5	2.4	5.0	5.3	5.5
Vaccinium corymbosum L. (northern highbush)						
Bluecrop	18.6	15.2	3.3	5.9	6.1	9.1
Duke	20.9	3.9	3.8	6.5	7.9	8.9
Mean	19.8	9.5	3.6	6.2	7.0	9.0

Columbia, MD) at 510 and 700 nm in buffers at pH 1.0 and 4.5, using $A = [(A_{510} - A_{700})_{pH\ 1.0} - (A_{510} - A_{700})_{pH\ 4.5}]$, with a molar extinction coefficient of cyanidin 3-glucoside (26,900) for blueberries. Results were expressed as milligrams of cyanidin 3-glucoside equivalent per 100 g dw.

2.4. Peroxyl radicals assay (ROO·)

The peroxyl radicals (ROO·) 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) and a microplate fluorescence reader (FL800; Bio-Tek Instrument, Winooski, VT) (Huang, Ou, Hampsch-Woodill, Flanagan, & Prior, 2002). Final ORAC values were calculated using the regression equation between Trolox concentration and the net area under the curve (AUC) and were expressed as μmol Trolox equivalents (TE) per gram of dw.

2.5. Hydroxyl radical scavenging capacity (·OH; HOSC) assay

The HOSC assay was conducted with acetone solutions according to a previously published protocol (Moore, Yin, & Yu, 2006), with some modifications. The ·OH in aqueous media is generated through the Fenton reaction. The assay was carried out using a high-throughput instrument platform consisting of a robotic eight-channel liquid handling system and a microplate fluorescence reader with a FL800 microplate fluorescence reader (Bio-Tek Instruments, Inc., Winooski, VT). Fluorescence was measured every minute for 3 h with an excitation wavelength of 485 nm and emission wavelength of 535 nm. The plate reader was controlled by software KC4 3.0 (revision 29). Sample dilution was accomplished by a Precision 2000 automatic pipetting system, managed by precision power software (version 1.0) (Bio-Tek Instruments, Inc.). 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 0.199 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 for HOSC quantification. The HOSC values were determined by calculating the net area under the curve (AUC) of the standards and samples. The standard curve was obtained by plotting Trolox (TE) concentrations against the average net AUC of the three measurements for each concentration. Final HOSC values were calculated using the regression equation between TE concentration and the net AUC and were expressed as μmol TE/g dw.

2.6. 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, MacRae, and Ferguson (1984). The antioxidant capacity of fruit extract against H_2O_2 value was expressed as mg ascorbate equivalent/10 g dw.

2.7. Singlet oxygen ($^1O_2$) assay

The production of singlet oxygen ($^1O_2$) by sodium hypochloride and H_2O_2 was quantified by using a spectrophotometric method according to Chakraborty and 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 mg ascorbate equivalent/10 g dw.

2.8. Purification of blueberry extracts and α-glucosidase (EC 3.2.1.20) inhibition assay

The 40 ml of supernatants from above extracts were concentrated to dryness using a Buchler Evapomix (Fort Lee, NJ) in a water bath at 35 °C, dissolved in 10 ml of acidified water (3% formic acid). This was then passed through a C_{18} Sep–Pak cartridge (Waters), which was previously activated with methanol followed by water and then 3% aqueous formic acid. Anthocyanins and other phenolics were adsorbed onto the column, while sugars, acids, and other water-soluble compounds were diluted with 15 ml of 3% formic acid. Anthocyanins and other phenolics were then recovered with 10.0 ml of acidified methanol containing 3% formic acid and then lypophilised and used for α-glucosidase inhibitory assay.

The α-glucosidase inhibitory effects of the aqueous extracts of the 33 blueberry cultivars were assayed according to the procedure described previously by Babu et al. (2004) and Zhang et al. (2010). Briefly, the enzyme reaction was performed using

Table 4
Percentage contribution of dry weight (dw), total anthocyanins (TA), total phenolics (TP), and scavenging capacity for peroxyl radicals (ROO·; ORAC), hydroxyl radicals (·OH), hydrogen peroxidase (H_2O_2), and singlet oxygen ($^1O_2$) from peel and flesh extracts of 33 blueberry cultivars (29 *V. ashei*, two *V. ashei* derivative hybrids, and two northern highbush standards) to whole fruit.

Blueberry cultivar	Fruit dw		TA		TP		ORAC		·OH		H_2O_2		$^1O_2$	
	Peel	Flesh	Peel	Flesh	Peel	Flesh	Peel	Flesh	Peel	Flesh	Peel	Flesh	Peel	Flesh
Vaccinium ashei Reade (rabbiteye)														
Aliceblue	38.5	61.5	90.0	10.0	83.5	16.5	56.4	43.6	70.6	29.4	74.8	25.2	79.5	20.5
Austin	36.2	63.8	87.8	12.2	81.8	18.2	54.0	46.0	67.0	33.0	67.8	32.2	80.4	19.6
Beckyblue	29.2	70.8	95.4	4.6	85.4	14.6	57.2	42.8	63.0	37.0	77.5	22.5	79.8	20.2
Bluebelle	26.0	74.0	93.6	6.4	80.3	19.7	55.0	45.0	58.3	41.7	69.0	31.0	82.4	17.6
Bluegem	31.8	68.2	87.4	12.6	82.0	18.0	50.8	49.2	69.1	30.9	69.8	30.2	81.7	18.3
Briteblue	23.8	76.2	82.6	17.4	79.9	20.1	51.1	48.9	61.1	38.9	60.5	39.5	81.2	18.8
Callaway	29.2	70.8	84.5	15.5	82.5	17.5	53.3	46.7	67.7	32.3	63.4	36.6	80.2	19.8
Centurion	29.8	70.2	86.5	13.5	89.4	10.6	64.6	35.4	69.2	30.8	69.6	30.4	76.5	23.5
Chaucer	22.9	77.1	88.8	11.2	86.1	13.9	62.6	37.4	80.7	19.3	67.0	33.0	80.9	19.1
Choice	36.4	63.6	87.8	12.2	81.6	18.4	55.3	44.7	64.2	35.8	64.4	35.6	80.8	19.2
Clara	26.9	73.1	83.9	16.1	84.2	15.8	55.9	44.1	66.6	33.4	69.7	30.3	80.1	19.9
Coastal	35.4	64.6	88.3	11.7	85.0	15.0	51.5	48.5	69.9	30.1	70.6	29.4	79.3	20.7
Delite	32.8	67.2	88.1	11.9	79.0	21.0	56.3	43.7	68.1	31.9	72.3	27.7	78.5	21.5
Early May	31.3	68.7	89.5	10.5	83.6	16.4	58.9	41.1	72.3	27.7	72.9	27.1	81.0	19.0
Ethel	36.7	63.3	89.4	10.6	82.4	17.6	50.6	49.4	69.7	30.3	69.4	30.6	78.8	21.2
Garden Blue	33.0	67.0	87.7	12.3	87.1	12.9	50.2	49.8	75.0	25.0	72.3	27.7	83.5	16.5
Homebell	37.0	63.0	89.0	11.0	79.8	20.2	57.1	42.9	66.0	34.0	72.0	28.0	77.7	22.3
Ira	32.0	68.0	91.5	8.5	84.2	15.8	54.0	46.0	65.0	35.0	71.6	28.4	79.4	20.6
Menditoo	34.9	65.1	85.2	14.8	88.4	11.6	54.5	45.5	72.0	28.0	69.8	30.2	80.5	19.5
Myers	30.1	69.9	83.3	16.7	79.0	21.0	52.0	48.0	59.8	40.2	70.6	29.4	80.9	19.1
Owen	26.2	73.8	83.8	16.2	75.3	24.7	56.4	43.6	61.9	38.1	67.7	32.3	71.7	28.3
Premier	33.1	66.9	93.4	6.6	90.0	10.0	67.6	32.4	79.4	20.6	81.9	18.1	80.6	19.4
Satilla	27.4	72.6	89.2	10.8	85.7	14.3	62.6	37.4	73.1	26.9	73.3	26.7	81.2	18.8
Southland	26.9	73.1	86.0	14.0	78.7	21.3	50.8	49.2	60.9	39.1	65.8	34.2	78.6	21.4
Suwanee	22.0	78.0	84.2	15.8	80.6	19.4	53.1	46.9	58.8	41.2	62.1	37.9	84.3	15.7
Tifblue	29.8	70.2	89.4	10.6	83.4	16.6	51.1	48.9	69.7	30.3	69.1	30.9	84.2	15.8
Walker	32.3	67.7	86.2	13.8	79.0	21.0	60.1	39.9	56.1	43.9	66.3	33.7	84.8	15.2
Windy	33.7	66.3	91.0	9.0	81.1	18.9	57.6	42.4	62.5	37.5	71.2	28.8	85.4	14.6
Woodard	30.4	69.6	89.0	11.0	78.4	21.6	50.8	49.2	64.5	35.5	70.6	29.4	83.0	17.0
Mean	30.9	69.1	88.0	12.0	82.7	17.3	55.6	44.4	67.0	33.0	69.8	30.2	80.6	19.4
Vaccinium ashei hybrid derivatives														
Pearl River	31.5	68.5	93.8	6.2	87.5	12.5	66.8	33.2	75.2	24.8	76.7	23.3	79.1	20.9
Pink Lemonade	29.5	70.5	54.6	45.4	53.7	46.3	50.1	49.9	58.2	41.8	53.1	46.9	53.6	46.4
Mean	30.5	69.5	74.2	25.8	70.6	29.4	58.5	41.5	66.7	33.3	64.9	35.1	66.3	33.7
Vaccinium corymbosum L. (northern highbush)														
Bluecrop	27.8	72.2	87.7	12.3	84.3	15.7	56.3	43.7	69.3	30.7	70.0	30.0	77.9	22.1
Duke	31.2	68.8	90.5	9.5	86.5	13.5	63.6	36.4	74.7	25.3	78.3	21.7	80.2	19.8
Mean	29.5	70.5	89.1	10.9	85.4	14.6	60.0	40.0	72.0	28.0	74.2	25.8	79.0	21.0

p-nitrophenyl-α-D-glucopyranoside (*p*NPG) as the substrate, which was hydrolysed by α-glucosidase to release *p*-nitrophenol, a colour agent that can be monitored at 405 nm (Babu et al., 2004). The assay was conducted by mixing 80 μl the sample solution (1 mg/ml) with 20 μl the enzyme solution (1 U/ml) and incubated at 37 °C for 3 min under shaking. After incubation, 100 μl of 4 mM *p*NPG solution in 0.1 M phosphate buffer (pH 6.8) was added and the reaction was performed at 37 °C. The release of *p*-nitrophenol from *p*NPG was monitored at 405 nm every minute for 45 min by a plate reader (Bio-Tek Instruments, Inc., Winooski, VT). The plate reader was controlled by software KC4 3.0 (revision 4.0). The α-glucosidase activity was determined by measuring the area under the curve (0–45 min) for each sample and compared with that of the control (buffer solution in place of the extract). The results were then expressed as percentage of α-glucosidase activity compared with that of control.

2.9. Statistical analysis

Quantitative values for total anthocyanins (TA), total phenolics (TP), free radical scavenging capacity against peroxyl free radicals (ROO·), hydroxyl radicals (·OH), hydrogen peroxide (H_2O_2), and singlet oxygen ($^1O_2$) radicals and α-glucosidase inhibitory activities were analysed as two-factor linear models using Proc Mixed (SAS Institute Inc., 2010a) with cultivar and tissue as the factors on dw. The assumptions of the model were checked and the variance grouping technique was used to correct for heterogenous variances. Means comparisons were done with Šidák adjusted *p*-values so that the experiment-wise error was 0.05. The variables were also analysed jointly in a multivariate analysis using the centroid hierarchical cluster method (SAS Institute Inc., 2010b). The correlation coefficients between scavenging activities of ROO· radicals *vs.* other free radical scavenging activities of H_2O_2, ·OH, $^1O_2$, and α-glucosidase inhibitory activities were also calculated.

3. Results

3.1. Total anthocyanin (TA) and total phenolic (TP) content

The TA and TP contents from 33 blueberry cultivars are listed in Table 1. The content of TA and TP in peel and flesh of blueberries varied significantly among 33 cultivars tested. In peel, TA ranged from 693.0 ('Pink Lemonade') to 8814.9 mg/100 g dw ('Suwanee'). In flesh, TA ranged from 93.8 ('Beckyblue') to 528.6 mg/100 g dw ('Southland'). In peel, TP content ranged from 2509.2 ('Ira') to 6987.3 mg/100 g dw ('Suwanee'), and in flesh, ranged from 151.1 ('Chaucer') to 692.1 mg/100 g dw ('Choice'). Relatively, the TA amount in cultivar 'Suwanee' was approximately 12.7 times greater than that in the pink-fruited cultivar 'Pink Lemonade' (Table 1).

Table 5
Percentage inhibition of α-glucosidase activity and percentage contribution to α-glucosidase inhibitory activity in whole fruit from peel and flesh extracts of 33 blueberry cultivars (29 *V. ashei*, two *V. ashei* derivative hybrids, and two northern highbush standards).

Blueberry cultivar	(%) Inhibition of α-glucosidase activity[a] (100 μg dw)			(%) Contribution to α-glucosidase inhibitory activity in whole fruit	
	Flesh	Peel	Peel/flesh	Flesh	Peel
Vaccinium ashei Reade (rabbiteye)					
Aliceblue	16.9 d-j[b]	66.8 klm	4.0	28.8	71.2
Austin	18.2 d-g	56.3 o	3.1	36.3	63.7
Beckyblue	12.1 l	58.2 no	4.8	33.5	66.5
Bluebelle	13.9 i-l	65.2 lm	4.7	37.7	62.3
Bluegem	16.4 g-h	78.3 fg	4.8	30.9	69.1
Briteblue	21.9 bcd	95.1 ab	4.3	42.6	57.4
Callaway	25.3 ab	97.6 ab	3.9	38.6	61.4
Centurion	18.4 c-i	83.4 ef	4.5	34.1	65.9
Chaucer	12.5 kl	62.2 mno	5.0	40.2	59.8
Choice	28.9 a	95.2 ab	3.3	34.7	65.3
Clara	20.1 b-g	87.4 cde	4.3	38.5	61.5
Coastal	21.9 bcd	82.3 ef	3.8	32.7	67.3
Delite	20.7 b-f	77.1 f-i	3.7	35.6	64.4
Early May	21.3 b-e	85.7 de	4.0	35.2	64.8
Ethel	17.0 d-j	76.9	4.5	27.6	72.4
Garden Blue	14.0 i-l	67.7 klm	4.8	29.6	70.4
Homebell	15.3 hij	62.0 mno	4.1	29.6	70.4
Ira	13.6 jkl	64.4 lmn	4.7	31.1	68.9
Menditoo	24.7 abc	78.3 fg	3.2	37.0	63.0
Myers	22.7 abc	83.9 ef	3.7	38.6	61.4
Owen	20.5 b-g	81.0 efg	4.0	41.6	58.4
Premier	14.9 e-l	71.1 ijk	4.8	29.7	70.3
Satilla	21.0 b-f	93.0 abc	4.4	37.4	62.6
Southland	22.6 abc	98.1 a	4.3	38.5	61.5
Suwanee	19.7 c-g	97.6 ab	5.0	41.7	58.3
Tifblue	15.6 d-l	67.9 j-m	4.4	35.0	65.0
Walker	17.0 f-i	63.1 mn	3.7	36.1	63.9
Windy	13.7 i-l	62.1 mno	4.5	30.3	69.7
Woodard	20.8 b-h	92.2 bcd	4.4	35.9	64.1
Mean	18.7	77.6	4.2	35.1	64.9
Vaccinium ashei hybrid derivatives					
Pearl River	15.3 hij	75.6 g-j	4.9	30.6	69.4
Pink Lemonade	10.2 l	20.2 p	2.0	45.8	55.2
Mean	12.8	47.9	3.5	38.2	62.3
Vaccinium corymbosum L. (northern highbush)					
Bluecrop	15.5 d-l	77.8 fgh	5.0	34.1	65.9
Duke	16.5 d-k	71 h-l.6	4.3	33.7	66.3
Mean	16.0	74.7	4.7	33.9	66.1

Values within the same column followed by different letters were significantly different at $p < 0.05$.
[a] The α-glucosidase activity was determined by measuring the area under the curve for each sample and compared with that of the control (buffer solution in place of the extract). The results were then expressed as percentage of α-glucosidase activity compared with that of control.
[b] Data were expressed as means of three assays for each cultivars.

3.2. Antioxidant activity

The scavenging capacities against ROO$^{\cdot}$, $^{\cdot}$OH, H_2O_2, and $^1O_2$ in peel and flesh of various blueberry cultivars are shown in Tables 1 and 2. The scavenging capacities from the peel and flesh were significantly different. In blueberry peel, the scavenging capacities against ROO$^{\cdot}$ ranged from 401.4 ('Pink Lemonade') to 2243.7 μmol TE/g dw ('Suwanee'). For flesh tissue, the scavenging capacities against ROO$^{\cdot}$ ranged from 143.3 ('Chaucer') to 600.4–607.4 μmol TE/g dw ('Southland' and 'Callaway') (Table 1).

In peel of blueberries, the scavenging capacities for $^{\cdot}$OH radicals ranged from 453.4 ('Pink Lemonade') to 2429.8 μmol TE/g dw ('Suwanee'), reflecting a 5.4-fold difference among cultivars (Table 2). The scavenging capacities of flesh in blueberry cultivars against $^{\cdot}$OH ranged from 108.3 ('Chaucer') to 663.0 μmol TE/g dw ('Choice'), reflecting a 6.1-fold difference among cultivars (Table 2).

In peel of blueberries, the scavenging capacity for H_2O_2 expressed as ascorbate equivalents (asc-eq) ranged from 17.0 ('Pink Lemonade') to 78.0 mg asc-eq/10 g dw ('Callaway'). In flesh tissue, 'Choice' had highest scavenging activity for H_2O_2 with with 21.8 mg asc-eq/10 g fw (mg asc-eq/10 g dw), while 'Beckyblue' had the lowest H_2O_2 scavenging activity in peel with 5.9 mg asc-eq/10 g dw (Table 2).

The scavenging capacity of peel against $^1O_2$ ranged from 3.8 ('Pink Lemonade') to 162.9 ('Callaway') mg asc-eq/10 g dw. The flesh tissue of all tested blueberry cultivars had low scavenging activities for $^1O_2$ (Table 2).

In general, the peel of rabbiteye cultivars 'Suwanee', and 'Callaway' had superior scavenging capacities for the reactive oxygen species, not only for ROO$^{\cdot}$ and $^{\cdot}$OH, but also for H_2O_2, and $^1O_2$ (Tables 1 and 2). For flesh tissue, 'Southland' and 'Choice' had the highest scavenging capacities for ROO$^{\cdot}$, $^{\cdot}$OH, H_2O_2, and $^1O_2$.

3.3. Relative contributions of peel and flesh to fresh weight, dry weight, TA, TP, and antioxidant activities of blueberry cultivars

There were substantial differences between peel and flesh with respect to dry weight, and their contribution to TA, TP, and antioxidant activities (Tables 3 and 4). Among the tested blueberry cultivars, 'Suwanee' and 'Chaucer' had the highest flesh/peel ratios, whereas 'Aliceblue' had the lowest ratio (Table 4). This indicated that blueberry fruit consisted of much more flesh than peel.

The peel always contained higher TA and TP than the flesh. For rabbiteyes, the peel to flesh ratio value for TA were from 1.8 ('Pink Lemonade') to 50.2 ('Beckyblue') (Table 3). On a whole fruit basis, the relative percentage contribution to TA in the peel and flesh of blueberries ranged from 82.6% and 17.4 % ('Briteblue') to 95.4% and 4.6 % ('Beckyblue'), respectively (Table 4). For TP, the ratios of peel to flesh were 3.9 ('Pink Lemonade' and 'Duke') to 20.9 ('Chaucer') (Table 3). On whole fruit basis, peel and flesh contributed 53.7% and 46.3 % ('Pink Lemonade') to 90.0% and 10.0 % ('Premier') for TP (Table 4). These data indicated that even though peel only takes up a small amount of fruit weight compared to flesh in blueberries, it makes a greater contribution to TA and TP than flesh in all blueberries tested.

The free radical scavenging capacities of peel and flesh were also significantly different. The scavenging capacities against ROO$^{\cdot}$, $^{\cdot}$OH, H_2O_2, and $^1O_2$ in peel were higher than in flesh for all blueberry cultivars tested in this study (Tables 3 and 4). The ratio of peel to flesh, with respect to scavenging capacities against ROO$^{\cdot}$, $^{\cdot}$OH, H_2O_2, and $^1O_2$, ranged from as low as 1.3 (ROO$^{\cdot}$) to as high as 19.0 ($^1O_2$). On weight basis, rabbiteye 'Chaucer' had the highest ratio of peel to flesh for scavenging ROO$^{\cdot}$ and $^{\cdot}$OH, while 'Beckyblue' had highest ratio for scavenging H_2O_2, and 'Suwanee' the highest ratio for scavenging $^1O_2$ (Table 3). The percentage contributions of peel and flesh to whole berry on scavenging capacities against ROO$^{\cdot}$, $^{\cdot}$OH, H_2O_2, and $^1O_2$ are shown in Table 4. 'Premier' had highest scavenging capacities against ROO$^{\cdot}$, $^{\cdot}$OH, and H_2O_2 in peel tissue whereas 'Windy' had highest for $^1O_2$ in peel tissue (Table 4).

3.4. α-Glucosidase (EC 3.2.1.20) inhibitory activity

The peel of all blueberry cultivars tested had a high α-glucosidase inhibitory effect compared to flesh tissue. This indicates that the α-glucosidase inhibitory effect in blueberry fruit is derived mainly from peel. Compared to the enzyme standard, the percentage inhibitory activity in peel ranged from 20.2% ('Pink Lemonade') to 98.1% ('Southland'), whereas values in flesh ranged 10.2% ('Pink Lemonade') to 28.9% ('Choice'). Compared across the rabbiteye cultivars, the relative magnitude of inhibitory activities of α-glucosidase activity in peel over flesh was 4.2-fold (Table 5). On a per fruit basis, which accounts for cultivar variations in peel to flesh ratios, the contribution to inhibitory effects in peel ranged from 55.2% ('Pink Lemonade') to 72.4 % ('Ethel'), and in flesh, from 27.6% ('Ethel') to 45.8% ('Pink Lemonade').

3.5. Interactions between cultivars and tissues

Cultivars and tissues (peel and flesh) were evaluated for several fruit quality parameters: total anthocyanin, total phenolic content, antioxidant capacities, and α-glucosidase inhibitory activity. For all parameters evaluated, significant cultivar × tissue interactions were observed (Table 6). Despite these interaction effects, peel tissues were significantly higher in value for the measured parameters. In the conventionally pigmented cultivars, the interaction effects represented a non-parallel variation between peel and flesh tissue, but never a reversal of rank. Due to the interaction effect, cultivars were statistically compared within tissues, and for tissue assessment, tissues were compared within cultivars.

The variant results for the pink-fruited cultivar (such as 'Pink Lemonade') were not unexpected. This cultivar was included to represent a counterpoint to the conventional cultivars, and represents a valuable contrast to the chemistry and physiology in the

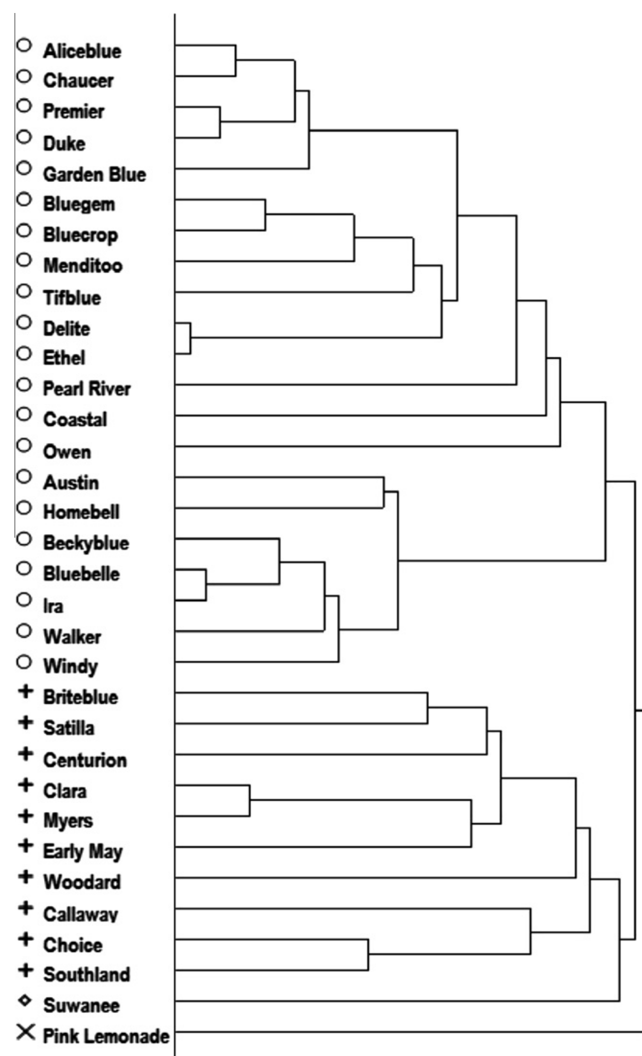

Fig. 1. The centroid hierarchical cluster analysis of seven variables: TA, TP, oxygen radical scavenging capacity against ROO$^{\cdot}$, $^{\cdot}$OH, H_2O_2, $^1O_2$, and α-glucosidase inhibitory activities from peel extracts of 33 blueberry cultivars.

Table 6
Analysis of variance results for variables: total anthocyanins (TA), total phenolics (TP), oxygen radical scavenging capacity against peroxyl radicals (ROO$^{\cdot}$), hydroxyl radicals ($^{\cdot}$OH), hydrogen peroxide (H_2O_2), and singlet oxygen ($^1O_2$), and α-glucosidase inhibitory activities from peel and flesh extracts of 33 blueberry cultivars (29 *V. ashei*, two *V. ashei* derivative hybrids, and two northern highbush standards).

Analysis of variance for Tables 1 and 2															
Source	DF	TA		TP		ROO$^{\cdot}$ (ORAC)		$^{\cdot}$OH		H_2O_2		$^1O_2$		α-Glucosidase inhibitory activity	
		F-value	*p*-value	*F*-value	*p*-value	*F*-value	*p*-value	*F*-value	*p*-value	*F*-value	*p*-value	*F*-value	*p*-value	*F*-value	*p*-value
Tissue (T)	1	3.277E7	<0.0001	1.826E7	<0.0001	683650	<0.0001	3352346	<0.0001	25036	<0.0001	116214	<0.0001	49860	<0.0001
Cultivar (C)	32	155752	<0.0001	95974	<0.0001	16137	<0.0001	18751	<0.0001	108	<0.0001	2492	<0.0001	179	<0.0001
T × C	32	143704	<0.0001	81517	<0.0001	9016	<0.0001	9493	<0.0001	62	<0.0001	1693	<0.0001	78	<0.0001

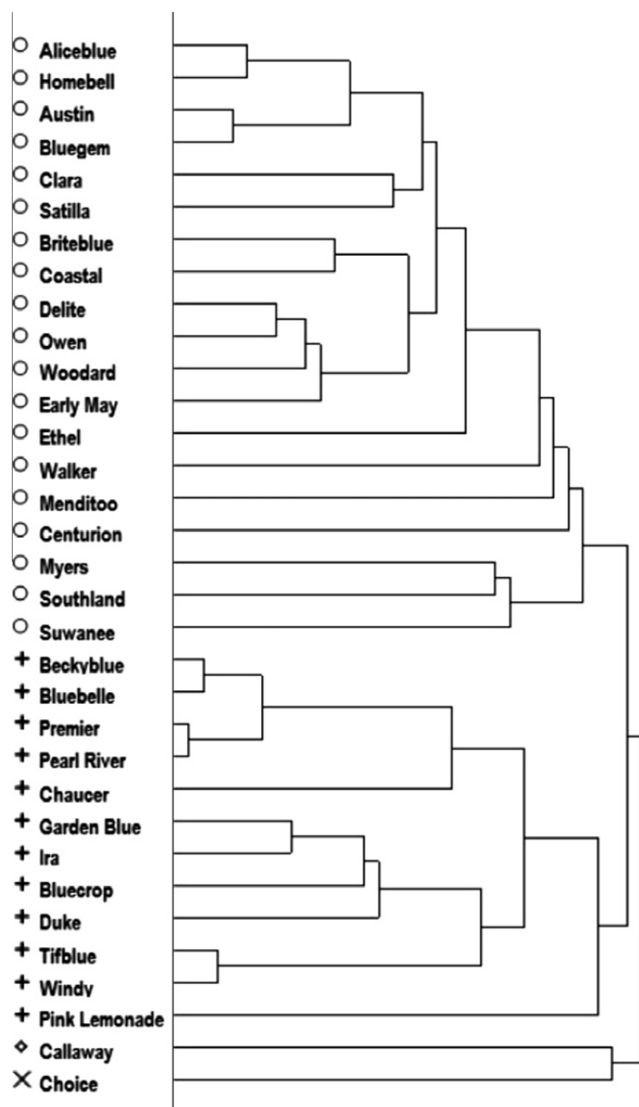

Fig. 2. The centroid hierarchical cluster analysis of seven variables: TA, TP, oxygen radical scavenging capacity against ROO$^{\cdot}$, $^{\cdot}$OH, H_2O_2, $^1O_2$, and α-glucosidase inhibitory activities from flesh extracts of 33 blueberry cultivars.

standard cultivars. We discuss this cultivar here so that the remaining discussion can deal with results applicable to typically pigmented blueberry cultivars. As might be expected, 'Pink Lemonade' was exceptional with respect to peel parameters. In peel tissue, 'Pink Lemonade' was lowest in TA, TP, ORAC, H_2O_2, $^1O_2$, $^{\cdot}$OH, and α-glucosidase activity. These low values were often 50% or less of the average values for pigmented cultivars. By contrast, in flesh tissue, 'Pink Lemonade' was typically within the low, but normal range compared to standard cultivars for all parameters except $^1O_2$ and α-glucosidase activity, where it was again the lowest.

3.6. Evaluations of parameters across cultivars

Centroid clustering of values for TA, TP, ORAC, H_2O_2, $^1O_2$, $^{\cdot}$OH, and α-glucosidase inhibitory activity indicated that four clusters were sufficient to group the variation for our measured parameters. The dendrogram for peel tissue (Fig. 1) separates the cultivars into four clusters of 21, 10, 1, and 1, respectively (top to bottom). The dendrogram, at the highest difference level, separates 'Pink Lemonade' into an unit group based upon its low values for all of these parameters. 'Suwanee', by contrast, appears to be separated into its own group because of its consistently high values for most of these parameters. The dendrogram for flesh tissue (Fig. 2) separates the cultivars into four clusters of 19, 12, 1, and 1, respectively (top to bottom). This dendrogram, at the highest difference level, separates 'Choice' and 'Callaway' into unit groups based upon high values for many of these parameters. Within the uppermost group (beginning with 'Aliceblue'), only 10 cultivars are common with the similarly positioned group in the previous figure for peel tissue, and notably, in this figure, the cultivar 'Pink Lemonade' clusters with a set of 12 conventionally pigmented cultivars.

In this study, a significant positive correlation ($p \leqslant 0.05$) was found between TA or TP and antioxidant activity (Table 7). For peel, the correlations for TA *vs*. ORAC, $^{\cdot}$OH, H_2O_2, and $^1O_2$ were 0.85, 0.79, 0.87, and 0.85, respectively. In flesh tissue, the correlations for TA *vs*. ORAC, were largely similar with $^{\cdot}$OH, H_2O_2, and $^1O_2$ values of r = 0.87, 0.80, 0.85, and 0.67, respectively. Significant positive correlations ($p \leqslant 0.05$) were also found for TP *vs*. oxygen free radical scavenging capacities of ORAC, $^{\cdot}$OH, H_2O_2, and $^1O_2$ in flesh with r = 0.91, 0.91, 0.78, and 0.69, respectively, and for peel r = 0.93, 0.93, 0.93, and 0.91, respectively.

Table 7
Correlation coefficients (r) among total anthocyanins (TA), total phenolics (TP), and different free radical (ORAC, $^{\cdot}$OH, H_2O_2, and $^1O_2$) scavenging activities and α-glucosidase inhibitory activity from peel and flesh extracts of 33 blueberry cultivars (29 *V. ashei*, two *V. ashei* derivative hybrids, and two northern highbush standards).[a]

	Correlation coefficients (r) for peel extracts						
	TA	TP	ORAC	$^{\cdot}$OH	H_2O_2	$^1O_2$	α-Glucosidase inhibitory activity
TA	1						
TP	0.81	1					
ORAC	0.85	0.94	1				
$^{\cdot}$OH	0.79	0.93	0.88	1			
H_2O_2	0.87	0.93	0.87	0.89	1		
$^1O_2$	0.85	0.91	0.87	0.83	0.93	1	
α-Glucosidase inhibitory activity	0.92	0.88	0.88	0.89	0.92	0.87	1
Correlation coefficients (r) for flesh extracts							
TA	1						
TP	0.84	1					
ORAC	0.87	0.91	1				
$^{\cdot}$OH	0.80	0.91	0.87	1			
H_2O_2	0.85	0.78	0.86	0.81	1		
$^1O_2$	0.67	0.69	0.66	0.72	0.76	1	
α-Glucosidase inhibitory activity	0.82	0.80	0.82	0.80	0.79	0.73	1

[a] Correlation is significant at the $p < 0.05$ level (2-tailed).

4. Discussion

We found that peel had much higher scavenging capacities than flesh against ROO$^{\cdot}$, $^{\cdot}$OH, H_2O_2, and $^1O_2$ in blueberry cultivars. The scavenging capacities of peel averaged 2.6-fold higher than that of flesh for ROO$^{\cdot}$, 5.0-fold higher for $^{\cdot}$OH, 5.3-fold higher for H_2O_2, and 9.5-fold higher for $^1O_2$ for all tested blueberries. Accordingly, the percentage contribution of peel and flesh on scavenging capacities against ROO$^{\cdot}$, $^{\cdot}$OH, H_2O_2, and $^1O_2$ also varied, with peel contributing a higher percentage of free radical scavenging capacity than did flesh. The peel contributed on average 56.0% for ROO$^{\cdot}$ scavenging; 67.3% for $^{\cdot}$OH scavenging; 69.7% to H_2O_2 scavenging, and 79.6% for $^1O_2$ scavenging in all tested blueberry fruit, even though peel only comprised average 30.8%. This indicates that most of the antioxidant activity in whole blueberry fruit was derived from peel. In other berry fruit, Aaby, Skrede, and Wrolstad (2005) reported that thalamus of strawberries contributed about 86% of the antioxidant activity and the achenes about 14%, even though achenes were only about 1% of the total fresh weight.

This study showed that antioxidant scavenging capacity for ROO$^{\cdot}$, $^{\cdot}$OH, H_2O_2, and $^1O_2$ radicals varied significantly with genetic composition (cultivars) and tissue (peel *vs.* flesh). This supports reports that the ORAC values among 18 blueberry cultivars ranged from a low of 20.5 μmol TE/g to a high of 60.3 μmol TE/g, reflecting a 2.9-fold difference (Howard, Clark, & Brownmiller, 2003). Other studies 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, Akoh, & Krewer, 2002), 4.7-fold (Connor et al., 2002), 5.2-fold (Moyer et al., 2002), and 6.8-fold (Ehlenfeldt & Prior, 2001).

Despite these numerous studies, no information has been given comparing different antioxidant capacities between peel and flesh tissues of blueberries.

The multivariate centroid hierarchical cluster analysis indicated that for both peel and flesh, four clusters were sufficient to group the cultivars for the measured variables (Figs. 1 and 2). The clustering suggests that for peel tissue 'Pink Lemonade' was compositionally distinct from the pigmented berries, but for flesh tissue, even such an exotic type is relatively similar to most other blueberries.

There are many studies on the control of blood glucose level for the prophylaxis of diabetic disease by physiologically functional food materials (Takii, Matsumoto, Kometani, Okada, & Fushiki, 1997). In this study, we have proved that anthocyanins and phenolics from peel and flesh of blueberry fruit extracts posses potent α-glucosidase inhibitory activity. In general, peel extracts contained higher TA and TP and also showed higher inhibitory effect than flesh. In our study, the highest correlation (significant at $p \leqslant 0.05$) was seen between TA and α-glucosidase inhibitory activity in both peel extracts ($r = 0.92$) and flesh ($r = 0.82$) (Table 7). TA values were marginally better indicators than TP.

α-Glucosidase catalyses the final step in the digestive process of carbohydrates and its inhibitors can retard the uptake of dietary carbohydrates and suppress postprandial hyperglycaemia and could be useful for treating diabetes (Toeller, 1994). Inhibition of α-glucosidase is considered an effective measure for regulating type II diabetes by controlling glucose uptake (Puls et al., 1977). Generation of reactive oxygen species and free radicals is accelerated in diabetes (Asayama, 1990). Therefore, free radical scavenging capacity of fruits, such as blueberries, may be beneficial in minimising damages induced by diabetes. There was also a positive correlation (significant at $p \leqslant 0.05$) between α-glucosidase inhibitory activity and scavenging capacities against ROO$^{\cdot}$, $^{\cdot}$OH, H_2O_2, and $^1O_2$ in various cultivars of blueberry peel tissues, averaging $r = 0.89$. Positive correlations, but at a lower levels, were also noted for α-glucosidase inhibitory activity and scavenging capacities for ORAC, $^{\cdot}$OH, H_2O_2, and $^1O_2$ in blueberry flesh, with r values averaging $r = 0.79$ (Table 7). The inhibitory effectiveness of anthocyanin against α-glucosidase has also been reported in currant, strawberry, and raspberry fruit extracts (McDougall et al., 2005). Polyphenolic extracts from sweet potato roots (Matsui et al., 2001a) and morning glory flowers (Matsui et al., 2001b) enriched in anthocyanins were reported as effective inhibitors of rat intestinal α-glucosidase. Pinto et al. (2008) reported that the strawberry cultivars Dover, and Oso Grande had high TP contents and a good correlation was observed between α-glucosidase inhibitory activity and TP content ($r = 0.95$). Positive correlation between α-glucosidase inhibitory activity and phenolic compounds has also been reported in sea buckthorn leaf extracts (Kim, Kwon, Sa, & Kim, 2011) and edible vegetables (Mai, Thu, Tien, & Chuyen, 2007). Polyphenolic compounds in plants have been shown to inhibit the activities of digestive enzymes due to their ability to bind with protein (McDougall & Stewart, 2005). Cheplick, Kwon, Bhowmik, and Shetty (2007) reported that, in general, darker-pigmented black and purple raspberry cultivars had higher TA and TP content, antioxidant activity, and higher α-glucosidase inhibitory effect than the lighter-coloured yellow cultivars. However, one yellow-pigmented raspberry cultivar had better inhibitory activity than most red raspberry cultivars (Cheplick et al., 2007). Zhang et al. (2010) found 'Dinkum' (red) and 'Josephine' (yellow) raspberry varieties possess higher total phenolic content, ORAC, DPPH radical scavenging activity, and α-glucosidase inhibitory activity than other five cultivars ('Nova', 'Heritage', 'Autumn Britten', 'Anne', and 'Fall Gold'). However, overall, the enzyme inhibition and antioxidant properties were not consistently correlated with their total phenolic, anthocyanin, and antioxidant activity in all seven tested raspberry cultivars (Zhang et al., 2010). Therefore, in raspberry, high antioxidant activity may not be used for predicting good α-glucosidase inhibition. It may be that α-glucosidase is influenced more by specific individual anthocyanins and phenolics rather than the overall amount of plant phenolics. Studies have shown that anthocyanins are effective inhibitors of α-glucosidase with acylated anthocyanins being much more efficient than deacylated ones (Matsui et al., 2001b). The nature of the sugar conjugate and the aglycone are also important determinants of anthocyanin absorption and function in both humans and rats (McGhie, Ainge, Barnett, Cooney, & Jensen, 2003).

In summary, this study demonstrated that peel tissue possessed higher levels of antioxidant capacities and α-glucosidase inhibitory activities compared to flesh tissue in all blueberries tested. Peel contributed a higher percentage of free radical scavenging capacity than flesh tissue even though the fruit contained much higher amounts of flesh than peel in terms of dw. This indicates that the antioxidant activity and α-glucosidase inhibitory activity were mainly derived from peel of the blueberry fruit. Different cultivars of blueberries possessed varied amounts of total anthocyanins, total phenolics, antioxidant capacities, and potent α-glucosidase inhibitory activities in peel and flesh tissues. Studies on the health benefits of fruits have attracted the interest of scientists seeking to prevent disease and promote health. Therefore, specific blueberry cultivars with high phenolic compounds, antioxidant capacity, and α-glucosidase inhibitory activity could be selected for use in blueberry breeding programs. Further investigation is needed to determine the individual polyphenol components present in blueberry cultivars that may be responsible for improvements in health benefits such as regulation of α-glucosidase inhibitory activity.

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