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Short Communication

α-Glucosidase inhibitory activity and antioxidant capacity in the peel and pulp of mixed-species blueberry hybrids

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Abstract

Inhibition of α-glucosidase activity is considered an effective means for controlling diabetes by regulating glucose uptake, and blueberries have been shown to possess high levels of inhibitory activity. In the present study, we examined the variations in α-glucosidase inhibition, phenolic and anthocyanin levels, and antioxidant capacity in the peel and pulp of 16 mixed-species rabbiteye hybrids (*Vaccinium ashei* Reade × *Vaccinium* spp.), one rabbiteye cultivar (*V. ashei*) and two highbush hybrids (*Vaccinium corymbosum*). Peel tissue had, on average, about four times higher levels of α-glucosidase inhibitory activity than pulp, and exhibited significantly higher levels of all other measured activities, even though the peel comprised only a small portion of the fruit. Significant variations in the levels of antioxidant activity were observed; however, no consistent differences were observed between the hybrids with various species composition. Significant positive correlations ($r \geq 0.84$) were found among α-glucosidase inhibitory activity, total anthocyanin (TA) and phenolic levels, and scavenging activity against ROO·, ·OH, $^1O_2$ and H_2O_2 radicals in the extracts from the peel and pulp. There was a high correlation observed between α-glucosidase inhibitory activity levels and ROO·(ORAC)$_{peel}$ ($r = 0.95$). A similarly high correlation with TA$_{peel}$ ($r = 0.93$) suggests that TA would be a suitable assay proxy if a broader genotypic evaluation of blueberry genotypes is desired.

Keywords: anthocyanins; highbush; phenolics; rabbiteye; *Vaccinium*

Introduction

Blueberries contain abundant amounts of anthocyanins, and their extracts are efficient inhibitors of α-glucosidase activity (McDougall and Stewart, 2005; McDougall *et al.*, 2005). Inhibition of carbohydrate-hydrolysing enzymes, such as α-glucosidase, is one of the therapeutic approaches to decrease postprandial hyperglycaemia (Rabasa-Lhoret and Chiasson, 2004; Bhandari *et al.*, 2008), and is considered an effective measure for regulating diabetes by controlling glucose uptake (Puls *et al.*, 1977).

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We previously reported on the levels of α-glucosidase inhibitory activity and antioxidant activity in rabbiteye and highbush blueberry cultivars (Wang *et al.*, 2012) and extend herein that work by evaluating the peel and pulp of 16 mixed-species hexaploid rabbiteye hybrids (*Vaccinium ashei* Reade × *Vaccinium* spp.) in comparison with rabbiteye (*V. ashei*) and highbush (*Vaccinium corymbosum* L.) genotypes, and also evaluating phenolic and anthocyanin levels, and their antioxidant activity.

Experimental methods

Fruit were grown at the Marucci Center for Blueberry and Cranberry Research and Extension (Rutgers University), Chatsworth, NJ, USA. Fully ripe (100% blue) berries were harvested at an overall bush ripeness of 45–100%, with the most typical value being about 60%. Fruit (500–900 g) were sampled from 19 genotypes with various species composition (Table 1). They were selected for uniform size and colour, and frozen to −80°C until processed.

Each berry was separated into peel and pulp prior to the analysis. For dry weight (dw) measurements, tissues were dessicated at 70°C for 72 h. Antioxidant activity from fresh weight was converted to dw based on weight loss upon drying. Triplicate samples were made from 4 g of the peel or 10 g of the pulp (25–35 fruit per genotype). These samples were homogenized and extracted four times with acetone–formic acid (1:1). The extracts were centrifuged at 14,000 ***g*** for 20 min at 4°C. The supernatants were combined to a final volume of 50 ml and stored at −80°C until assayed.

Experimental procedures have been reported previously (Wang *et al.*, 2012) and included assays for α-glucosidase inhibition (α-Gluc-i; Babu *et al.*, 2004; Zhang *et al.*, 2010), total phenolic (TP; Slinkard and Singleton, 1997) and total anthocyanin (TA) levels (Cheng and Breen, 1991), and scavenging capacity for peroxyl radicals (ROO•; Huang *et al.*, 2002), hydroxyl radicals (•OH; Moore *et al.*, 2006; Wang *et al.*, 2012), singlet oxygen radicals ($^1O_2$; Chakraborty and Tripathy, 1992) and hydrogen peroxide radicals (H_2O_2; Patterson *et al.*, 1984).

Variables were statistically analysed by tissue type using a one-factor linear model in PROC MIXED (SAS/STAT, 2011) with genotype as the factor. The variables TA_{peel}, $\alpha\text{-glucosidase}_{peel}$ and $\alpha\text{-glucosidase}_{pulp}$ met all model variance assumptions. For other variables, a variance grouping technique was used to correct for heterogeneous variances. Mean values of individual genotypes were analysed as simple comparisons against the standard cultivar Legacy, using Šidák-adjusted *P* values. Correlation coefficients were calculated using SPSS (SPSS Statistics, 2008).

Results and discussion

There were significant differences in the levels of α-Gluc-i activity exhibited between the peel and the pulp ($P \leq 0.0001$). The peel showed higher levels of inhibitory activity, with a mean value 4.3 times that of the pulp (range 2.6–5.8 × Table 2). In peel tissue, the mean level of α-Gluc-i activity was found to be 74.4% of the control (20 µl α-glucosidase, 1 U/ml, range 65.7–89.3%). Higher levels of inhibitory activity observed in peel tissue did not consistently correlate with those in pulp tissue. The three clones with highest inhibitory activities (US 1247, 'Legacy' and US 1218; >80% of inhibitory activity) in peel tissue did not rank highest in pulp tissue (Table 2).

The range of the genotypes evaluated was limited; however, overall, the levels of α-Gluc-i activity in the hybrids with mixed-species components did not appear to be demonstrably different from that in either the pure rabbiteye or highbush genotypes. Nonetheless, some selections containing specific combinations of germplasm expressed higher levels of α-Gluc-i in peel tissue. The clone that expressed the highest level of inhibition (US 1247) was comprised of about 20% *Vaccinium boreale* Hall & Aalders ancestry (a low-growing, cold-hardy, diploid species). However, two selections with equivalent percentages of *V. boreale* ancestry (US 1144 and US 1145) were not unusual in their inhibition levels. Similarly, in pulp tissue, three selections with *Vaccinium constablaei* Gray ancestry (a cold-hardy, high-altitude, hexaploid species) had elevated levels of α-Gluc-i (US 1216, US 1382, US 1384); however, again, the levels of α-Gluc-i activity in similarly comprised selections were not different from those in the overall group. Nonetheless, these data mildly suggest that if one were looking for enhanced levels of α-Gluc-i in the peel, a broader range of clones particularly with *V. boreale* ancestry could be a useful place to begin.

For the levels TA, TP and antioxidant activity, significant differences were observed between the peel and pulp ($P \leq 0.0001$). On a dry-weight basis, an average of 30.2 times higher levels for TA (range 6.7–76.6 ×) and 17.7 times those for TP (range 10.5–26.5 ×) were observed between the peel and pulp. Significant differences were also observed among the genotypes (Table 2).

The blueberry genotypes exhibited high levels of antioxidant activity against the ROO•, •OH and $^1O_2$ radicals and against the oxidant H_2O_2, and significant differences were found between the peel and pulp tissue for each of these parameters ($P \leq 0.0001$). Significant differences were also observed between several genotypes compared with 'Legacy' (Table S1, available online). All the extracts from peel tissue exhibited

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Table 1. Evaluation of blueberry genotypes for α-glucosidase inhibitory activity and antioxidant capacity [two highbush hybrids (*Vaccinium corymbosum* L.), 16 mixed-species rabbiteye hybrids (*Vaccinium ashei* Reade × *Vaccinium* spp.) and one rabbiteye hybrid (*V. ashei*)]

		Species composition (%)[a]						
Genotype	Parentage and ploidy level	*ash*	*cor*	*con*	*dar*	*ang*	*bor*	Other
Highbush hybrids (*V. corymbosum*)								
US 1252	US 813 × US 1025 (4*x*)	–	78	–	14	10	–	–
Legacy	Elizabeth × US 75 (4*x*)	–	75	–	25	–	–	–
Mixed-species rabbiteye hybrids (*V. ashei* Reade × *Vaccinium* spp.)								
US 1048, US 1049	US 871 × T 286 (6*x*)	50	18	25	5	–	–	3
US 1055	US 874 × JU 8 (6*x* × 5*x*)	27	37	27	5	–	–	4
US 1057, US 1060	US 874 × Premier (6*x*)	50	18	25	5	–	–	3
US 1144, US 1145	US 876 × Delite (4*x* × 6*x*)	60	20	–	–	–	20	<1
US 1216	Delite × Little Giant (6*x*)	75	–	25	–	–	–	–
US 1218, US 1219	Delite × Sierra (6*x* × 4*x*)	66	19	6	8	–	–	<1
US 1247	US 960 × Climax (4*x* × 6*x*)	60	18	–	–	–	20	<3
US 1382	Centurion × Nocturne (6*x*)	75	9	13	–	–	–	<4
US 1384	Climax × Little Giant (6*x*)	75	–	25	–	–	–	–
US 1385	Climax × Nocturne (6*x*)	75	9	13	–	–	–	<4
US 1638, US 1639	NJ 89-158-6 × Montgomery (6*x*)	50	35	–	9	5	–	<2
Rabbiteye hybrid (*V. ashei* Reade)								
Columbus	Tifblue × Menditoo (6*x*)	100	–	–	–	–	–	–

ang = *V. angustifolium*; *ash* = *V. ashei*; *bor* = *V. boreale*; *con* = *V. constablaei*; *cor* = *V. corymbosum*; *dar* = *V. darrowii*.
[a] Species composition was calculated from pedigree diagrams; fractions were rounded to the nearest whole percent.

Table 2. α-Glucosidase inhibitory activity, total anthocyanin (TA) and total phenolic (TP) levels from the peel and pulp extracts of blueberries compared with 'Legacy'[a]

	α-Glucosidase inhibitory activity[b] (% vs. standard)		TA (mg/g dw)		TP (mg/g dw)	
Genotype	Peel	Pulp	Peel	Pulp	Peel	Pulp
Highbush hybrid (*Vaccinium corymbosum* L.)						
Legacy	83	14	93.4	1.2	64.2	2.4
US 1252	74	15	64.6*	3.1*	59.5	3.3
Mixed-species rabbiteye hybrids (*Vaccinium ashei* Reade × *Vaccinium* spp.)						
US 1048	**66***	18	**30.2***	3.1*	**36.6***	3.3
US 1049	68	20*	34.7*	3.3*	39.4*	3.6*
US 1055	70	14	54.3*	2.5*	37.8*	2.3
US 1057	75	18	48.8*	3.1*	42.5*	2.4
US 1060	69	15	45.9*	3.0*	39.1*	2.6
US 1144	71	15	55.6*	1.4	48.3*	3.0
US 1145	72	14	58.6*	**1.0**	50.8*	2.8
US 1216	67*	23*	47.5*	5.9*	43.9*	4.0*
US 1218	81	18	76.4*	1.9	71.6	3.3
US 1219	78	17	74.9*	1.9	67.1	3.3
US 1247	**89**	18	**96.6**	3.8*	**83.7***	3.3
US 1382	72	**28***	64.7*	**9.7***	47.6*	**4.5***
US 1384	75	24*	74.1*	8.6*	50.4*	3.7*
US 1385	78	17	74.9*	1.6	54.6	2.9
US 1638	74	16	50.0*	1.4	47.4*	2.4
US 1639	72	**13**	53.5*	1.2	45.4*	**2.2**
Rabbiteye hybrid (*V. ashei* Reade)						
Columbus	79	16	82.4	1.9	50.9*	2.2
Means	74	18	62.2	3.1	51.6	3.0

dw, dry weight.
[a] Values given in boldface represent the minimum and maximum values in each category. Values with asterisks are significantly different ($P \leq 0.05$) from those of the standard highbush cultivar Legacy. [b] α-Glucosidase inhibitory activity level was determined by measuring the area under the curve for each sample compared with that of the control. The results are expressed as a percentage of α-glucosidase inhibitory activity.

stronger antioxidant activities (Table S1, available online). The mean ratios and ranges between the peel and pulp for radical-scavenging capacity were as follows: ROO• (4.6×, range 1.4–6.6×); •OH (9.2×, range 3.9–12.6×); $^1O_2$ (4.0×, range 1.6–9.4×); H_2O_2 (4.8×, range 2.2–7.0×). For the parameters evaluated, overall, the hybrids with mixed-species components did not appear demonstrably different from either the pure rabbiteye or highbush genotypes.

The correlations (r) among the levels of α-Gluc-i activity, TA, TP and scavenging activity against ROO•, •OH, $^1O_2$ and H_2O_2 radicals in the extracts from the peel and pulp were found to be positive and significant ($P \leq 0.05$; Table S2, available online). For peel extracts, the correlation coefficients ranged from 0.84 to 0.97. For pulp extracts, similar correlations existed, with most (but not all) of the correlation values being slightly less than those observed in peel extracts (Table S2, available online). The highest correlations were observed between the levels of α-Gluc-i activity and radical-scavenging activity against ROO• in both peel and pulp (0.95 and 0.93, respectively). However, particularly important were the correlations between the levels of α-Gluc-i activity and TA ($r = 0.93$ and 0.92 for the peel and pulp, respectively). The ease of assaying for TA levels suggests that it would be the best proxy for α-Gluc-i activity if a broader genotypic evaluation of blueberry genotypes is desired.

Supplementary material

To view supplementary material for this article, please visit http://dx.doi.org/10.1017/S1479262114000690

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