Correlation of Antioxidant Capacities to Oxygen Radical Scavenging Enzyme Activities in Blackberry

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The activities of the oxygen radical scavenging enzymes [glutathione-peroxidase (GSH-POD), superoxide dismutase (SOD), and guaiacol peroxidase (G-POD)], hydrogen peroxide scavenging enzymes in the ascorbate-glutathione cycle [ascorbate peroxidase (AsA-POD), monodehydroascorbate reductase (MDAR), dehydroascorbate reductase (DHAR), and glutathione reductase (GR)], the nonenzyme components [ascorbate (AsA), dehydroascorbate (DHAsA), glutathione (GSH), and oxidized glutathione (GSSG)], and their antioxidant capacity [oxygen radical absorbance capacity (ORAC)] were measured in the juice of six different thornless blackberry (*Rubus* sp.) cultivars. The 'Hull Thornless' cultivar contained the highest levels, whereas 'Black Satin' consistently had the lowest activities for all the enzymes tested in this study. ORAC values were also the highest in 'Hull Thornless' and lowest in 'Black Satin'. The highest levels of AsA and DHAsA were in the juice of 'Hull Thornless' blackberries with 1.09 and 0.15 μmol/g fresh wt, respectively. 'Hull Thornless' also had the highest ratio of AsA/DHAsA among the six blackberry cultivars studied. The 'Smoothstem' cultivar contained the lowest amounts of AsA and DHAsA. 'Hull Thornless' had the highest GSH content with 78.7 nmol/g fresh wt, while 'Chester Thornless' contained the largest amount of GSSG. The highest GSH/GSSG ratio was 4.90 which was seen in the 'Hull Thornless' cultivar. The correlation coefficient between ORAC values and AsA/DHAsA ratios was as high as 0.972. A correlation ($r = 0.901$) was also detected between ORAC values and GSH content. The antioxidant activity in blackberry juice was positively correlated to the activities of most antioxidant enzymes ($r = 0.902$ with SOD; $r = 0.858$ with GSH-POD; $r = 0.896$ with ASA-POD; and $r = 0.862$ with GR).

Keywords: *Antioxidant; free radicals; oxygen scavenging enzymes; blackberry*

INTRODUCTION

Blackberries are good sources of natural antioxidants (Wang et al., 1996). In addition to vitamins and minerals, extracts of blackberries are also rich in anthocyanin, other flavonoids, and phenolic acids (Heinonen et al., 1998). Berry fruits have shown a remarkably high scavenging activity toward chemically generated radicals (Heinonen et al., 1998). They are effective in inhibiting oxidation of human low-density lipoproteins and thus have potential effects in preventing various human diseases (Ames, 1983; Gey et al., 1991; Steinberg, 1991).

Our previous study showed that blackberries have high oxygen radical absorbance activity against peroxyl radicals ($ROO^{•}$), superoxide radicals ($O_2^{•-}$), hydrogen peroxide (H_2O_2), hydroxyl radicals ($OH^{•}$), and singlet oxygen ($'O_2$) (Wang and Lin, 2000; Wang and Jiao, 2000). Antioxidant activities differ among cultivars and correlate positively with total phenolic or anthocyanin content. However, no information is available about the enzymatic system for active oxygen detoxification in blackberry. The present study was undertaken to determine the involvement of superoxide dismutase (SOD), guaiacol peroxidase (G-POD), glutathione-peroxidase (GSH-POD), catalase, and enzymes in the ascorbate-glutathione cycle [ascorbate peroxidase (AsA-POD), monodehydroascorbate reductase (MDAR), dehydroascorbate reductase (DHAR), and glutathione reductase (GR)] and the nonenzyme components [ascorbate (AsA), dehydroascorbate (DHAsA), glutathione (GSH), and oxidized glutathione (GSSG)] which might be actively involved in oxygen detoxification in blackberry juice. The relationship of these oxygen radical scavenging enzyme activities to the antioxidant capacity in blackberry juice was also investigated.

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MATERIALS AND METHODS

Chemicals. *N*-Ethylmaleimide, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), and trichloroacetic acid were obtained from Aldrich Chemical Co. (Milwaukee, WI). EDTA (ethylenediaminetetracetic acid, disodium salt, dihydrate-Na_2 EDTA·$2H_2O$) was obtained from Life Technologies (Rockville, MD). Ascorbate oxidase, bathophenanthroline, dicoumarol, dithiothreitol (DDT), glutathione (oxidized form), glutathione (GSH, reduced form), glutathione reductase, guaiacol, hydrogen peroxide (30% w/w), methionine, β-nicotinamide adenine dinucleotide (β-NADH, reduced form), β-nicotinamide adenine dinucleotide phosphate (β-NADPH, reduced form), nitro blue tetrazolium, phenylmethylsulfonyl fluoride, R-phycoerythrin (R-PE, from *Porphydium cruentum*), polyvinylpolypyrrolidone, and riboflavin (vitamin B_2) were purchased from Sigma Chemical Co. (St. Louis, MO). 2′,2′-Azobis(2-amidinopropane) dihydrochloride (AAPH) was purchased from Wako Chemicals USA Inc. (Richmond, VA).

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Fruit Sample Preparation. Thornless blackberries ('Black Satin', 'Chester Thornless', 'Hull Thornless', 'Smoothstem', 'Thornfree', and 'Triple Crown') used in this study were grown at the Beltsville Agricultural Research Center. Blackberry fruits were harvested from six to eight bushes of each cultivar at the commercially ripe stage. Undamaged berries were selected and mixed, and sample juices were obtained by pulverizing three 100 g composite samples of berries from each cultivar. All juice samples were centrifuged at 20 000*g* for 20 min at 2 °C and the supernatant was used for enzyme and oxygen radical absorbance capacity (ORAC) assays.

Glutathione-Peroxidase (GSH-POD, EC 1.11.1.9). Blackberry juice (2 mL) was diluted with 8 mL of 50 mM of sodium phosphate buffer (pH 6.1). GSH-POD activity was determined using the method of Tappel (1978) with a slight modification. The reaction mixture contained 0.1 M Tris-HCl buffer (pH 8.0), 0.4 mM EDTA, 1.0 mM NaN_3, 1.0 mM glutathione (GSH), 0.15 mM NADPH, 1 unit of glutathione reductase, and 0.1 mL of diluted juice. The total reaction volume was 1.0 mL. The reaction was started by adding 0.1 mL of 1.0 mM H_2O_2 . GSH-POD activity was determined via a spectrophotometer (Shimadzu UV-160A, Shimadzu Scientific Instruments, Columbia, MD) on the rate of NADPH oxidation at 340 nm. Enzyme activity was expressed as nanomoles of NADPH oxidized per milligram of protein per minute.

Glutathione Reductase (GR, EC 1.6.4.2). GR activity was assayed according to Smith et al. (1988). The assay mixture contained 50 mM Tris-HCl buffer (pH 7.5), 3 mM $MgCl_2$, 0.5 mM oxidized glutathione (GSSG), 2 mM EDTA, 0.15 mM NADPH, and 0.1 mL of diluted fruit juice (2 mL of fruit juice was diluted with 8 mL of 50 mM Tris-HCl buffer, pH 7.5). The total reaction volume was 1.0 mL. The activity of GR was determined by monitoring the glutathione-dependent oxidation of NADPH at 340 nm. The reaction was started by adding GSSG, and the rate of oxidation was calculated using the extinction coefficient of NADPH (6.22 mM^{-1} cm^{-1}). GR activity was expressed as nanomoles of NADPH oxidized per milligram of protein per minute.

Superoxide Dismutase (SOD, EC 1.15.1.1). Blackberry juice (2 mL) was diluted with 8 mL Na−phosphate buffer (50 mM, pH 6.1) and was purified according to Wang et al. (1991) before assaying the SOD enzyme activity. Total SOD activity was assayed photochemically (Monk et al. 1987), based on the photoreduction of nitro blue tetrazolium (NBT) by light in the presence of riboflavin and methionine. NBT is reduced to blue diformazan, which has a strong absorbance at the 560 nm wavelength. Under aerobic assay conditions, SOD inhibits the formation of blue diformazan. Nitro blue tetrazolium can be reduced by either pyridine nucleotide or O_2^- (Thayer, 1990). Dicoumarol was included in the reaction mixture to inhibit reduction by pyridine nucleotide (Thayer, 1990) and to obtain a completely O_2^- -dependent reduction of NBT. The reaction mixture (1 mL) contained 100 M dicoumarol, 1.3 M riboflavin, 13 mM methionine, 0.05 M Na_2CO_3, 0.01 M Na-phosphate buffer (pH 7.8), and 0.1 mL of the enzyme extract; after 3 min, 63 μM NBT was added. The mixtures were illuminated by fluorescent lamp (light intensity was 170 $\mu E \cdot m^{-2}\, s^{-1}$) for 3 min. Identical solutions held in the dark served as blanks. One unit of SOD was defined as the amount of enzyme which produced a 50% inhibition of NBT reduction under assay conditions. Since inhibition is not linearly correlated with SOD concentration, a V/v transformation was used to obtain linearity (V = basic reaction rate without blackberry juice extract, v = reaction rate with extract). Linear correlation gave the equation: SOD units/mL = $(0.467\,V/v - 0.039) \times$ dilution factor. The correlation coefficient for this line was 0.987.

Ascorbate Peroxidase (AsA-POD, EC 1.11.1.11). AsA-POD activity was assayed according to the method of Amako et al. (1994) by measuring the oxidation of ascorbate at 290 nm; the reaction mixture contained 50 mM potassium phosphate, pH 7.0, 0.5 mM ascorbate, 0.1 mM EDTA, 0.5 mM H_2O_2, and 0.1 mL of potassium phosphate diluted fruit juice (5-time dilution) in a total volume of 1.0 mL. The reaction was started by adding H_2O_2. Enzyme activity was expressed as nanomoles of ascorbate oxidized per milligram of protein per min.

Dehydroascorbate Reductase (DHAR, EC 1.8.5.1). DHAR activity was assayed by measuring the rate of NADPH oxidation at 340 nm (Shigeoka et al. 1980). The reaction mixture contained 50 mM potassium phosphate, pH 6.1, 0.2 mM NADPH, 2.5 mM dehydroascorbate, 2.5 mM glutathione, 0.6 unit glutathione reductase (GR; from spinach, EC 1.6.4.2), and 0.1 mL of diluted fruit juice (2 mL of juice was diluted with 2 mL of 50 mM potassium phosphate, pH 6.1). The reaction was started by adding dehydroascorbate. Enzyme activity was expressed as nanomoles of NADPH oxidized per milligram of protein per min.

Monodehydroascorbate Reductase (MDAR, EC 1.6.5.4). MDAR activity was assayed by measuring the rate of NADH oxidation at 340 nm (Nakagawara and Sagisaka, 1984). The reaction mixture contained 50 mM K−phosphate buffer (pH 7.3), 0.2 mM NADH, 1.0 mM ascorbate, 1.0 unit of ascorbate oxidase, 0.1 mL of 50 mM K−phosphate buffer (pH 7.3) diluted fruit juice (2-time dilution) in a total volume of 1.0 mL. The reaction was started by adding ascorbate oxidase (from *Cucurbita*, EC 1.10.3.3). Enzyme activity was expressed as nanomoles of NADH oxidized per milligram of protein per min.

Guaiacol Peroxidase (G-POD, EC 1.11.1.7). The G-POD assay mixture contained 0.1 M phosphate buffer (pH 6.1), 4 mM guaiacol as donor, 3 mM H_2O_2 as substrate, and 1.0 mL of 0.1 M phosphate buffer (pH 6.1) diluted juice (5-time dilution). The total reaction volume was 3.0 mL. The rate of change in absorbance at 420 nm was measured, and the level of enzyme activity was expressed as the difference in absorbance (OD) per mg protein, per min.

Determination of Ascorbate (AsA) and Dehydroascorbate (DHAsA). AsA and DHAsA were determined using the methods of Arakawa et al. (1981) and Nakagawara and Sagisaka (1984). This assay is based on the reduction of ferric ion to ferrous ion with ascorbic acid in acid solution followed by formation of the red chelate between ferrous ion and 4,7-diphenyl-1,10-phenanthrolin (bathophenanthroline) that absorbs at 534 nm. Total ascorbate (ascorbate plus dehydroascorbate) was determined through a reduction of dehydroascorbate to ascorbate by dithiothreitol. The ascorbate assay mixture contained 0.1 mL of the fruit juice, 0.5 mL of absolute ethanol, 0.6 M trichloroacetic acid, 3 mM bathophenanthroline, 8 mM H_3PO_4, 2 mM *N*-ethylmaleimide, and 0.17 mM $FeCl_3$. The final total volume was 1.5 mL, and the solution was allowed to stand at 30 °C for 90 min for the Fe^{2+}-batho-phenanthroline complex to develop. The absorbance of the colored solution was read at 534 nm. The total ascorbate assay mixture contained 0.1 mL of the fruit juice, 0.15 mL of 3.89 mM dithiothreitol, and 0.35 mL of absolute ethanol in a total volume of 0.6 mL. Then, the reaction mixture was left standing at room temperature for 10 min. After reduction of dehydroascorbate to ascorbate, 0.15 mL of 0.24% *N*-ethylmaleimide in ethanol and 0.15 mL of 20% trichloroacetic acid were added. The color was developed by adding the following reagents in this sequence: 0.15 mL of 0.4% (v/v) H_3PO_4−ethanol, 0.3 mL of 0.5% (w/v) bathophenanthroline−ethanol, and 0.15 mL of 0.03% (w/v) $FeCl_3$−ethanol. The final volume was 1.5 mL, and after incubation at 30 °C for 90 min, the absorbance at 534 nm was recorded. Dehydroascorbate concentrations were estimated from the difference of "total ascorbate" and "ascorbate" concentrations. A standard curve in the range 0−10 μmol ascorbate or dehydroascorbate was used.

Measurement of Glutathione (GSH) and Oxidized Glutathione (GSSG). GSH and GSSG were assayed using the method described by Castillo and Greppin (1988). Total glutathione equivalents were determined by reacting 0.1 mL of juice with 60 mM KH_2PO_4−2.5 mM EDTA buffer (pH 7.5), 0.6 mM DTNB [5,5′-dithiobis(2-nitrobenzoic acid)] in 200 mM Tris-HCl, pH 8.0, 1 unit of glutathione reductase (GR; from spinach, EC 1.6.4.2), and 0.2 mM NADPH. The reaction was followed as the rate of change in absorbance at 412 nm by a spectrophotometer (Shimadzu UV-160A), and the total glutathione content was calculated from a standard curve. Oxidized glutathione (GSSG) was determined after removal of reduced glutathione (GSH) from the sample juice. GSH was determined from the reaction mixture by mixing 0.1 mL of

Table 1. Activities of Superoxide Dismutase (SOD), Guaiacol Peroxidase (G-POD), and Glutathione Peroxidase (GSH-POD) in Juice of Thornless Blackberry[a]

cultivar	SOD (U/mg protein)	G-POD (ΔA/mg protein·min)	GSH-POD (nmol/mg protein·min)
Black Satin	8.62 ± 0.5	1.63 ± 0.06	20.44 ± 0.6
Chester Thornless	13.49 ± 0.4	2.84 ± 0.10	27.27 ± 0.8
Hull Thornless	13.98 ± 0.3	3.25 ± 0.13	29.87 ± 1.1
Smoothstem	11.66 ± 0.2	1.26 ± 0.08	24.80 ± 0.7
Thornfree	11.30 ± 0.3	3.08 ± 0.05	25.73 ± 0.6
Triple Crown	11.76 ± 0.1	2.58 ± 0.07	26.98 ± 0.4
$LSD_{0.05}$	0.351	0.254	1.105
significance[b]			
cultivar	*	*	*

[a] Data expressed as mean ± SEM ($n = 3$). [b] * significant at $p \leq 0.05$.

Table 2. Activities of Ascorbate Peroxidase (Ascorbate-POD), Monodehydroascorbate Radical Reductase (MDAR), Dehydroascorbate Reductase (DHAR), and Glutathione Reductase (GR) in Juice of Thornless Blackberry[a]

	(nmol/mg protein·min)			
cultivar	AsA-POD	MDAR	DHAR	GR
Black Satin	102.5 ± 12	16.8 ± 1.9	6.2 ± 1.0	10.8 ± 1.6
Chester Thornless	145.6 ± 9	25.1 ± 2.1	10.4 ± 0.6	17.4 ± 1.2
Hull Thornless	158.5 ± 11	28.0 ± 2.5	11.8 ± 1.1	21.3 ± 1.4
Smoothstem	128.7 ± 4	23.9 ± 1.8	8.2 ± 0.7	15.2 ± 1.1
Thornfree	123.2 ± 3	19.9 ± 2.0	9.1 ± 0.5	16.5 ± 0.3
Triple Crown	145.7 ± 8	24.9 ± 1.0	10.2 ± 0.1	16.9 ± 0.2
$LSD_{0.05}$	8.12	0.82	0.60	0.95
significance[b]				
cultivar	*	*	*	*

[a] Data expressed as mean ± SEM ($n = 3$). [b] * significant at $p \leq 0.05$.

juice with 60 mM KH_2PO_4−2.5 mM EDTA buffer (pH 7.5) and 0.6 mM DTNB [5,5′-dithiobis(2-nitrobenzoic acid)] in 200 mM Tris-HCl, pH 8.0. The mixture was incubated at 30 °C for 10 min, and the reaction was followed as the rate of change in absorbance at 412 nm. GSSG was determined by subtraction of GSH from total glutathione.

Oxygen Radical Absorbance Capacity (ORAC) Assay. ORAC assays for blackberry juice were carried out following procedures modified from a method previously described by Cao et al. (1993). The reaction mixture contained 1.7 mL of 75 mM phosphate buffer (pH 7.0), 100 μL of R-PE (3.4 mg/l), 100 μL of 320 mM AAPH, and 100 μL of sample. Phosphate buffer was used as a blank and 1 μM of Trolox (a water-soluble α-tocopherol analogue) as a standard during each run. The final volume of 2 mL was used in a 10 mm wide fluorometer cuvette. R-PE, phosphate buffer, and samples were preincubated at 37 °C for 15 min. The reaction was started by the addition of AAPH. Fluorescence was measured and recorded every 5 min at the emission of 570 nm and excitation of 540 nm using a Shimadzu RF-Mini 150 Recording Fluorometer (Columbia, MD) until the fluorescence of the last reading declined to less than 5% of the first reading. This usually took approximately 70 min. One blank, one standard, and a maximum of 10 samples were analyzed at the same time. Each sample was repeated three times. The ORAC value refers to the net protection area under the quenching curve of R-PE in the presence of an antioxidant. The final results (ORAC value) were calculated and expressed using Trolox equivalents per gram fresh weight (WM) basis (Cao et al., 1993).

Protein Determination. Protein was determined according to Bradford (1976), using bovine serum albumin (BSA) as a standard.

Statistical Analysis. Correlation and regression analyses of ORAC versus oxygen scavenging enzymes (SOD, G-POD, GSH-POD, AsA-POD, MDAR, DHAR, and GR) and the nonenzyme components (AsA, DHAsA, GSH, GSSG) were performed using NCSS (NCSS 97 Kaysville, UT). Data were subjected to analysis of variance, and the effect of cultivars on ORAC values and activities of oxygen scavenging enzymes or nonenzyme components were evaluated by the Tukey-Kramer Multiple-Comparison test used in NCSS. Differences at $p < 0.05$ were considered significant.

RESULTS

Oxygen Scavenging Enzymes. The activities of the oxygen scavenging enzymes superoxide dismutase (SOD), guaiacol peroxidase (G-POD), and glutathione peroxidase (GSH-POD) in the juice of six different thornless blackberry cultivars are shown in Table 1. 'Hull Thornless' contained the highest levels of activity for all of the enzymes with 13.98 U/mg protein SOD, 3.25 ΔA/mg protein·min G-POD, and 29.87 nmol/mg protein·min GSH-POD. Among all of the cultivars tested, 'Black Satin' had the lowest activities of SOD with 8.62 U/mg protein and GSH-POD with 20.44 nmol/mg protein·min. Meanwhile Smoothstem had the lowest G-POD activity with only 1.26 ΔA/mg protein·min.

Enzymes in Ascorbate-Glutathione Cycle. Activities of enzymes in the ascorbate-glutathione cycle including ascorbate peroxidase (AsA-POD), monodehydroascorbate radical reductase (MDAR), dehydroascorbate reductase (DHAR), and glutathione reductase (GR) in various thornless blackberry cultivars are shown in Table 2. The 'Hull Thornless' cultivar had the highest enzyme activities for these enzymes in the ascorbate-glutathione cycle with 158.5 nmol/mg protein·min AsA-POD, 28.0 nmol/mg protein·min MDAR, 11.8 nmol/mg protein·min DHAR, and 21.3 nmol/mg protein·min GR. Juice from the 'Chester Thornless' cultivar had the second highest levels of enzyme activity in MDAR, DHAR, and GR. 'Black Satin' consistently had the lowest activities for all four enzymes in the ascorbate-glutathione cycle.

Nonenzyme Components. Ascorbic acid (AsA), dehydroascorbic acid (DHAsA), reduced glutathione (GSH), and oxidized glutathione (GSSG) were found in blackberry juice (Table 3). The highest levels of AsA and DHAsA were observed in the juice of 'Hull Thornless' fruit with 1.09 and 0.15 μmol/g fresh wt, respectively. 'Hull Thornless' also had the highest ratio of AsA/DHAsA among the six blackberry cultivars studied. 'Chester Thornless' fruit juice had the next highest content of AsA. The 'Smoothstem' cultivar contained the lowest amounts with 0.64 μmol/g fresh wt AsA and 0.10 μmol/g fresh wt DHAsA. 'Hull Thornless' had the highest GSH content with 78.7 nmol/g fresh wt, while 'Chester Thornless' contained the largest amount of GSSG with 16.3 nmol/g fresh wt. 'Black Satin' contained the least GSH at 63.9 nmol/g fresh wt, while 'Thornfree' had the lowest levels of GSSG with 15.5 nmol/g fresh wt. The highest GSH/GSSG ratio was 4.90 which was found in the 'Hull Thornless' cultivar.

$ORAC_{ROO\cdot}$ Activity in Blackberry Juice. Antioxidant capacity (expressed as an ORAC value) in fruit juice of blackberry varied greatly among various cultivars (Figure 1). The ORAC values for blackberry juice ranged from 14.8 to 22.6 μmol TE/g of fresh berries, with the cv. Hull Thornless yielding the highest ORAC value followed by 'Chester Thornless', 'Triple Crown', 'Smoothstem', and 'Thornfree'. 'Black Satin' had the lowest ORAC value (Figure 1).

Table 3. Ascorbic Acid (AsA), Dehydroascorbic Acid (DHAsA), Reduced Glutathione (GSH), Oxidized Glutathione (GSSG), and Ratios of AsA/DHAsA and GSH/GSSG in Juice of Thornless Blackberry[a]

cultivar	AsA (μmol/g fwt)	DHAsA (μmol/g fwt)	GSH (nmol/g fwt)	GSSG (nmol/g fwt)	AsA/DHAsA (ratio)	GSH/GSSG (ratio)
Black Satin	0.71 ± 0.04	0.12 ± 0.01	63.9 ± 1.7	15.7 ± 1.1	5.79 ± 0.10	4.07 ± 0.06
Chester Thornless	1.02 ± 0.04	0.14 ± 0.01	76.3 ± 1.4	16.3 ± 1.2	7.11 ± 0.18	4.69 ± 0.03
Hull Thornless	1.09 ± 0.06	0.15 ± 0.01	78.7 ± 2.2	16.0 ± 1.4	7.30 ± 0.12	4.90 ± 0.11
Smoothstem	0.64 ± 0.05	0.10 ± 0.02	64.1 ± 1.2	15.5 ± 0.3	6.22 ± 0.37	4.14 ± 0.05
Thornfree	0.76 ± 0.04	0.13 ± 0.01	65.9 ± 1.3	14.8 ± 0.6	5.92 ± 0.64	4.44 ± 0.17
Triple Crown	0.84 ± 0.06	0.12 ± 0.01	72.7 ± 2.5	15.6 ± 0.3	6.62 ± 0.15	4.65 ± 0.02
$LSD_{0.05}$	0.077	0.016	4.21	0.79	0.528	0.264
significance[b]						
cultivar	*	*	*	*	*	*

[a] Data expressed as mean ± SEM (n = 3). [b] * significant at $p \leq 0.05$.

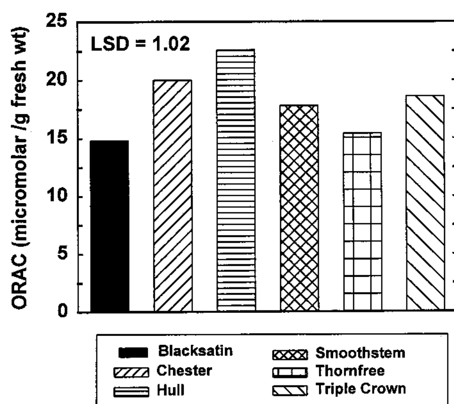

Figure 1. Antioxidant activity (ORAC) of juices in various cultivars of thornless blackberry.

DISCUSSION

High levels of free radicals or active oxygen species create oxidative stress which leads to a variety of biochemical and physiological lesions often resulting in metabolic impairment and cell death. Various activities of antioxidant and oxygen scavenging enzymes were detected in blackberry juice. 'Hull Thornless' blackberry juice had the highest activities, and 'Black Satin' contained the least activities of antioxidant and oxygen scavenging enzymes, enzymes in the ascorbate-glutathione cycle, and also nonenzyme components in the ascorbate-glutathione cycle among the six different cultivars used in this study.

SOD catalyzes the breakdown of $O_2^{\bullet -}$ to O_2 and H_2O_2, removes singlet oxygen as well as $O_2^{\bullet -}$, prevents formation of OH^- (Fridovich, 1973), and has been implicated as an essential defense against the potential toxicity of oxygen (McCord, 1979). The SOD activities in blackberry juice range from 8.62 to 13.98 U/mg protein. High SOD activity also correlated to high antioxidant activity with r = 0.902 in blackberry juice.

G-POD is involved in a large number of biochemical and physiological processes. The mode of action of G-POD on the H_2O_2 substrate is to liberate free radicals. These free radicals are highly phytotoxic. The accumulation of H_2O_2 may cause changes in plant metabolism. The H_2O_2 formed by G-POD may be scavenged by catalase and GSH-POD. However, the catalase activity in blackberry juice was not detectable (data not shown). GSH-POD may be responsible for scavenging H_2O_2, catalyzing the peroxidation of reduced glutathione (GSH), and forming the oxidized disulfide form of glutathione (GSSG) as a product. Blackberry juice showed activities of G-POD and GSH-POD. The activity of GSH-POD is correlated to antioxidant activity in blackberry juice with r equal to 0.858, whereas G-POD had a low correlation (r = 0.489) to antioxidant activity.

Blackberry juice from all six cultivars contained enzyme activity in the ascorbate-glutathione cycle (AsA-POD, DHAR, MDAR, and GR) and had high contents of AsA, GSH, and high ratios of AsA/DHAsA, GSH/GSSG. 'Hull Thornless' blackberry juice had higher antioxidant content, ratios of AsA/DHAsA, GSH/GSSG, and enzyme activities than other blackberry cultivars.

AsA-POD is a heme-containing protein and is highly specific for ascorbate as the electron donor. The basic properties of AsA-POD are very different from those of G-POD with regards to their amino sequences and other molecular properties (Asada, 1992). Ascorbic acid is an essential compound in plant tissues and has been the focus of numerous studies in relation to the enzymatic and nonenzymatic oxidations in the biological system. It serves as an excellent antioxidant and plays a fundamental role in removal of hydrogen peroxide via the ascorbate-glutathione cycle and produces DHAsA. DHAsA is reduced to ascorbic acid by MDAR or DHAR at the expense of NADH and glutathione (GSH) (Halliwell, 1982). AsA-POD activity positively correlated with ascorbic acid content in blackberry juice (r = 0.834). The close correlations between ORAC values and AsA content (r = 0.837), ASA-POD (r = 0.896), and ratio of AsA/DHAsA (r = 0.972) were also evident.

Glutathione is the major low molecular weight thiol compound in most plants and exists in both a reduced form (GSH) and an oxidized form, glutathione disulfide (GSSG). The reduced form of glutathione plays an important role in the stabilization of many enzymes. It also serves as a substrate for DHAR and reacts directly with free radicals including hydroxyl radical to prevent the inactivation of enzymes by oxidation of the essential thiol group. The majority of glutathione in the cell is maintained in the reduced state (Kosower and Kosower, 1978). GSH in blackberry juice ranges from 63.9 to 78.7 nmol/g fresh wt. GSSG was found to be present in low quantities with a concentration below 17.0 nmol/g fresh wt. A high GSH/GSSG ratio is necessary for several physiological functions. This includes activation and inactivation of redox-dependent enzyme systems (Ziegler, 1985). Cultivar fruit juices with high ORAC values also had high GSH contents (r = 0.901) and high GSH/GSSH ratios (r = 0.827).

Glutathione reductase (GR) is an ubiquitous NADPH-dependent enzyme and is present in cells of both plants and animals (Jocelyn, 1972). It has been suggested that in higher plants, GR may be a rate-limiting enzyme for defense against active O_2 toxicity (Gossett et al., 1996; Tanaka, 1994). In blackberry juice, we found GR activity ranged from 10.8 to 21.3 nmol/mg protein·min with 'Hull

Thornless' having the highest activity and 'Black Satin' the lowest. Cultivars with high activity of GR were also found to have high antioxidant activity in the juice ($r = 0.862$).

Collectively, our data showed that blackberry juice of 'Black Satin', 'Chester Thornless', 'Hull Thornless', 'Smoothstem', 'Thornfree', and 'Triple Crown' show considerable antioxidant activity. High ascorbic acid content and activities of SOD, G-POD, GSH-POD, AsA-POD, MDAR, and DHAR could increase the capacity for free-radical scavenging. The high activity of GR and a high level of GSH may play an important role as antioxidants and could prevent the formation of S−S bonds, therefore providing high antioxidant activity in juice.

ABBREVIATIONS USED

AAPH, 2′,2′-azobis(2-amidinopropane) dihydrochloride; AsA, ascorbate; AsA-POD, ascorbate peroxidase; DHAsA, dehydroascorbate; DHAR, dehydroascorbate reductase; GSH, glutathione; GSH-POD, glutathione-peroxide; GR, glutathione reductase; G-POD, guaiacol peroxidase; superoxide dismutase (SOD), MDAR, nonodehydroascorbate reductase; GSSG, oxidized glutathione; ORAC, oxygen radical absorbance capacity; R-PE, R-phycoerythrin; Trolox, 6- hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; TE, trolox equivalents.

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