Antioxidant Activity in Fruits and Leaves of Blackberry, Raspberry, and Strawberry Varies with Cultivar and Developmental Stage

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Fruits and leaves from different cultivars of thornless blackberry (*Rubus* sp.), red raspberry (*Rubus idaeus* L.), black raspberry (*Rubus occidentalis* L.), and strawberry (*Fragaria* × *ananassa* D.) plants were analyzed for total antioxidant capacity (oxygen radical absorbance capacity, ORAC) and total phenolic content. In addition, fruits were analyzed for total anthocyanin content. Blackberries and strawberries had the highest ORAC values during the green stages, whereas red raspberries had the highest ORAC activity at the ripe stage. Total anthocyanin content increased with maturity for all three species of fruits. Compared with fruits, leaves were found to have higher ORAC values. In fruits, ORAC values ranged from 7.8 to 33.7 μmol of Trolox equivalents (TE)/g of fresh berries (35.0−162.1 μmol of TE/g of dry matter), whereas in leaves, ORAC values ranged from 69.7 to 182.2 μmol of TE/g of fresh leaves (205.0−728.8 μmol of TE/g of dry matter). As the leaves become older, the ORAC values and total phenolic contents decreased. The results showed a linear correlation between total phenolic content and ORAC activity for fruits and leaves. For ripe berries, a linear relationship existed between ORAC values and anthocyanin content. Of the ripe fruits tested, on the basis of wet weight of fruit, cv. Jewel black raspberry and blackberries may be the richest source for antioxidants. On the basis of the dry weight of fruit, strawberries had the highest ORAC activity followed by black raspberries (cv. Jewel), blackberries, and red raspberries.

Keywords: *Antioxidant; anthocyanin; phenolics; free radical; blackberry; raspberry; strawberry*

INTRODUCTION

Natural antioxidants occur in all parts of plants. These antioxidants include carotenoids, vitamins, phenols, flavonoids, dietary glutathionine, and endogenous metabolites (Larson, 1988). Plant-derived antioxidants have been shown to function as singlet and triplet oxygen quenchers, free radical scavengers, peroxide decomposers, enzyme inhibitors, and synergists (Larson, 1988).

Fruits and vegetables contain many different antioxidant components (Cao et al., 1996; Velioglu et al., 1998; Wang et al., 1996). The consumption of fruits and vegetables has been associated with low incidences and mortality rates of cancer (Ames et al., 1993; Dragsted et al., 1993; Willett, 1994) and heart disease (Verlangieri et al., 1985). Eating fruits and vegetables also reduces blood pressure, boosts the immune system, detoxifies contaminants and pollutants, and reduces inflammation (Ascherio et al., 1992; Sacks and Kass, 1988).

The phytochemicals in plant tissues responsible for the antioxidant capacity can largely be attributed to the phenolics, anthocyanins, and other flavonoid compounds (Cao et al., 1997). Prior et al. (1998) found different antioxidant capacities in various species and cultivars of *Vaccinium*. Increasing maturity of blueberries at harvest yielded higher antioxidant, anthocyanin, and total phenolic contents (Prior et al., 1998). However, little information is available on antioxidant capacities in leaves and fruits of other berry crops at different developmental stages. The present investigation evaluated the antioxidant capacities in different genotypes and developmental stages of the fruits and leaves of blackberry, raspberry, and strawberry plants.

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MATERIALS AND METHODS

Chemicals. (*R*)-Phycoerythrin (R-PE) from *Porphydium cruentum* was purchased from Sigma (St. Louis, MO). 2′,2′-Azobis(2-amidinopropane) dihydrochloride (AAPH) was purchased from Wako Chemicals USA Inc. (Richmond, VA). 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) was obtained from Aldrich (Milwaukee, WI).

Fruit and Leaf Sample Preparation. Thornless blackberry (cv. Chester Thornless, Hull Thornless, and Triple Crown Thornless), black raspberry (cv. Jewel), red raspberry (cv. Autumn Bliss, Canby, Sentry, and Summit), and June-bearing strawberry (cv. Allstar, Delmarvel, Earliglow, Latestar, Lester, Mohawk, Northeaster, and Red Chief) plants used in this study were grown at the Beltsville Agricultural Research Center. Strawberry plants used in this study were grown in a greenhouse. Collections of berries and leaves were performed over a period of 2 weeks during fruit-bearing season. Strawberry fruits were harvested from 10 plants of each cultivar, and samples of blackberry and raspberry fruits were harvested from 6 to 8 bushes of each cultivar at various maturity stages (the determination of fruit maturity was based on fruit surface color and divided as green, pink, and commercially ripe). Undamaged berries were selected and mixed, and sample juices were obtained by pulverizing three 100 g composite samples of berries from each cultivar at each maturity stage. All juice samples were centrifuged at 14000*g* for 20 min at 4 °C. Healthy, young leaves (from the upper part of shoots or stems) and old leaves (from the lower part of shoots or stems) were selected for the samples used in this study. Triplicate

10.1021/jf9908345 Published 2000 by the American Chemical Society
Published on Web 01/14/2000

Table 1. Antioxidant Activity (ORAC), Anthocyanin Content, and Total Phenolic Content of Juices in Various Cultivars of Thornless Blackberry Fruit at Different Maturities[a]

cultivar	maturity	ORAC[b] (μmol of TE/g) WM basis	ORAC[b] (μmol of TE/g) DM basis	anthocyanin[c] (mg/100 g) WM basis	anthocyanin[c] (mg/100 g) DM basis	total phenolic[d] (mg/100 g) WM basis	total phenolic[d] (mg/100 g) DM basis
Chester Thornless	green	25.1 ± 1.1	177.8 ± 3.1	0.5 ± 0.4	3.9 ± 1.1	308 ± 9.7	2182 ± 13.4
	pink	15.4 ± 0.5	97.3 ± 3.5	8.8 ± 0.7	55.4 ± 4.9	247 ± 6.8	1556 ± 11.5
	ripe	22.2 ± 0.9	132.1 ± 5.3	153.3 ± 10.6	912.5 ± 27.3	226 ± 4.5	1345 ± 8.9
Hull Thornless	green	28.8 ± 0.5	204.1 ± 10.4	1.3 ± 0.7	9.2 ± 2.0	326 ± 6.5	2310 ± 18.6
	pink	17.6 ± 1.7	111.3 ± 11.9	10.7 ± 1.1	67.7 ± 7.7	262 ± 3.2	1657 ± 13.2
	ripe	24.6 ± 1.0	146.4 ± 8.5	171.6 ± 5.3	1020.8 ± 31.5	248 ± 5.9	1476 ± 17.3
Triple Crown	green	23.4 ± 0.5	165.8 ± 1.4	1.0 ± 0.6	7.1 ± 1.7	252 ± 8.4	1786 ± 15.7
	pink	13.7 ± 1.0	86.8 ± 7.0	7.9 ± 1.5	50.1 ± 10.5	227 ± 6.0	1438 ± 12.4
	ripe	20.3 ± 0.4	120.8 ± 12.3	133.5 ± 8.2	794.6 ± 12.5	204 ± 2.0	1214 ± 11.9
$LSD_{0.05}$		1.13	18.98	5.90	18.48	7.59	16.93
significance[e]							
cultivar [C]		*	*	*	*	*	*
maturity [M]		*	*	*	*	*	*
C × M		ns	ns	*	*	*	*

[a] Data expressed as mean ± SEM. [b] Data expressed as micromoles of Trolox equivalents per gram of fresh weight (WM basis) or dry matter (DM basis). [c] Data expressed as milligrams of cyanidin 3-glucoside per 100 g of fresh weight (WM basis) or dry matter (DM basis). [d] Data expressed as milligrams of gallic acid equivalents (GAE) per 100 g of fresh weight (WM basis) or dry matter (DM basis). [e] ns, *, nonsignificant or significant at $p \leq 0.05$, respectively.

composite leaf samples (2 g) from each cultivar were extracted in 15 mL of phosphate buffer (75 mM, pH 7.0) using a homogenizer and centrifuged at 20000*g* at 4 °C for 30 min. The supernatant (juice fractions and leaf extracts) was transferred to vials, stored at −80 °C, and then used for analyses after suitable dilution with a phosphate buffer (75 mM, pH 7.0).

Oxygen Radical Absorbance Capacity (ORAC) Assay. ORAC assays for fruits and leaves of blackberry, raspberry, and strawberry were carried out following procedures modified from a method previously described by Cao et al. (1993). This assay measures the ability of antioxidant components in test materials to inhibit the decline in R-PE fluorescence that is induced by a peroxyl radical generator, AAPH. 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 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 <5% of the first reading. This usually takes ~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 of fresh weight (WM) or dry matter (DM) (Cao et al., 1993). Dry matter was determined after lyophilization.

$$\text{ORAC value } (\mu\text{M}) = 20K(S_{\text{sample}} - S_{\text{blank}})/(S_{\text{Trolox}} - S_{\text{blank}})$$

K is a sample dilution factor, and S is the area under the fluorescence decay curve of the sample, Trolox or blank, which is calculated as

$$S = (0.5 + f_5/f_0 + f_{10}/f_0 + f_{15}/f_0 + f_{20}/f_0 + f_{25}/f_0 + f_{30}/f_0 + \ldots + f_{60}/f_0 + f_{65}/f_0 + f_{70}/f_0) \times 5$$

where f_0 is the initial fluorescence at 0 min and f_i the fluorescence measurement at time i.

Analysis of Total Anthocyanin Content. Total anthocyanin contents in fruit juice were determined using the pH differential method (Cheng and Breen, 1991). Absorbance was measured in a Shimadzu spectrophotometer (Shimadzu UV-160) at 510 and 700 nm in buffers at pH 1.0 and 4.5, using $A = [(A_{510} - A_{700})_{\text{pH1.0}} - (A_{510} - A_{700})_{\text{pH4.5}}]$ with molar extinction coefficients of pelargonidin 3-glucoside (22400) for strawberry fruit juices and of cyanidin 3-glucoside (29600) for blackberry and raspberry fruit juices. Results were expressed as milligrams of pelargonidin 3-glucoside (strawberry juices) or cyanidin 3-glucoside (blackberry and raspberry juices) equivalents per 100 g of fresh weight (WM) or dry matter (DM).

Total Phenolic Compound Analysis. Total soluble phenolics in the fruit juices and leaf extracts were determined with Folin−Ciocalteu reagent according to the method of Slinkard and Singleton (1977) using gallic acid as a standard. Results were expressed as milligrams of gallic acid equivalent (GAE) per gram (for leaves) or per 100 g (for fruits) of fresh weight (WM) or dry matter (DM).

Statistical Analysis. Correlation and regression analyses of ORAC versus total phenolics or total anthocyanin were performed using NCSS (NCSS 97, 1997). Data were subjected to analysis of variance, and means were compared by least significant difference (LSD). The effect of species, cultivar, and maturity on ORAC values and anthocyanin and total phenolic content were evaluated by Fisher's LSD multiple-comparison test used in NCSS. Differences at $p < 0.05$ were considered to be significant.

RESULTS

ORAC, Total Anthocyanin Content, and Total Phenolic Content in Fruits. The influences of species, cultivar, and maturity on antioxidant capacity (expressed as an ORAC value), total anthocyanin, and total phenolic contents in fruits of blackberry, raspberry, and strawberry were significant (Tables 1−5). Blackberry, cv. Jewel black raspberry, and strawberry fruits harvested during their green stage consistently yielded the highest ORAC values. Red raspberry fruit had their highest ORAC value at the ripe stage. In all fruits, the pink stage had the lowest ORAC value. The ORAC values for blackberries ranged from 13.7 to 28.8 μmol of Trolox equivalents (TE)/g of fresh berries (86.6−204.1 μmol of TE/g of dry matter), with the cv. Hull Thornless yielding the highest ORAC value, anthocyanin content, and total phenolic content at all stages of maturity (Table 1). For raspberry fruits, ORAC values ranged from 7.8 to 33.7 μmol of TE/g of fresh berries (37.1−162.1 μmol of TE/g of dry matter) (Table 2). Cv. Jewel,

Table 2. Antioxidant Activity (ORAC), Anthocyanin Content, and Total Phenolic Content of Juices from Various Cultivars of Raspberry Fruits at Different Maturities[a]

cultivar	maturity	ORAC[b] (μmol of TE/g) WM basis	ORAC[b] (μmol of TE/g) DM basis	anthocyanin[c] (mg/100 g) WM basis	anthocyanin[c] (mg/100 g) DM basis	total phenolic[d] (mg/100 g) WM basis	total phenolic[d] (mg/100 g) DM basis
Jewel	green	33.7 ± 4.0	162.1 ± 9.5	1.7 ± 0.6	27.9 ± 1.4	338 ± 7.1	1625 ± 24.7
(black raspberry)	pink	16.1 ± 0.6	66.4 ± 2.8	22.8 ± 5.4	93.9 ± 6.7	190 ± 3.5	783 ± 15.9
	ripe	28.2 ± 1.4	136.2 ± 8.1	197.2 ± 3.5	952.4 ± 20.1	267 ± 4.3	1530 ± 16.7
Autumn Bliss	green	15.4 ± 1.0	44.0 ± 2.4	1.6 ± 0.5	4.6 ± 1.2	174 ± 3.7	497 ± 11.2
(red raspberry)	pink	8.5 ± 0.3	35.0 ± 1.4	6.4 ± 1.2	26.4 ± 5.7	104 ± 1.5	428 ± 7.6
	ripe	18.5 ± 0.7	106.3 ± 4.0	75.0 ± 3.8	431.1 ± 21.8	245 ± 6.1	1408 ± 35.4
Canby	green	14.0 ± 0.5	40.0 ± 1.2	0.1 ± 0.0	0.3 ± 0.1	145 ± 4.9	414 ± 11.7
(red raspberry)	pink	7.8 ± 0.5	37.1 ± 2.4	1.9 ± 1.0	7.8 ± 4.8	57 ± 1.2	371 ± 5.7
	ripe	15.9 ± 1.0	91.4 ± 5.7	45.4 ± 2.1	260.9 ± 12.1	208 ± 2.6	1195 ± 14.9
Sentry	green	17.0 ± 0.5	48.6 ± 4.2	0.1 ± 0.1	0.3 ± 0.2	189 ± 5.3	540 ± 12.6
(red raspberry)	pink	11.8 ± 0.7	40.8 ± 3.3	4.4 ± 1.5	15.2 ± 7.1	116 ± 1.1	401 ± 8.2
	ripe	18.2 ± 0.3	104.6 ± 9.7	52.8 ± 2.4	303.4 ± 13.8	226 ± 6.0	1298 ± 34.5
Summit	green	19.4 ± 1.2	55.5 ± 2.9	2.2 ± 0.6	6.2 ± 1.4	215 ± 4.1	615 ± 9.7
(red raspberry)	pink	15.4 ± 0.7	50.8 ± 3.3	15.9 ± 1.2	52.4 ± 5.7	122 ± 2.1	402 ± 10.0
	ripe	20.0 ± 1.1	114.9 ± 6.3	99.5 ± 3.8	571.8 ± 21.8	258 ± 5.6	1482 ± 32.2
$LSD_{0.05}$		1.08	6.15	2.85	13.20	7.48	63.24
significance[e]							
cultivar [C]		*	*	*	*	*	*
maturity [M]		*	*	*	*	*	*
C × M		*	*	*	*	*	*

[a] Data expressed as mean ± SEM. [b] Data expressed as micromoles of Trolox equivalents per gram of fresh weight (WM basis) or dry matter (DM basis). [c] Data expressed as milligrams of cyanidin 3-glucoside per 100 g of fresh weight (WM basis) or dry matter (DM basis). [d] Data expressed as milligrams of gallic acid equivalents (GAE) per 100 g of fresh weight (WM basis) or dry matter (DM basis). [e] *, significant at $p \leq 0.05$.

Table 3. Antioxidant Activity (ORAC), Anthocyanin Content, and Total Phenolic Content of Juices from Strawberry (Cv. Allstar) Fruits at Different Stages of Maturity[a]

stage (maturity)	ORAC[b] (μmol of TE/g) WM basis	ORAC[b] (μmol of TE/g) DM basis	anthocyanin[c] (mg/100 g) WM basis	anthocyanin[c] (mg/100 g) DM basis	total phenolic[d] (mg/100 g) WM basis	total phenolic[d] (mg/100 g) DM basis
small green	22.7 ± 0.6	160.9 ± 4.3	0.2 ± 0.1	1.4 ± 0.7	278 ± 9.5	1971 ± 20.1
large green	19.9 ± 1.0	144.2 ± 7.2	0.4 ± 0.2	2.9 ± 1.4	234 ± 6.5	1696 ± 21.1
white	12.2 ± 0.8	96.8 ± 6.3	3.3 ± 0.8	26.2 ± 6.3	178 ± 3.8	1394 ± 19.8
pink	9.8 ± 0.7	82.4 ± 5.9	5.5 ± 0.6	46.2 ± 5.0	129 ± 2.0	1083 ± 12.6
50% red	9.7 ± 0.2	81.8 ± 1.8	16.2 ± 2.1	143.4 ± 18.6	91 ± 1.9	916 ± 11.5
80% red	10.4 ± 0.3	95.4 ± 2.7	23.6 ± 2.3	216.5 ± 21.1	94 ± 0.8	971 ± 6.9
full red	12.0 ± 0.5	118.8 ± 4.9	38.9 ± 1.1	385.1 ± 10.9	96 ± 0.9	946 ± 8.9
$LSD_{0.05}$	0.43	6.54	1.62	14.64	5.81	29.99
significance[e]						
maturity [M]	*	*	*	*	*	*

[a] Data expressed as mean ± SEM. [b] Data expressed as micromoles of Trolox equivalents per gram of fresh weight (WM basis) or dry matter (DM basis). [c] Data expressed as milligrams of pelargonidin-3-glucoside per 100 g of fresh weight (WM basis) or dry matter (DM basis). [d] Data expressed as milligrams of gallic acid equivalents (GAE) per 100 g of fresh weight (WM basis) or dry matter (DM basis). [e]*Significant at $p \leq 0.05$.

a black raspberry, had the highest ORAC value at the green, pink, and ripe stages and also yielded the highest anthocyanin and total phenolic contents compared to the other red raspberry cultivars (Table 2). The small green stage of strawberry fruit had the highest ORAC value and total phenolic content, both of which steadily decreased at the 50% red stage, which had the lowest ORAC value and total phenolic content. Beyond the 50% red stage, the ORAC value and anthocyanin content steadily increased with fruit maturity, but the total phenolic content remained at relatively low levels (Table 3). ORAC, anthocyanin content, and total phenolic content were also different among the eight different cultivars of strawberry fruits (Table 4). ORAC values for the different cultivars of strawberries ranged from 12.2 to 17.4 μmol of TE/g of fresh berries. Cv. Earliglow had the highest ORAC value (17.4 μmol of TE/g of fresh berries), anthocyanin content (45.3 mg/100 g of fresh berries), and total phenolic content (152.0 mg/100 g of fresh berries).

On the basis of the wet weight of fruit, ripe fruit of cv. Jewel black raspberry had higher ORAC values than the three cultivars of ripe thornless blackberry fruit, (Chester Thornless, Hull Thornless, and Triple Crown) and four red raspberry cultivars (Autumn Bliss, Canby, Sentry, and Summit) (Tables 1 and 2). With all fruits, the anthocyanin content increased as the berries matured. However, for total phenolic content, raspberry fruit showed a decrease from the green to the pink stages followed by a significant increase from the pink stage to the ripe stages. In blackberries and strawberries, total phenolic content significantly decreased as the fruit matured from the green to ripe stages (Tables 1 and 3). Compared to blackberries and raspberries, strawberries had generally lower mean values of total antioxidant capacity, anthocyanin content, and total phenolic contents on the basis of the wet weight of ripe fruit. Black raspberries and blackberries had the highest mean values of total antioxidant capacity, anthocyanin content, and total phenolic content. On the basis

Table 4. Antioxidant Activity (ORAC), Anthocyanin Content, and Total Phenolic Contents of Juices from Various Cultivars of Ripe Strawberry Fruits[a]

cultivar	ORAC[b] (μmol of TE/g) WM basis	ORAC[b] (μmol of TE/g) DM basis	anthocyanin[c] (mg/100 g) WM basis	anthocyanin[c] (mg/100 g) DM basis	total phenolic[d] (mg/100 g) WM basis	total phenolic[d] (mg/100 g) DM basis
Allstar	12.2 ± 0.3	120.8 ± 2.9	23.3 ± 1.3	230.7 ± 12.8	95 ± 1.1	943 ± 10.4
Delmarvel	13.2 ± 0.1	130.7 ± 0.9	26.9 ± 0.8	266.3 ± 7.9	119 ± 2.3	1180 ± 22.2
Earliglow	17.4 ± 0.2	172.3 ± 2.0	45.3 ± 2.6	448.5 ± 25.7	152 ± 2.1	1507 ± 20.9
Latestar	13.4 ± 0.4	132.7 ± 3.9	24.7 ± 0.4	244.6 ± 3.9	107 ± 1.4	1061 ± 13.4
Lester	15.9 ± 0.3	157.4 ± 3.0	35.7 ± 1.0	353.3 ± 9.9	125 ± 3.2	1239 ± 20.7
Mohawk	16.2 ± 0.4	160.4 ± 3.9	31.5 ± 0.6	311.9 ± 5.9	137 ± 2.8	1358 ± 26.7
Northeaster	16.8 ± 0.1	166.3 ± 1.0	38.6 ± 1.5	382.2 ± 14.8	150 ± 4.0	1388 ± 25.7
Red Chief	14.2 ± 0.2	140.6 ± 2.0	28.7 ± 0.3	284.2 ± 2.9	121 ± 1.7	1195 ± 10.9
$LSD_{0.05}$	0.34	3.17	1.61	16.65	8.18	24.19
significance[e]						
cultivar [C]	*	*	*	*	*	*

[a] Data expressed as mean ± SEM. [b] Data expressed as micromoles of Trolox equivalents per gram of fresh weight (WM basis) or dry matter (DM basis). [c] Data expressed as milligrams of pelargonidin 3-glucoside per 100 g of fresh weight (WM basis) or dry matter (DM basis). [d] Data expressed as milligrams of gallic acid equivalents (GAE) per 100 g of fresh weight (WM basis) or dry matter (DM basis). [e] *, significant at $p \leq 0.05$.

Table 5. Comparison of the Mean Values of Antioxidant Activity (ORAC), Anthocyanin Content, and Total Phenolic Content in Fruit Juice of Different Maturities in Various Berry Species (Thornless Blackberry, Black Raspberry, Red Raspberry, and Strawberry)[a]

species	maturity	ORAC[b] (μmol of TE/g) WM basis	ORAC[b] (μmol of TE/g) DM basis	anthocyanin[c] (mg/100 g) WM basis	anthocyanin[c] (mg/100 g) DM basis	total phenolic[d] (mg/100 g) WM basis	total phenolic[d] (mg/100 g) DM basis
blackberry	green	25.7 ± 1.2	182.6 ± 5.4	0.9 ± 0.7	6.7 ± 1.6	295 ± 8.2	2166 ± 15.9
	pink	15.6 ± 0.8	98.5 ± 7.3	9.1 ± 1.1	57.7 ± 7.7	245 ± 5.3	1550 ± 12.4
	ripe	22.4 ± 0.6	133.3 ± 8.6	152.8 ± 8.0	909.3 ± 23.8	226 ± 4.1	1347 ± 12.7
black raspberry	green	33.7 ± 4.0	162.1 ± 9.5	1.7 ± 0.6	27.9 ± 1.4	338 ± 7.1	1625 ± 24.7
	pink	16.1 ± 0.6	66.4 ± 2.8	22.8 ± 1.4	93.9 ± 6.7	190 ± 3.5	783 ± 15.9
	ripe	28.2 ± 1.4	136.2 ± 8.1	197.2 ± 8.5	952.4 ± 20.1	267 ± 4.3	1535 ± 16.7
red raspberry	green	16.5 ± 0.8	47.0 ± 2.7	1.0 ± 0.2	2.9 ± 0.9	181 ± 5.0	517 ± 8.6
	pink	10.9 ± 0.6	40.9 ± 2.6	7.2 ± 1.2	25.5 ± 5.8	99 ± 1.5	400 ± 7.7
	ripe	18.2 ± 0.8	104.3 ± 6.4	68.0 ± 3.0	391.8 ± 17.4	234 ± 5.1	1346 ± 21.3
strawberry	green	21.3 ± 1.2	152.6 ± 5.8	0.3 ± 0.1	2.2 ± 1.1	256 ± 6.1	1834 ± 20.6
	pink	9.7 ± 0.6	82.4 ± 5.9	5.5 ± 0.9	46.2 ± 7.6	129 ± 1.5	1083 ± 12.6
	ripe	14.9 ± 0.8	147.7 ± 7.9	31.9 ± 4.1	315.2 ± 15.8	103 ± 2.0	1033 ± 15.0
$LSD_{0.05}$		1.82	7.73	4.47	14.28	5.79	19.17
significance[e]							
species [S]		*	*	*	*	*	*
maturity [M]		*	*	*	*	*	*
[S] × [M]		*	*	*	*	*	*

[a] Data expressed as mean ± SEM. Data for the thornless blackberry was the mean value ± SEM calculated from three cultivars (Chester Thornless, Hull Thornless, Triple Crown); black raspberry mean value ± SEM was calculated from cultivar Jewel. Red raspberry mean value ± SEM was calculated from four cultivars (Autumn Bliss, Canby, Sentry, and Summit); strawberry mean value ± SEM was calculated from eight cultivars (Northeaster, Earliglow, Lester, Delmarvel, Allstar, Mohawk, Latestar, and Red Chief). [b] Data expressed as micromoles of Trolox equivalents per gram of fresh weight (WM basis) or dry matter (DM basis). [c] Data expressed as milligrams of cyanidin 2-glucoside (blackberry and raspberry) or pelargonidin 3-glucoside (strawberry) per 100 g of fresh weight (WM basis) or dry matter (DM basis). [d] Data expressed as milligrams of gallic acid equivalents (GAE) per 100 g of fresh weight (WM basis) or dry matter (DM basis). [e] *, significant at $p \leq 0.05$.

Table 6. Correlation Coefficients of Antioxidant Activity (ORAC) and Anthocyanin or Total Phenolic Content at Different Maturities of Various Berry Crops[a]

species	maturity	correlation coefficient: ORAC vs anthocyanins WM basis	ORAC vs anthocyanins DW basis	ORAC vs phenolics WM basis	ORAC vs phenolics DW basis
blackberry	green	0.557	0.578	0.878	0.876
	pink	0.892	0.883	0.896	0.993
	ripe	0.986	0.937	0.996	0.995
raspberry	green	0.459	0.293	0.995	0.994
	pink	0.898	0.960	0.843	0.876
	ripe	0.957	0.988	0.907	0.904
strawberry	green	−0.976	−0.984	0.994	0.948
	pink	0.840	0.837	0.991	0.990
	ripe	0.912	0.941	0.862	0.884

[a] Data expressed on fresh weight (WM) basis or dry matter (DM) basis.

of the dry weight of ripe fruit, however, strawberries had the highest ORAC activity followed by black raspberries, blackberries, and red raspberries (Table 5). In blackberry, raspberry, and strawberry fruit, the ORAC value correlated with the total phenolic content at the green, pink, and ripe stages (Table 6). A negative correlation was found in strawberries at the green stage between ORAC value and anthocyanin content. No significant correlation existed between the ORAC value and the anthocyanin content at the green stage for blackberry and raspberry fruit. However, there appeared to be an increasing correlation between the

Table 7. Antioxidant Acitivity (ORAC) and Total Phenolic Content of Leaf Extracts from Young Leaves of Different Cultivars of Thornless Blackberries, Black Raspberry, Red Raspberries, and Strawberries[a]

		ORAC[b] (μmol of TE/g)		total phenolic[c] (mg/g)	
species	cultivar	WM basis	DM basis	WM basis	DM basis
blackberry	Chester Thornless	103.5 ± 1.5	414.0 ± 6.0	20.7 ± 0.8	82.8 ± 3.2
	Hull Thornless	95.8 ± 1.1	383.2 ± 4.4	18.5 ± 1.1	74.0 ± 4.4
	Triple Crown	115.2 ± 2.3	460.8 ± 9.2	22.9 ± 0.5	91.6 ± 2.0
black raspberry	Jewel	122.7 ± 3.2	490.8 ± 12.8	24.2 ± 0.7	96.8 ± 2.8
red raspberry	Autumn Bliss	139.8 ± 4.1	559.2 ± 16.4	30.1 ± 0.7	120.4 ± 2.8
	Canby	95.6 ± 1.5	382.4 ± 6.0	16.9 ± 0.4	67.6 ± 1.6
	Sentry	91.5 ± 1.8	366.0 ± 5.7	11.8 ± 0.5	47.2 ± 1.3
	Summit	182.2 ± 5.7	728.8 ± 22.8	32.3 ± 1.2	129.2 ± 4.8
strawberry	Allstar	136.0 ± 3.2	544.0 ± 12.8	13.8 ± 0.9	55.2 ± 3.6
$LSD_{0.05}$		3.69	14.72	0.97	6.97
significance[d]					
cultivar [C]		*	*	*	*

[a] Data expressed as mean ± SEM. [b] Data expressed as micromoles of Trolox equivalents per gram of fresh weight (WM basis) or dry matter (DM basis). [c] Data expressed as milligrams of gallic acid equivalents (GAE) per gram of fresh weight (WM basis) or dry matter (DM basis). [d] *, significant at $p \leq 0.05$.

Table 8. Antioxidant Acitivity (ORAC) and Total Phenolic Content of Leaf Extract from Chester Thornless Blackberry, Autumn Bliss Red Raspberry, and Allstar Strawberry at Young and Old Leaf[a]

		ORAC[b] (μmol of TE/g)		total phenolic[c] (mg/g)	
species	leaf age	WM basis	DM basis	WM basis	DM basis
Chester Thornless (blackberry)	young	104.9 ± 2.1	419.6 ± 8.4	20.9 ± 0.9	83.6 ± 3.6
	old	69.7 ± 1.5	205.0 ± 4.4	16.5 ± 0.2	48.5 ± 5.1
Autum Bliss (raspberry)	young	142.2 ± 2.6	568.8 ± 10.4	31.7 ± 0.6	126.8 ± 2.4
	old	110.3 ± 1.1	324.4 ± 3.2	18.6 ± 1.3	54.7 ± 3.8
Allstar (strawberry)	young	131.8 ± 2.1	527.2 ± 8.4	13.6 ± 0.5	54.6 ± 2.0
	old	94.7 ± 1.6	278.5 ± 4.7	10.5 ± 0.4	30.9 ± 3.1
$LSD_{0.05}$		2.52	8.91	0.93	4.38
significance[d]					
species [S]		*	*	*	*
maturity [M]		*	*	*	*
S × M		ns	ns	*	*

[a] Data expressed as mean ± SEM. [b] Data expressed as micromoles of Trolox equivalents per gram of fresh weight (WM basis) or dry matter (DM basis). [c] Data expressed as milligrams of gallic acid equivalents (GAE) per gram of fresh weight (WM basis) or dry matter (DM basis). [d] ns, *, nonsignificant or significant at $p \leq 0.05$, respectively.

ORAC value and anthocyanin content as the fruit matured from the pink stage to the ripe stage (Table 6). On a dry matter basis, the same patterns of correlation between ORAC versus total phenolics or total anthocyanins were also seen (Table 6).

ORAC and Total Phenolic Content in Leaves. Cultivars showed significant differences in ORAC values and total phenolic content in leaves of blackberry, raspberry, and strawberry (Table 7). Leaf age (young versus old) also significantly affected ORAC values and total phenolic content (Table 8). Leaves from blackberry (Chester Thornless, Hull Thornless, and Triple Crown), black raspberry (Jewel), red raspberry (Autumn Bliss, Canby, Sentry, and Summit), and strawberry (Allstar) plants were found to have higher antioxidant capacities than the respective fruit tissues, with ORAC values ranging from 69.7 to 182.2 μmol of TE/g of fresh leaves (205.0−728.8 μmol of TE/g of dry matter) (Tables 1−5, 7, and 8). In thornless blackberry, cv. Triple Crown had the highest antioxidant activity and total phenolic content, wheras cv. Hull Thornless had the lowest. In raspberry, cv. Autumn Bliss and Summit leaves also had high ORAC values and total phenolics content (Table 7). There appeared to be significant differences among the antioxidant activities of young versus old leaves of Chester Thornless blackberry, Autumn Bliss red raspberry, and Allstar strawberry. Young leaves had higher ORAC values and total phenolic content than old leaves (Table 8).

Results from the leaf analysis consistently indicated a significant correlation between total antioxidant content (ORAC) and total phenolic content. In thornless blackberry, raspberry, and strawberry leaves, the correlations between ORAC values and total phenolic content were 0.961, 0.911, and 0.996, respectively.

DISCUSSION

Blackberries, raspberries, and strawberries are good sources of natural antioxidants (Wang et al., 1996; Heinonen et al., 1998). Extracts of berries of several cultivars of blackberries, black and red currants, blueberries, and black and red raspberries showed a remarkably high scavenging activity toward chemically generated superoxide radicals (Heinonen et al., 1998). High levels of antioxidants have been shown to have multiple benefits to human health (Ames et al., 1993; Gey et al., 1991). Synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) have been used as antioxidants for food preservation since the beginning of this century. However, these compounds are suspected of causing liver damage and carcinogenicity (Ito et al., 1983). Thus, the interest in natural antioxidants has increased considerably (Löliger, 1991). On the basis of our results from the ORAC assays, fruits and leaves of blackberry, raspberry, and strawberry plants had high antioxidant capacities relative to Trolox, α-tocopherol, or vitamin C. For example, 100 g (fresh weight) of ripe blackberry cv. Hull Thornless has an ORAC activity (2460 μmol of TE) equal to that of ~0.60 g of Trolox, 1.06 g of α-tocopherol (the ORAC activity of 1 mol of α-tocopherol = 1 mol of TE; Cao et al., 1993), or 0.69 g of vitamin C (the ORAC activity of 1 μmol of vitamin C = 0.52 μmol of TE; Cao et al., 1993). Prior et al. (1998) estimated that normal intake in humans of antioxidants as measured by ORAC within the United States is in the range of 1.2−1.7 mmol of ORAC/day.

Blackberries, raspberries, and strawberries, in addition to the usual nutrients such as vitamins and minerals, are also rich in anthocyanin, flavonoids, and phenolic acids (Heinonen et al., 1998; Rice-Evans and Miller, 1996; Wang et al., 1997). Berry fruits were shown to be effective in inhibiting oxidation of human low-density lipoproteins, which may have potential health effects (Heinonen et al., 1998).

Anthocyanins are probably the largest group of phenolic compounds in the human diet, and the daily intake of anthocyanins in human has been estimated to be as much as 180−215 mg/day in the United States (Kühnau, 1976). Supplementing these berries with a balanced diet could be more effective and economical than consuming an individual antioxidant such as ascorbic acid or vitamin A or E in protecting the body against various oxidative stresses. Rice-Evans and Miller (1996) state that the total antioxidant potential of fruits and vegetables is more important than levels of any individual specific antioxidant constituent.

A linear correlation exists between total phenolic content and ORAC activity for fruits and leaves of blackberry, raspberry, and strawberry. For ripe berries, there was also a linear relationship between ORAC values and anthocyanin content. Prior et al. (1998) found a linear relationship between ORAC and anthocyanin or total phenolic content in fruit of *Vaccinium* species. Kalt et al. (1998) also reported that phenolic composition, anthocyanin, and antioxidant capacities in fruit of strawberry, raspberry, and highbush and lowbush blueberries changed during postharvest storage treatments, and the antioxidant capacity was correlated with the content of phenolics and anthocyanins.

The antioxidant capacity of anthocyanins may be one of their most significant biological properties in human (Wang et al., 1996). Biomedical and epidemiological research suggests that the dietary antioxidant contained in fruits and vegetables may play an important role in preventing disease (Wang et al., 1996). Delphinidin, cyanidin, pelargonidin, malvidin, and peonidin are the major anthocyanins found in berries. It has been shown that anthocyanins are strong antioxidants with free radical scavenging properties attributed to the phenolic hydroxy groups attached to ring structures (Rice-Evans et al., 1996; Yoshiki et al., 1995; Wang et al., 1997). They offer protection against free radical damage and low-density lipoprotein oxidation, platelet aggregation, and endothelium-dependent vasodilatation of arteries (Cao et al., 1997; Heinonen et al., 1998; Kandaswami and Middleton, 1994; Rice-Evans and Miller, 1996; Wang et al., 1997).

On the basis of the wet weight of ripe fruit, the results of our experiments showed that black raspberries and blackberries had higher antioxidant activities than red raspberries and that strawberries generally had lower mean values of total antioxidants than red raspberries. Black raspberries and blackberries contain high amounts of cyanidin glycosides, a strong antioxidant (Macheix et al., 1990), whereas strawberries are rich in pelargonidin 3-glucoside (Gil et al., 1997) and ascorbic acid, which are weak antioxidants. The ORAC values for cyanidin glycosides and pelargonidin 3-glucoside were 2.24 and 1.54, respectively (Wang et al., 1997). Antioxidant efficacy in preventing oxidation of human low-density lipoprotein among anthocyanins is as follows: delphinidin > cyanidin > malvidin > pelargonidin (Satué-Gracia et al., 1997) and is influenced by the number of hydroxyls on the B ring of the anthocyanin molecule (Rajalakshmi and Narasimhan, 1996). On the basis of the dry weight of ripe fruit, strawberries had the highest ORAC activity followed by black raspberries (cv. Jewel), blackberries, and red raspberries.

The total phenolic content of berries and leaves investigated in this study varied from 91 to 338 mg/100 g for fresh fruits (916−2310 mg/100 g of DM) and from 10.5 to 32.3 mg/g for fresh leaves (30.9−129.2 mg/g of DM). A positive and highly significant relationship between total phenolic and antioxidant activity was found. Leaves from thornless blackberry (Chester Thornless, Hull Thornless, and Triple Crown), black raspberry (Jewel), red raspberry (Autumn Bliss, Canby, Sentry, and Summit), and strawberry (Allstar) plants were found to have high antioxidant capacities and total phenolic content compared to their fruit tissues. However, there appears to be no correlation between the ORAC values of fruits and leaves from the same cultivar of blackberry, raspberry, and strawberry (on fresh weight or dry matter basis). This lack of relationship may indicate that antioxidant capacity can be highly specific for tissue type. Analyzing leaves does not seem to predict ORAC values in fruits and, thus, cannot be used for preselection in fruit breeding. Also, the components in fruit and leaf tissues, which contribute to ORAC activity, changed with maturity; for example, phenolic content decreases while anthocyanins and other flavonoids increase with maturity in fruit tissue of blackberries and strawberries (Tables 1 and 2).

Young leaves from berry crops had higher ORAC values and total phenolic content compared to older leaves. In fresh tea leaves, young leaves contained higher polyphenols, especially (−)-epigallocatechin 3-gallate, and had higher ORAC values than older tea leaves (Lin et al., 1996). High antioxidant activity was also found in the leaves of barley, *Prunus*, and *Eucalytus*, although the chemical properties and physiological role of their active principles are not yet fully understood (Osawa, 1994).

Flavonoids, vitamin E, phenolic acids, phenols (rosmaridiphenol, curcumin, butein, ubiquinol), nitrogen compounds (alkaloids, chlorophyll derivatives, amino acids, and amines), carotenoids, and vitamin C are found in berry crops and other plant materials and indeed play an important part in the function of antioxidation and antiproliferation (Larson, 1988). The antioxidant-rich photochemicals in strawberry have been shown to reduce or retard the central nervous system deficits seen in aging rats (Bickford et al., 1997) and to protect against oxidative stress (Sofic et al., 1997). The antioxidant capacity varies considerably in different types of berry crops and also in different levels of maturity and different plant parts (Tables 1−4, 6, and 7). Because raspberry and blackberry fruits contain higher levels of antioxidant capacities compared to strawberry fruit, health benefits from consuming these small fruits are also possible. Because of their high antioxidant content, berry leaves can also be added to tea mix to increase the antioxidant capacity level in the beverages for greater benefits to human health.

ABBREVIATIONS USED

AAPH, 2′,2′-azobis(2-amidinopropane) dihydrochloride; ORAC, oxygen radical absorbance capacity; R-PE, (*R*)-phycoerythrin; Trolox, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; TE, Trolox equivalents.

ACKNOWLEDGMENT

We thank Dr. Guohua Cao for his valuable suggestions on the ORAC assay and critical review of the manuscript. We express our appreciation to Ms. Ya Shian Li and Ms. Katrina M. Wojcik for technical assistance.

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Received for review July 26, 1999. Revised manuscript received November 16, 1999. Accepted November 16, 1999. Use of a company or product name by the USDA does not imply approval or recommendation of the product to the exclusion of others which also may be suitable.

JF9908345