Scavenging Capacity of Berry Crops on Superoxide Radicals, Hydrogen Peroxide, Hydroxyl Radicals, and Singlet Oxygen

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The antioxidant activities against superoxide radicals ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), hydroxyl radicals ($OH^{\bullet}$), and singlet oxygen ($'O_2$) was evaluated in fruit juice from different cultivars of thornless blackberries (*Rubus* sp.), blueberries (*Vaccinium* spp.), cranberries (*Vaccinium macrocarpon* Aiton), raspberries (*Rubus idaeus* L. and *Rubus occidentalis* L.), and strawberries (*Fragaria* x *ananassa* Duch.). Among the different cultivars, juice of 'Hull Thornless' blackberry, 'Earliglow' strawberry, 'Early Black' cranberry, 'Jewel' raspberry, and 'Elliot' blueberry had the highest antioxidant capacity against superoxide radicals ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), hydroxyl radicals ($OH^{\bullet}$), and singlet oxygen ($'O_2$). In general, blackberries had the highest antioxidant capacity inhibition of $O_2^{\bullet -}$, H_2O_2, and $OH^{\bullet}$. Strawberry was second best in the antioxidant capacity assay for these same free radicals. With regard to $'O_2$ scavenging activity, strawberry had the highest value, while blackberry was second. Cranberries had the lowest inhibition of H_2O_2 activity. Meanwhile, blueberries had the lowest antioxidant capacity against $OH^{\bullet}$ and $'O_2$. There were interesting and marked differences among the different antioxidants in their abilities to scavenge different reactive oxygen species. β-Carotene had by far the highest scavenging activity against $'O_2$ but had absolutely no effect on H_2O_2. Ascorbic acid was the best at inhibiting H_2O_2 free radical activity. For $OH^{\bullet}$, there was a wide range of scavenging capacities from a high of 15.3% with α-tocopherol to a low of 0.88% with ascorbic acid. Glutathione had higher $O_2^{\bullet -}$ scavenging capacity compared to the other antioxidants.

Keywords: *Active oxygen species; superoxide radicals; hydrogen peroxide; hydroxyl radicals; singlet oxygen; berry crops*

INTRODUCTION

Reactive oxygen species, including superoxide radicals ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), hydroxyl radicals ($OH^{\bullet}$), and singlet oxygen ($'O_2$), are generated as byproducts of normal metabolism. Increased levels of these reactive oxygen species or free radicals create oxidative stress, which lead to biochemical and physiological lesions which may impair metabolism, cause oxidative damage to lipids, proteins, and nucleic acids, and eventually result in cell death (Heinonen et al., 1998; Rice-Evans and Miller, 1996; Satué-Gracia et al., 1997). Cumulative oxidative damage leads to numerous diseases and disorders (Halliwell, 1991; Halliwell and Aruoma, 1992; Steinberg, 1991). Physiological defenses against oxidative stress include small molecule antioxidants and antioxidant proteins (Frei et al., 1992).

Diets rich in fruits and vegetables significantly reduce the incidence and mortality rates of cancer, cardiovascular disorders, and other degenerative diseases caused by oxidative stress (Ames et al., 1993; Dragsted et al., 1993). Fruits and vegetables contain many different antioxidant components (Cao et al., 1996; Velioglu et al.,1998; Wang et al., 1996) which provide protection against harmful free radicals (Ames et al., 1993; Gey, 1990). Eating fruits and vegetables also reduces blood pressure, enhances the immune system, detoxifies contaminants and pollutants, and reduces inflammation (Ascherio et al., 1992).

Most studies on determining the relative importance of antioxidants against lipid peroxidation in human plasma have used aqueous peroxyl radicals as oxidants and measured the total radical-trapping antioxidant parameter of plasma by exposing diluted plasma *in vitro* to aqueous peroxyl radicals generated by thermal decomposition of the water-soluble azo-compound 2′,2′-azobis (2-amidinopropane) dihydrochloride (Wayner et al., 1985). However, relatively little information is available on the scavenging of superoxide radicals ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), hydroxyl radicals, and singlet oxygen. Our previous studies have shown that strawberry, thornless blackberry, and red and black raspberry have high oxygen radical absorbance activity against peroxyl radicals (Wang and Lin, 2000). Antioxidant activities differ among cultivars. A positive correlation was found between antioxidant activity and total phenolic or anthocyanin contents**.** No information is available on the scavenging capacity of these small fruit crops against radicals other than peroxyl radicals. The present study evaluated the different genotypes of blackberry, blueberry, cranberry, raspberry, and strawberry on their antioxidant activities against radicals of superoxide radicals ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), hydroxyl radicals ($OH^{\bullet}$), and singlet oxygen ($'O_2$,).

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MATERIALS AND METHODS

Chemicals. Ascorbate, chlorogenic acid, β-carotene, ferrous sulfate (heptahydrate), histidine, hydrogen peroxide (30% w/w), hydroxylamine hydrochloride, *N*,*N*-dimethyl-*p*-nitrosoaniline, α-naphthyamine, sodium nitrite, sodium tungstate dihydrate, sulfanilic acid, xanthine, and xanthine oxide were purchased from Sigma (St. Louis, MO). Ether, sodium hypochlorite, α-tocopherol, and titanium(IV) chloride were purchased from Aldrich (Milwaukee, WI). Salicylic acid was purchased from Fisher (Pittsburgh, PA).

Sample Preparation. Thornless blackberries, raspberries, and strawberries used in this study were grown at the Henry A. Wallace Research Center, U.S. Department of Agriculture in Beltsville, MD. Cranberry fruits and cultivated blueberries ("Bluecrop" and "Elliot") were obtained from the Rutgers Blueberry and Cranberry Research Center in Chatsworth, NJ. Wild blueberries were from Jonesboro, ME. Fruit samples of strawberry were harvested from 10 plants of each cultivar. Blackberry, blueberry, and raspberry fruits were harvested from six to eight bushes for each cultivar. Wild blueberry cultivars (#1, #2, #3) were obtained from three different fields of native wild blueberry plants. Cranberries were harvested from four different plots for each cultivar. All berries were harvested at commercial maturity. Undamaged berries were selected, the seeds were removed (for cranberry only), and the berries were cut into small slices and mixed. To prepare the juice samples, three 100 g samples of berries from each cultivar were pulverized and then centrifuged at 14 000*g* for 20 min at 4 °C. The supernatants were transferred to vials, stored at −80 °C, and then used for analyses.

Superoxide Radical ($O_2^{\bullet-}$) Assay. The assay for superoxide radical ($O_2^{\bullet-}$) was determined using the methods of Elstner and Heupel (1976) with slight modifications. The $O_2^{\bullet-}$ was generated by xanthine−xanthine oxidase systems (Richmond et al., 1981). The generated $O_2^{\bullet-}$ was then oxidized by hydroxylammonium chloride. Nitrite formation from hydroxylammonium chloride was determined at 530 nm in the spectrophotometer. The reaction mixture contained 1.0 mL of 65 mM Na-phosphate buffer (pH 7.8), 0.1 mL of 7.5 mM xanthine, 0.1 mL of 10 mM hydroxylammonium chloride, 0.1 mL of fruit juice, and 0.4 mL of double-distilled H_2O. The reaction was started by an additional 0.3 mL of xanthine oxidase (containing 60 μg of protein). The total reaction volume was 2.0 mL and incubated at 25 °C for 20 min. Then 0.5 mL was removed from the above reaction mixture, 0.5 mL of 19 mM sulfanilic acid and 0.5 mL of 1.0% α-naphthylamine were added, and the mixture was shaken for 5 min. After standing at room temperature for 20 min, the optical density of the mixture was determined at 530 nm (Shimadzu UV-160A, Shimadzu Scientific Instruments, Columbia, MD) against blanks which had been prepared similarly but without fruit juice. The final results were expressed as percent inhibition of $O_2^{\bullet-}$ production in the presence of fruit juice. The scavenging capacity of α-tocopherol at various concentrations (1−10 μg) on superoxide radical ($O_2^{\bullet-}$) was measured and used for determining the $O_2^{\bullet-}$ scavenging capacity of fruit juice. The antioxidant capacity of fruit juice against $O_2^{\bullet-}$ value was expressed as μmol of α-tocopherol equivalent per 10 g of fresh weight.

Hydrogen Peroxide (H_2O_2) Assay. The assay for hydrogen peroxide in fruit juices of blackberry, raspberry, cranberry, blueberry, and strawberry was carried out following procedures previously described by Patterson et al. (1984). This assay measures the direct reaction of hydrogen peroxide and Ti(IV). Titanium reagent (135 μL of 20% $TiCl_4$ in concentrated HCl) was added to 100 μL of fruit juice, 0.815 μL of Na-phosphate buffer (0.17 M, pH 7.4), 200 μL of NH_4OH (17.0 M), and 100 μL of H_2O_2 to give a Ti−H_2O_2 complex (precipitated). The precipitate was dissolved in 3 mL of 1 M H_2SO_4. The reaction was measured at 410 nm (Shimadzu UV-160A) against blanks which had been prepared similarly but without fruit juice. The final results were expressed as percent inhibition of H_2O_2 production in the presence of fruit juice. The scavenging capacity of ascorbate at various concentrations (1−10 μg) on hydrogen peroxide (H_2O_2) was measured and used for determining the H_2O_2 scavenging capacity of fruit juice. The antioxidant capacity of fruit juice against H_2O_2 value was expressed as μmol of ascorbate equivalent per 10 g of fresh weight.

Hydroxyl Radical ($OH^{\bullet}$) Assay. The assay for hydroxyl radical ($OH^{\bullet}$) was determined using the methods of Richmond et al. (1981) with slight modifications. The $OH^{\bullet}$ in aqueous media is generated through the Fenton reaction. The reaction mixture contained 0.24 M K-phosphate buffer (pH 7.4), 1.0 mM salicyclic acid, 0.3 mM $FeSO_4$/EDTA (4 mM), 0.8 mM H_2O_2, and 100 μL of fruit juice. The total reaction volume was 5.0 mL and incubated at 25 °C for 90 min. Then 120 μL of 6 M HCl was added, followed by 4 mL of chilled ether extraction. Ether was evaporated to dryness in a water bath at 40 °C, and the residue was dissolved in 1 mL of cold double-distilled water, to which the following was added: 0.5 mL of 10% (w/v) trichloroacetic acid in 0.5 M HCl, 1 mL of 10% (w/v) sodium tungstate, and 1 mL of 0.5% (w/v) $NaNO_2$. After standing for 5 min, absorbance at 510 nm was read immediately after adding 2 mL of 0.5 M KOH. Relative scavenging efficiency (% inhibition of hydroxylation) of fruit juice was estimated from the difference in absorbance (OD) with and without addition of the fruit juice. The scavenging capacity of chlorogenic acid at various concentrations (1−10 μg) on hydroxyl radical ($OH^{\bullet}$) was measured and used for determining the $OH^{\bullet}$ scavenging capacity of fruit juice. The antioxidant capacity of fruit juice against $OH^{\bullet}$ value was expressed as μmol of chlorogenic acid equivalent per 10 g of fresh weight.

Singlet Oxygen ($'O_2$) Assay. The production of singlet oxygen ($'O_2$) by sodium hypochloride and H_2O_2 was determined 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 $'O_2$ and histidine as a selective acceptor of $'O_2$. The bleaching of *N*,*N*-dimethyl-*p*-nitrosoaniline was monitored spectrophotometrically at 440 nm. The assay mixture contained 45 mM Na-phosphate buffer (pH 7.1), 10 mM histidine, 10 mM NaOCl, 10 mM H_2O_2, 50 mM of *N*,*N*-dimethyl-*p*-nitrosoaniline, and 0.1 mL of fruit juice. The total reaction volume was 2.0 mL and incubated at 30 °C for 40 min. The extent of $'O_2$ production was determined by measuring the decrease in absorbance of *N*,*N*-dimethyl-*p*-nitrosoaniline at 440 nm. Relative scavenging efficiency (% inhibition production of $'O_2$) of fruit juice was estimated from the difference in absorbance of *N*,*N*-dimethyl-*p*-nitrosoaniline with and without the addition of fruit juice. The scavenging capacity of β-carotene at various concentrations (1−10 μg) on singlet oxygen ($'O_2$) was measured and used for determining the $'O_2$ scavenging capacity of fruit juice. The antioxidant capacity of fruit juice against $'O_2$ value was expressed as μmol of β-carotene equivalent per 10 g of fresh weight.

Statistical Analysis. Data were subjected to analysis of variance using NCSS (NCSS 97, Kaysville, UT). The values of superoxide radicals ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), hydroxyl radicals ($OH^{\bullet}$), and singlet oxygen ($'O_2$) radical absorbance capacity and their relative scavenging efficiency were evaluated by the Tukey-Kramer Multiple-Comparison test used in NCSS. Differences at $p < 0.05$ were considered significant.

RESULTS

Among the different blackberry cultivars, juice of 'Hull Thornless' had the highest antioxidant capacity against $O_2^{\bullet-}$, H_2O_2, $OH^{\bullet}$, and $'O_2$ value (Figure 1). 'Hull Thornless' blackberry had the highest percent inhibition of the free radical activity of $O_2^{\bullet-}$ at 72.0% and the highest ability to inhibit H_2O_2 free radical activity at 73.9%, while 'Black Satin' had the lowest capacity for each of these activities (Table 1). 'Hull Thornless' had the highest capacity for inhibition of $OH^{\bullet}$ at 76.7% and

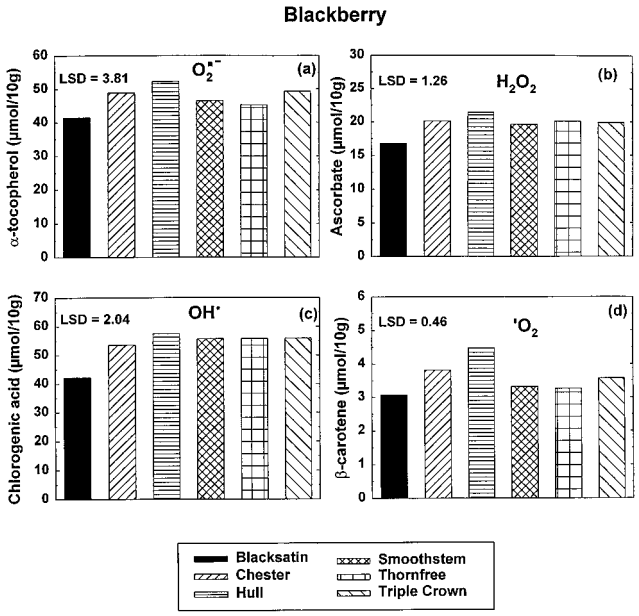

Figure 1. The scavenging capacity of juice from different cultivars of blackberry on active oxygen species ($O_2^{\bullet-}$, H_2O_2, OH•, and $^1O_2$). Data of $O_2^{\bullet-}$, H_2O_2, OH•, and $^1O_2$ are expressed as micromoles of α-tocophenol, ascorbate, chlorogenic acid, and β-carotene equivalents per 10 g of fresh weight (WM basis), respectively.

Table 1. Scavenging Capacity of Juice from Different Cultivars of Blackberry on Active Oxygen Species ($O_2^{\bullet-}$, H_2O_2, OH•, and $^1O_2$)

	% inhibition[a]			
cultivar	$O_2^{\bullet-}$	H_2O_2	OH•	$^1O_2$
Black Satin	43.3	57.9	57.3	10.9
Chester Thornless	67.2	69.3	73.9	13.5
Hull Thornless	72.0	73.9	76.7	15.8
Smoothstem	63.7	67.4	75.0	11.7
Thornfree	63.3	69.4	76.1	11.5
Triple Crown	67.8	68.5	76.3	12.6
$LSD_{0.05}$	1.83	1.52	1.22	1.21
significant[b] cultivar	**	**	**	**

[a] Data expressed as percent inhibition of radical ($O_2^{\bullet-}$, H_2O_2, OH•, or $^1O_2$) production in the present of 0.1 mL of fruit juice. [b] ** significant at $p \leq 0.05$.

also the greatest inhibition of $^1O_2$ at 15.8%, while 'Black Satin' were lowest capacities at 57.3% and 10.9%, respectively. Therefore, the 'Hull Thornless' had the best overall ability to inhibit the different reactive oxygen species, while 'Black Satin' consistently was the least able to cause inhibition of all the reactive oxygen species ($O_2^{\bullet-}$, H_2O_2, OH•, and $^1O_2$) (Figure 1 and Table 1).

In strawberry, the relative scavenging efficiency (% inhibition of radical production) of fruit juice is listed in Table 2. The antioxidant capacity value against $O_2^{\bullet-}$ for strawberries ranged from 40.4 to 51.4 μmol α-tocopherol/10 g of fresh weight (Figure 2). 'Earliglow' inhibited 73.6% of $O_2^{\bullet-}$ which was the highest level among all of the strawberry cultivars used in this study. Meanwhile, 'Allstar' had the lowest scavenging $O_2^{\bullet-}$ efficiency with only 57.9% inhibition of $O_2^{\bullet-}$ production. The antioxidant capacity against H_2O_2 values in strawberry ranged from 15.9 to 20.7 μmol ascorbate/10 g of fresh weight. 'Earliglow' also had the highest scavenging efficiency against H_2O_2 and OH•, while 'Allstar' had the lowest. Meanwhile, 'Red Chief' showed the highest antioxidant capacity values against $^1O_2$ and also had the highest

Table 2. Scavenging Capacity of Juice from Different Cultivars of Strawberry on Active Oxygen Species ($O_2^{\bullet-}$, H_2O_2, OH•, and $^1O_2$)

	% inhibition[a]			
cultivar	$O_2^{\bullet-}$	H_2O_2	OH•	$^1O_2$
Allstar	57.9	54.9	53.3	10.2
Delmarvel	60.9	64.1	62.4	12.5
Earliglow	73.6	71.4	76.2	16.7
Latestar	71.5	68.3	70.5	16.1
Lester	69.9	70.7	70.1	14.4
Red Chief	67.8	65.0	69.8	17.4
$LSD_{0.05}$	2.01	2.17	2.74	0.49
significant[b] cultivar	**	**	**	**

[a] Data expressed as percent inhibition of radical ($O_2^{\bullet-}$, H_2O_2, OH•, or $^1O_2$) production in the present of 0.1 mL of fruit juice. [b] ** significant at $p \leq 0.05$.

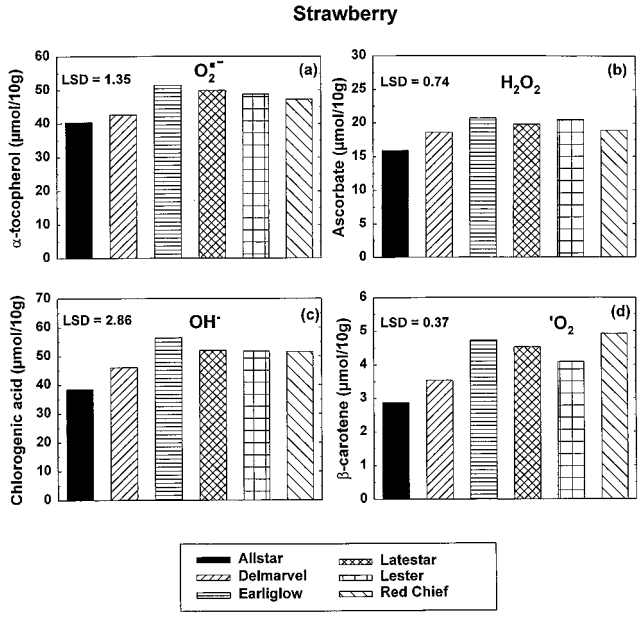

Figure 2. The scavenging capacity of juice from different cultivars of strawberry on active oxygen species ($O_2^{\bullet-}$, H_2O_2, OH•, and $^1O_2$). Data of $O_2^{\bullet-}$, H_2O_2, OH•, and $^1O_2$ are expressed as micromoles of α-tocophenol, ascorbate, chlorogenic acid, and β-carotene equivalents per 10 g of fresh weight (WM basis), respectively.

(17.4%) efficiency for inhibiting $^1O_2$, while 'Allstar' had the lowest.

The antioxidant capacity values against $O_2^{\bullet-}$, H_2O_2, OH•, and $^1O_2$ of cranberry cultivars are shown in Figure 3. Among the different cranberry cultivars, 'Early Black' stood out as having the highest antioxidant capacity values against $O_2^{\bullet-}$, H_2O_2, OH•, and $^1O_2$ and scavenging capacity to inhibit the reactive oxygen species (Table 3, Figure 3). Meanwhile, 'Howes' had the lowest scavenging capacity among all of the tested cranberry cultivars for $O_2^{\bullet-}$, OH•, and $^1O_2$ with 54.9%, 59.3%, and 7.21%, respectively. 'Ben Lear' had the lowest inhibition of H_2O_2 at 55.9%.

In raspberry juice, the values for antioxidant capacity against $O_2^{\bullet-}$, H_2O_2, OH•, and $^1O_2$ are shown in Figure 4. The antioxidant capacity values against $O_2^{\bullet-}$ for raspberries ranged from 28.5 to 46.7 μmol of α-tocopherol/10 g, and H_2O_2 values ranged from 12.6 to 20.8 μmol of ascorbate/10 g (Figure 4). 'Jewel' consistently had the best scavenging capacity for the reactive oxygen species $O_2^{\bullet-}$, H_2O_2, OH•, and $^1O_2$, with 66.9%, 71.5%, 77.3%, and

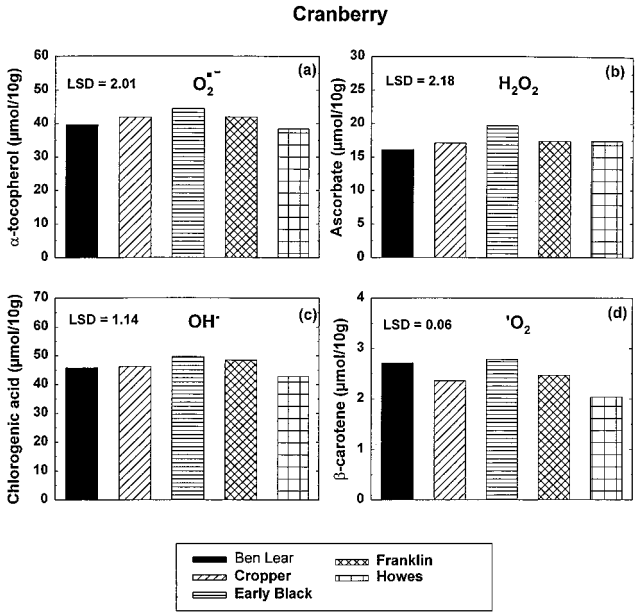

Figure 3. The scavenging capacity of juice from different cultivars of cranberry on active oxygen species ($O_2^{\bullet-}$, H_2O_2, OH•, and $^1O_2$). Data of $O_2^{\bullet-}$, H_2O_2, OH•, and $^1O_2$ are expressed as micromoles of α-tocophenol, ascorbate, chlorogenic acid, and β-carotene equivalents per 10 g of fresh weight (WM basis), respectively.

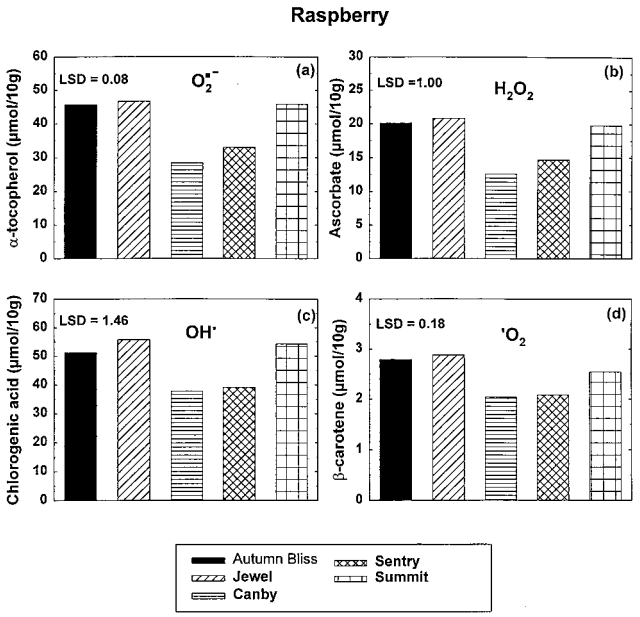

Figure 4. The scavenging capacity of juice from different cultivars of raspberry on active oxygen species ($O_2^{\bullet-}$, H_2O_2, OH•, and $^1O_2$). Data of $O_2^{\bullet-}$, H_2O_2, OH•, and $^1O_2$ are expressed as micromoles of α-tocophenol, ascorbate, chlorogenic acid, and β-carotene equivalents per 10 g of fresh weight (WM basis), respectively.

Table 3. Scavenging Capacity of Juice from Different Cultivars of Cranberry on Active Oxygen Species ($O_2^{\bullet-}$, H_2O_2, OH•, and $^1O_2$)

	% inhibition[a]			
cultivar	$O_2^{\bullet-}$	H_2O_2	OH•	$^1O_2$
Ben Lear	56.7	55.9	63.3	9.54
Cropper	59.9	58.7	63.9	8.34
Early Black	63.6	67.8	68.6	9.81
Franklin	59.9	60.1	66.9	8.73
Howes	54.9	59.9	59.3	7.21
$LSD_{0.05}$	2.09	2.80	1.23	0.66
significant[b] cultivar	**	**	**	**

[a] Data expressed as percent inhibition of radical ($O_2^{\bullet-}$, H_2O_2, OH•, or $^1O_2$) production in the present of 0.1 mL of fruit juice. [b] ** significant at $p \leq 0.05$.

Table 4. Scavenging Capacity of Juice from Different Cultivars of Raspberry on Active Oxygen Species ($O_2^{\bullet-}$, H_2O_2, OH•, and $^1O_2$)

	% inhibition[a]			
cultivar	$O_2^{\bullet-}$	H_2O_2	OH•	$^1O_2$
Autumn Bliss	65.3	69.3	70.9	9.88
Jewel	66.9	71.5	77.3	10.2
Canby	40.8	43.5	52.4	7.21
Sentry	47.5	50.7	54.3	7.36
Summit	65.6	68.3	75.2	8.98
$LSD_{0.05}$	1.20	2.11	1.85	0.31
significant[b] cultivar	**	**	**	**

[a] Data expressed as percent inhibition of radical ($O_2^{\bullet-}$, H_2O_2, OH•, or $^1O_2$) production in the present of 0.1 mL of fruit juice. [b] ** significant at $p \leq 0.05$.

10.2%, respectively (Table 4). Meanwhile, the 'Canby' raspberry cultivar had the lowest ability for inhibiting free radical activity for $O_2^{\bullet-}$, H_2O_2, OH•, and $^1O_2$, with 40.8%, 43.5%, 52.4%, and 7.2%, respectively.

In blueberry cultivars, 'Elliot' consistently had the highest antioxidant capacity against $O_2^{\bullet-}$, H_2O_2, OH•, and $^1O_2$ (Table 5, Figure 5). Wild blueberries have lower scavenging capacities against reactive oxygen species compared to 'Elliot' and 'Bluecrop'. Wild blueberry #3 had the lowest inhibition of $O_2^{\bullet-}$, H_2O_2, and $^1O_2$, with 52.5%, 49.2%, and 6.3%, respectively. Meanwhile, wild blueberry #2 was least effective in inhibiting the activities of OH• with 51.3%.

The five different types of small fruit (blackberry, strawberry, cranberry, raspberry, and blueberry) were then compared in terms of their mean antioxidant capacity against $O_2^{\bullet-}$, H_2O_2, OH•, and $^1O_2$ values and scavenging capacity for the different reactive oxygen species, and the result was shown in Figure 6 and Table 6. Blackberry had the highest antioxidant capacity against $O_2^{\bullet-}$, H_2O_2, and OH•. Meanwhile strawberry was second best in the antioxidant capacity assay for these same free radicals. With regards to $^1O_2$ scavenging

Table 5. Scavenging Capacity of Juice from Different Cultivars of Blueberry on Active Oxygen Species ($O_2^{\bullet-}$, H_2O_2, OH•, and $^1O_2$)

	% inhibition[a]			
cultivar	$O_2^{\bullet-}$	H_2O_2	OH•	$^1O_2$
Bluecrop	68.1	66.6	63.1	9.6
Elliot	69.3	67.5	67.3	10.8
wild blueberry #1	56.9	55.5	53.4	8.0
wild blueberry #2	54.6	61.2	51.3	7.0
wild blueberry #3	52.5	49.2	58.9	6.3
$LSD_{0.05}$	1.13	0.81	2.05	0.50
significant[b] cultivar	**	**	**	**

[a] Data expressed as percent inhibition of radical ($O_2^{\bullet-}$, H_2O_2, OH•, or $^1O_2$) production in the present of 0.1 mL of fruit juice. [b] ** significant at $p \leq 0.05$.

activity, strawberry had the highest value (4.36 μmol of β-carotene/10 g), while blackberry was second. Among the least effective scavengers was raspberry for 57.3% of $O_2^{\bullet-}$. Cranberry had the lowest inhibition of H_2O_2 activity at 59.8%. Meanwhile blueberry had the least antioxidant capacity for OH• and $^1O_2$ with 58.7% and 7.7%, respectively.

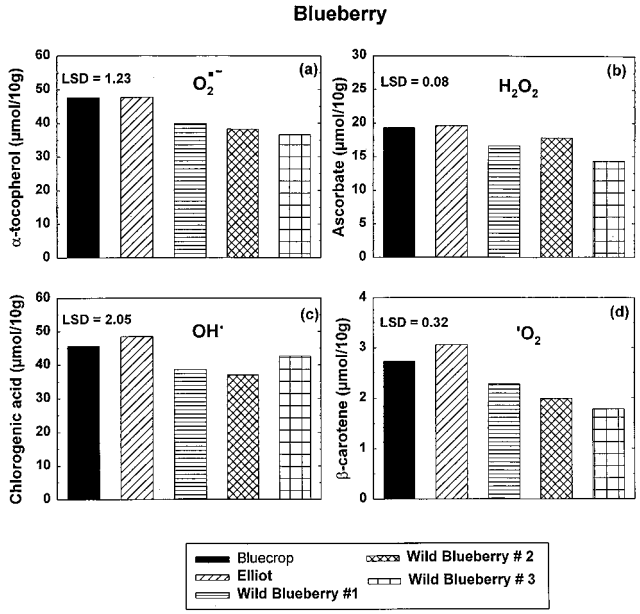

Figure 5. The scavenging capacity of juice from different cultivars of blueberry on active oxygen species ($O_2^{\bullet-}$, H_2O_2, OH•, and $^1O_2$). Data of $O_2^{\bullet-}$, H_2O_2, OH•, and $^1O_2$ are expressed as micromoles of α-tocophenol, ascorbate, chlorogenic acid, and *β*-carotene equivalents per 10 g of fresh weight (WM basis), respectively.

Table 6. Comparison of the Mean Values of the Scavenging Capacity of Juice from Different Berry Species (Blackberry, Blueberry, Cranberry, Raspberry, and Strawberry) on Active Oxygen Species ($O_2^{\bullet-}$, H_2O_2, OH•, and $^1O_2$)

	% inhibition[a]			
cultivar	$O_2^{\bullet-}$	H_2O_2	OH•	$^1O_2$
blackberry[b]	64.3	66.3	72.0	12.4
blueberry	60.1	61.2	58.7	7.71
cranberry	59.0	59.8	64.2	8.64
raspberry	57.3	60.9	66.9	8.88
strawberry	64.2	65.3	68.6	15.41
$LSD_{0.05}$	3.02	2.33	1.79	1.16
significant[c] cultivar	**	**	**	**

[a] Data expressed as percent inhibition of radical ($O_2^{\bullet-}$, H_2O_2, OH•, or $^1O_2$) production in the present of 0.1 mL of fruit juice. [b] Data for the thornless blackberry were derived from the mean value calculated from six cultivars (Black Satin, Chester Thornless, Hull Thornless, Smoothstem, Thornfree, Triple Crown); blueberry data were derived from the mean value calculated from five cultivars (Bluecrop, Elliot, wild blueberry #1, wild blueberry #2, wild blueberry #3); cranberry data were derived from the mean value calculated from five cultivars (Ben Lear, Cropper, Early Black, Franklin, Howes); raspberry data were derived from the mean value calculated from five cultivars (Autumn Bliss, Jewel, Canby, Sentry, Summit); strawberry data were derived from the mean value calculated from six cultivars (Allstar, Delmarvel, Earliglow, Latestar, Lester, Red Chief). [c] ** nonsignificant or significant at $p \leq 0.05$.

Different antioxidants were analyzed for their functional scavenging capacity against the four active oxygen species $O_2^{\bullet-}$, H_2O_2, OH•, and $^1O_2$ (Table 7). There were interesting and marked differences among the different antioxidants in their abilities to inhibit the different reactive oxygen species. For example *β*-carotene had by far the highest scavenging activity against $^1O_2$ at 35.3% but had absolutely no effect on H_2O_2. Ascorbic acid was the best at inhibiting H_2O_2 free radical activity at 34.5%. For OH•, there was a wide range of scavenging capacities from a high of 15.3% with

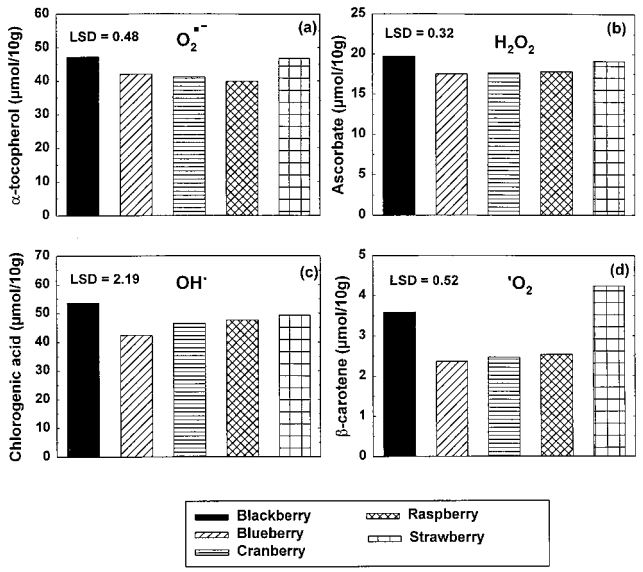

Figure 6. Comparison of the mean values of the scavenging capacity of juice from different berry species (blackberry, blueberry, cranberry, raspberry, and strawberry) on active oxygen species ($O_2^{\bullet-}$, H_2O_2, OH•, and $^1O_2$). Data for the thornless blackberry were derived from the mean value calculated from six cultivars (Black Satin, Chester Thornless, Hull Thornless, Smoothstem, Thornfree, Triple Crown); blueberry data were derived from the mean value calculated from five cultivars (Bluecrop, Elliot, wild blueberry #1, wild blueberry #2, wild blueberry #3); cranberry data were derived from the mean value calculated from five cultivars (Ben Lear, Cropper, Early Black, Franklin, Howes); raspberry data were derived from the mean value calculated from five cultivars (Autumn Bliss, Jewel, Canby, Sentry, Summit); strawberry data were derived from the mean value calculated from six cultivars (Allstar, Delmarvel, Earliglow, Latestar, Lester, Red Chief). Data of $O_2^{\bullet-}$, H_2O_2, OH•, and $^1O_2$ are expressed as micromoles of α-tocophenol, ascorbate, chlorogenic acid, and *β*-carotene equivalents per 10 g of fresh weight (WM basis), respectively.

Table 7. Scavenging Capacity of Different Antioxidants on Active Oxygen Species ($O_2^{\bullet-}$, H_2O_2, OH•, and $^1O_2$)

	relative scavenging rate (% of inhibition)			
antioxidant[a]	$O_2^{\bullet-}$	H_2O_2	OH•	$^1O_2$
ascorbic acid	10.6	34.5	0.88	6.18
β-carotene	14.1	0	1.58	35.3
chlorogenic acid	2.43	8.14	13.9	0.44
glutathione	20.1	19.9	5.19	3.61
α-tocopherol	14.3	1.42	15.3	22.5
$LSD_{0.05}$	0.61	0.57	0.24	1.16
significant[b] cultivar	**	**	**	**

[a] 0.1 mmol/L were used for each sample. [b] ** significant at $p \leq 0.05$.

α-tocopherol to a low of 0.88% with ascorbic acid. Glutathione has higher $O_2^{\bullet-}$ scavenging capacity (20.1%) compared to the other antioxidants.

DISCUSSION

Free radicals (ROO•, $O_2^{\bullet-}$, H_2O_2, OH•, and $^1O_2$) are formed during normal cell metabolism (Elstner, 1982). There are mechanisms to detoxify these free radicals in plant cells. Blackberries, raspberries, and strawberries are good sources of antioxidants and possess potent $ROO^{\bullet-}$ scavenging capacity (Heinonen et al., 1998; Wang et al., 1996; Wang and Lin, 2000). In the present study, we showed that small fruit crops such as black-

berries, blueberries, cranberries, raspberries, and strawberries also possess antioxidant activities against superoxide radicals ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), hydroxyl radicals ($OH^{\bullet}$), and singlet oxygen ($^1O_2$). Different cultivars showed varying degrees of scavenging capacity on different active oxygen species ($O_2^{\bullet-}$, H_2O_2, $OH^{\bullet}$, and $^1O_2$). The scavenging capacity among all of the tested small fruit crops ranged from 40.8 to 72.0% for $O_2^{\bullet-}$, from 50.7 to 73.9% for H_2O_2, from 52.4 to 77.3% for $OH^{\bullet}$, and from 6.3 to 17.4% for $^1O_2$. Among all the species and cultivars tested, blackberries and strawberries had the highest inhibition of active oxygen species ($O_2^{\bullet-}$, H_2O_2, $OH^{\bullet}$, and $^1O_2$) production. Among the different blackberry cultivars, juice of 'Hull Thornless' had the highest scavenging capacity of active oxygen species.

It has been reported that small fruit crops have high content of antioxidant compounds such as ascorbate, β-carotene, glutathione, α-tocopherol, anthocyanins, and phenolics. For example, 100 g of fresh blackberries contained 80 μg of β-carotene, 2.37 mg of α-tocopherol, and 15 mg of ascorbic acid; 100 g of strawberries contained 8 μg of β-carotene, 0.2 mg of α-tocopherol, and 77 mg of ascorbic acid; 100 g of raspberries contained 6 μg of β-carotene, 0.48 mg of α-tocopherol, and 32 mg of ascorbic acid (Holland et al., 1992). We found that 100 g of fresh blackberries (cv. Hull Thornless) contained 43 mg ascorbic acid, 2.5 mg glutathione (reduced form), 226 mg of total phenolics, and 153 mg of anthocyanins. These small molecules are involved in oxidative protective process and play an important role in scavenging harmful free radicals and preventing various human diseases (Ames et al., 1993; Gey, 1990).

Ascorbic acid is an essential compound in plant tissues and has been the focus of numerous studies in relation to enzymatic and nonenzymatic oxidation reactions in the biological system. Ascorbic acid has been reported to act as an antioxidant and functions as a cosubstrate of plant peroxidases, such as the ascorbate peroxidase system which produces dehydroascorbate (Larson, 1988; Polle and Rennenberg, 1994). Ascorbic acid acts as an effective quencher of superoxide radical ($O_2^{\bullet-}$) [with a rate constant of 2.7×10^5 M^{-1} s^{-1} ($2O_2^{\bullet-}$ + ascorbate + $2H^+$ → dehydroascorbate + $2H_2O_2$)] and the hydrogen peroxide (H_2O_2 + 2ascorbate → 2monodehydroascorbate + $2H_2O$). It has also been shown to scavenge $^1O_2$ at a relatively fast rate (about 10^7 M^{-1} s^{-1}) (Foyer, 1993; Larson, 1988; Polle and Rennenberg, 1994). We found that ascorbic acid was very effective in inhibiting H_2O_2 free radical activity at 34.5% which was 1.73, 24.26, and 4.23 times of the scavenging capacity of glutathione, α-tocopherol, and chlorogenic acid, respectively. The scavenging capacity of ascorbic acid on $O_2^{\bullet-}$ and $^1O_2$ was 10.6 and 6.18%, respectively. However, the scavenging capacity of ascorbic acid on $OH^{\bullet}$ was very weak (0.88%).

Singlet oxygen ($^1O_2$) is very powerfully quenched by β-carotene, with a rate constant of $2-3 \times 10^{10}$ M^{-1} s^{-1} (Larson, 1988; Pallett and Young, 1993). This rate constant exceeds that for the reaction of $^1O_2$ with most biologically important unsaturated fatty acids by 4−5 orders of magnitude, thus allowing a relatively low concentration of β-carotene to effectively protect membrane lipids from peroxidation reactions of $^1O_2$ radicals [$^1O_2$ + β-carotene → β-^{3}carotene* (triplet state) + O_2, β-^{3}carotene* (triplet state) → β-carotene + heat] (Larson, 1988; Pallett and Young 1993). β-Carotene also showed a capacity to scavenge $O_2^{\bullet-}$ with a rate constant of 2.5×10^8 M^{-1} s^{-1} (Krinsky, 1993; Palozza and Krinsky, 1992; Polle and Rennenberg, 1994). Our results showed that the scavenging capacity of β-carotene for $^1O_2$ radical was 35.3%, which equaled 9.78, 5.72, and 2.84 times the scavenging capacity of glutathione, ascorbate, and α-tocopherol, respectively. β-Carotene had scavenging activity against $O_2^{\bullet-}$ at 14.1% but had little effect on $OH^{\bullet}$ (1.58%) and no effect on the scavenging of H_2O_2.

Glutathione is generally considered to be ubiquitous sulhydryl-containing tripeptide in living cells. Glutathione often exists in both a reduced form (GSH) and an oxidized form, glutathione disulfide (GSSG). The majority of glutathione in the cell is maintained in the reduced state. The reduced form of glutathione plays an important role in the stabilization of many enzymes and a more general role as an oxidant scavenger in that it serves as a substrate for dehydroascorbate reductase and is also able to react directly with free radicals including the hydroxyl radical to prevent the inactivation of enzymes by oxidation of an essential thiol group (H_2O_2 + 2GSH → $2H_2O$ + GSSG) (Dalton et al., 1986). GSH also directly reduces $OH^{\bullet}$ radicals (Smirnoff and Cumbes, 1989) and scavenges $^1O_2$ with a rate constant of 10^6 M^{-1} s^{-1} (Devasagayam et al., 1991). We found that glutathione was very effective in inhibiting $O_2^{\bullet-}$ and H_2O_2 free radical activities at 20.1 and 19.9%, respectively. The scavenging capacity of glutathione on $OH^{\bullet}$ and $^1O_2$ was much less with 5.19 and 3.61%, respectively.

Vitamin E (α-tocopherol) is an exquisite, lipophilic, free radical scavenger which, like the carotenoids, exists within biological membranes. As a free radical trap, it directly interrupts free radical chain reactions. The normal mechanism of action for α-tocopherol is the inactivation of two equivalents of chain-carrying peroxy radicals ($ROO^{\bullet}$), terminating two potential radical chain reactions per molecule of inhibitor (Larson, 1988). α-Tocopherol can deactivate $^1O_2$ with a rate constant of $1-2.5 \times 10^8$ M^{-1} s^{-1} (Hess, 1993; Polle and Rennenberg, 1994), reduce $O_2^{\bullet-}$ (rate constant 10^6 M^{-1} s^{-1}), and terminate lipid peroxidation by reducing fatty acyl peroxy radicals (Polle and Rennenberg, 1994). Our results showed that the scavenging capacity of α-tocopherol for $^1O_2$ radical was 22.5%, which was slightly less than β-carotene (35.3%). α-Tocopherol had scavenging activity against $O_2^{\bullet-}$ and $OH^{\bullet}$ with 14.3 and 15.3%, respectively, but had little effect on H_2O_2 with only 1.42%.

Anthocyanins and phenolics are secondary plant metabolites. They protect the plant against damaging photodynamic reactions by quenching the excited state of active oxygen species (Larson, 1988; Lewis, 1993). Chlorogenic acid was found to be the most abundant phenolic acid in the plant extract and also the most active antioxidant. A 1.2×10^{-5} M solution of chlorogenic acid inhibited over 80% of peroxide formation in a linoleic acid test system (Larson, 1988). We found that chlorogenic acid was effective in inhibiting $OH^{\bullet}$ free radical activity at 13.9%. The scavenging capacity of chlorogenic acid on $O_2^{\bullet-}$ and H_2O_2 was 2.43 and 8.14%, respectively. However, the scavenging capacity of ascorbic acid on $^1O_2$ was very weak (0.44%).

Anthocyanins are probably the largest group of phenolic compounds in the human diet. Anthocyanins have been used for several therapeutic purposes including the treatment of diabetic retinopathy, fibrocystic disease,

and vision disorders (Leonardi, 1993; Scharrer and Ober, 1981). Anthocyanins also have the potential to serve as radiation-protective agents, vasotonic agents, and chemoprotective agents (Wang et al., 1997) and can act against carbon tetrachloride induced lipoperoxidation (Morazzoni and Bombardelli, 1996). Anthocyanins also decrease the fragility of capillaries, inhibit blood platelet aggregation, and strengthen the collagen matrix, which is the protein component of connective tissues (Morazzoni and Bombardelli, 1996).

In addition to providing the usual nutrients such as ascorbic acid, β-carotene, chlorogenic acid, glutathione, and α-tocopherol, small fruit crops such as blackberries, blueberries, cranberries, raspberries, and strawberries are also rich in anthocyanins, flavonoids, and phenolic acids (Heinonen et al., 1998; Rice-Evans and Miller, 1996; Wang et al., 1997; Wang and Lin, 2000). Based on our results, juices of small fruit crops showed a remarkably high scavenging activity of chemically generated active oxygen species. For example, 100 g (fresh weight) of juices of ripe blackberries cv. Hull Thornless have a scavenging capacity equal to 3.78 mg of ascorbic acid for hydrogen peroxide (H_2O_2), 11.0 mg of glutathione for superoxide radicals ($O_2^{\bullet -}$), 5.47 mg of α-tocopherol for singlet oxygen ($^1O_2$), 2.4 mg of β-carotene for $^1O_2$, and 19.1 mg of chlorogenic acid for hydroxyl radicals ($OH^{\bullet}$). The relevance of this information to the human diet is summarized by Rice-Evans and Miller (1996) who state that total antioxidant potential of fruits and vegetables is more important than levels of any individual specific antioxidant constituent.

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LITERATURE CITED

Ames, B. M.; Shigena, M. K.; Hagen, T. M. Oxidants, antioxidants and the degenerative diseases of aging. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 7915−7922.

Ascherio, A.; Rimm, E. B.; Giovannucci, E. L.; Colditz, G. A.; Rosner, B.; Willett, W. C.; Sacks, F.; Stampfer, M. J. A prospective study of nutritional factors and hypertension among US men. *Circulation* **1992**, *86*, 1475−1484.

Cao, G.; Sofic, E.; Prior, R. L. Antioxidant capacity of tea and common vegetables. *J. Agric. Food Chem.* **1996**, *44*, 3426−3441.

Chakraborty, N.; Tripathy, B. C. Involvement of singlet oxygen in 5-aminolevulinic acid-induced photodynamic damage of cucumber (*Cucumis sativus* L.) chloroplasts. *Plant Physiol.* **1992**, *98*, 7−11.

Dalton, D. A.; Russell, S. A.; Hanus, F. J.; Pascoe, G. A.; Evans, H. J. Enzymatic reactions of ascorbate and glutathione that prevent peroxide damage in soybean root nodules. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 3811−3813.

Devasagayam, T. P. A.; Sundquist, A.R; Di-Mascio, P.; Kaiser, S.; Sies, H. Activity of thios as singlet molecular oxygen quenches. *J. Photochem. Photobiol.* B: Biol. **1991**, *9*, 105−116.

Elstner, E. F. Oxygen activation and oxygen toxicity. *Annu. Rev. Plant Physiol.* **1982**, *33*, 73−82.

Elstner, E. F.; Heupel, A. Inhibition of nitrite formation from hydroxylammonium chloride: A simple assay for superoxide dismutase. *Anal. Biochem.* **1976**, *70*, 616−620.

Frei, B.; Stocker, R.; Ames, B. N. Small molecule antioxidant defenses in human extracellular fruids. In *Molecular biology of free radical scavenging systems*; Scandalios, J. G., Ed.; CSHL: New York, 1992; pp 23−28, 31−35, 37.

Foyer, C. H. Ascorbic acid. In *Antioxidants in higher plants*; Alscher, R. G., Hess, J. L., Eds.; CRC: Boca Raton, FL, 1993; pp 31−48, 51−52.

Gey, K. F. The antioxidant hypothesis of cardiovascular disease: epidemiology and mechanisms. *Biochem. Soc. Trans.* **1990**, *18*, 1041−1045.

Halliwell, B. Ascorbic acid and the illuminated chloroplast. In *Ascorbic acid: chemistry, metabolism and uses;* Seib, P. A., Tolbert, B. M., Eds.; Advances in Chemistry Series 200; American Chemical Society: Washington, DC, 1982; pp 263−274.

Halliwell, B. The biological toxicity of free radicals and other reactive oxygen species. In *Free radicals and food additives*; Aruona**,** O. I., Halliwell, B., Eds.; Taylor & Francis Inc.: PA, 1991; pp 41−45.

Halliwell, B.; Aruoma, O. I. DNA damage by oxygen-derived species: its mechanism, and measurement using chromatographic methods. In *Molecular biology of free radical scavenging systems*; Scandalios, J. G., Ed.; CSHL: New York, 1992; pp 23−27, 47−58.

Heinonen, I. M.; Meyer, A. S.; Frankel, E. N. Antioxidant activity of berry phenolics on human low-density lipoprotein and liposome oxidation. *J. Agric. Food Chem.* **1998**, *46*, 4107−4112.

Hess, J. L. Vitamin E, α-tocopherol. In *Antioxidants in higher plants*; Alscher, R. G., Hess, J. L., Eds.; CRC: Boca Raton, FL, 1993; pp 123−125.

Holland, B.; Welch, A. A.; Unwin, I. D.; Buss, D. H.; Paul, A. A.; South, D. A. T. *The composition of foods*. 5th. ed.; The Royal Society of Chemistry and Ministry of Agriculture, Fisheries and Food: 1992; pp 279−311.

Krinsky, N. I. Actions of carotenoids in biological systems. *Annu. Rev. Nutr.* **1993**, *13*, 561−573.

Larson, R. A. The antioxidants of higher plants. *Phytochemistry* **1988**, *27*, 969−978.

Leonardi, M. Treatment of fibrocystic disease of the breast with myrtillus anthocyanins. Our experience. *Minerva Ginecol.* **1993**, *45*, 617−621.

Lewis, N. G. Plant phenolics. In *Antioxidants in higher plants*; Alscher, R. G., Hess, J. L., Eds.; CRC: Boca Raton, FL, 1993; pp 135−60.

Morazzoni, P.; Bombardelli, E. *Vaccinium myrtillus* L. *Fitoterapia* **1996,** *LXVII*, 3−29.

NCSS 97. *Statistical System for Windows*; Kaysville, UT, 1997.

Pallett, K. E.; Young, A. J. Carotenoids. In *Antioxidants in higher plants*; Alscher, R. G., Hess, J. L., Eds.; CRC: Boca Raton, FL, 1993; pp 71−74.

Palozza, P.; Krinsky, N. I. Antioxidant effects of carotenoids in *vitro* and *vivo*: an overview. *Methods Enzymol.* **1992**, *213*, 403−420.

Patterson, B. D.; MacRae, E. A.; Ferguson, I. B. Estimation of hydrogen peroxide in plant extracts using titanium(IV). *Anal. Biochem.* **1984**, *139*, 487−492.

Polle, A. R.; Rennenberg, H. Photooxidative stress in trees. In *Causes of photooxidative stress and amelioration of defense systems in plants*; Foyer, C. H., Mullineaux, P. M., Eds.; CRC: Boca Raton, FL, 1994; pp 199−209.

Rice-Evans, C. A.; Miller, N. J. Antioxidant activities of flavonoids as bioactive components of food. *Biochemical Soc. Trans.* **1996,** *24*, 790−795.

Richmond, R.; Halliwell, B.; Chauhan, J.; Darbre, A. Superoxide-dependent formation of hydroxyl radicals: Detection of hydroxy radicals by the hydroxylation of aromatic compounds. *Anal. Biochem.* **1981**, *118*, 328−330.

Satué-Gracia, M. T.; Heinonen, I. M.; Frankel, E. N. Anthocyanins as antioxidants on human low-density lipoprotein and lecithin-liposome systems. *J. Agric. Food Chem.* **1997**, *45*, 3362−3367.

Scharrer, A.; Ober, M. Anthocyanosides in the treatment of retinopathies. *Klin. Monatsbl. Augenheikd*. **1981,** *178*, 386-389.

Smirnoff, N.; Cumbes, Q. J. Hydroxyl radical scavenging activity of compatible solutes. *Phytochemistry* **1989**, *28*, 1057−1060.

Steinberg, D. Antioxidants and atherosclerosis: a current assessment. *Circulation* **1991**, *84*,1420−1425.

Velioglu Y. S.; Mazza, G.; Gao, L.; Oomah, B. D. Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. *J. Agric. Food Chem*. **1998**, *46*, 4113−4117.

Wang, H.; Cao, G.; Prior. R. L. Total antioxidant capacity of fruits. *J. Agric. Food Chem*. **1996,** *44*, 701−705.

Wang, H.; Cao, G.; Prior, R. L. Oxygen radical absorbing capacity of anthocyanins. *J. Agric. Food Chem*. **1997,** *45*, 304−309.

Wang, S. Y.; Lin, H. S. Antioxidant activity in fruits and leaves of blackberry, raspberry, and strawberry is affected by cultivar and maturity. *J. Agric. Food Chem*. **2000,** *48*, 140−146.

Wayner, D. D. M.; Burton, G. W.; Ingold, K. U.; Locke, S. J. Quantitative measurement of total, peroxyl radical-trapping antioxidant capacity of human blood plasma by controlled peroxidation. The important contribution made by plasma proteins. *FEBS Lett*. **1985**, *187*, 33.

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