Effect of Plant Growth Temperature on Antioxidant Capacity in Strawberry

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The influence of four day/night growing temperature combinations (18/12, 25/12, 25/22, and 30/22 °C) on phenolic acid, flavonol, and anthocyanin content and their antioxidant activities against peroxyl radicals ($ROO^{\bullet}$), superoxide radicals ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), hydroxyl radicals ($OH^{\bullet}$), and singlet oxygen ($^1O_2$) in fruit juice of Earliglow and Kent strawberry (*Fragaria* × *ananassa* Duch.) cultivars was studied. Pelargonidin-based anthocyanins such as pelargonidin 3-glucoside (291.3−945.1 μg/g fresh wt.), pelargonidin 3-rutinoside (24.7−50.9 μg/g fresh wt.), and pelargonidin 3-glucoside-succinate (62.2−244.0 μg/g fresh wt.) were the predominant anthocyanins in strawberry fruit juice. The content of cyanidin-based anthocyanins, cyanidin 3-glucoside and cyanidin 3-glucoside-succinate, was much lower than that of pelargonidin-based anthocyanins. Strawberry growth in high temperature conditions significantly enhanced the content of *p*-coumaroylglucose, dihydroflavonol, quercetin 3-glucoside, quercetin 3-glucuronide, kaempferol 3-glucoside, kaempferol 3-glucuronide, cyanidin 3-glucoside, pelargonidin 3-glucoside, pelargonidin 3-rutinoside, cyanidin 3-glucoside-succinate, and pelargonidin 3-glucoside-succinate in strawberry juice. Plants grown in the cool day and cool night temperature (18/12 °C) generally had the lowest phenolic acid, flavonols, and anthocyanins. An increase in night temperature from 12 to 22 °C, with the day temperature kept constant at 25 °C, resulted in a significant increase in phenolic acid, flavonols, and anthocyanins. These conditions also resulted in a significant increase in antioxidant capacity. The highest day/night temperature (30/22 °C) yielded fruit with the most phenolic content as well as $ROO^{\bullet}$, $O_2^{\bullet-}$, H_2O_2, $OH^{\bullet}$, and $^1O_2$ radical absorbance capacity. Fruit of Kent cv. strawberry had higher values of phenolic acid, flavonols, anthocyanins, and antioxidant capacities than fruit of Earliglow cv. strawberry under all temperature regimes.

Keywords: *Antioxidant; anthocyanin; phenolics; free radical; strawberry; Fragaria* × *ananassa*

INTRODUCTION

Fruits and vegetables contain high levels of antioxidant compounds which provide protection against harmful free radicals and have been associated with lower incidence and mortality rates of cancer and heart disease in addition to a number of other health benefits (*1*−*7*). Our previous studies have shown that thornless blackberries (*Rubus* sp.), blueberries (*Vaccinium* spp.), cranberries (*Vaccinium macrocarpon* Aiton), raspberries (*Rubus idaeus* L. and *Rubus occidentalis* L.), and strawberries (*Fragaria* × *ananassa* Duch.) have high antioxidant capacity against peroxyl radicals ($ROO^{\bullet}$), superoxide radicals ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), hydroxyl radicals ($OH^{\bullet}$), and singlet oxygen ($^1O_2$), and their antioxidant activities were different among varieties (*8*, *9*). There is a positive correlation between antioxidant activity and total phenolic or anthocyanin content (*8*, *9*). The antioxidant capacity of blackberry was also found to correlate with oxygen radical scavenging enzyme activity (*10*). However, no information is available on the effect of environmental factors such as growing temperatures on scavenging capacity of strawberry against active oxygen species. The present study evaluated four different day/night growing temperatures (18/12, 25/12, 25/22, and 30/22 °C) on their antioxidant activities against $ROO^{\bullet}$, $O_2^{\bullet-}$, H_2O_2, $OH^{\bullet}$, and $^1O_2$ radicals, associated with changes in anthocyanins and other phenolic compounds of strawberry.

MATERIALS AND METHODS

Chemicals. Ascorbate, β-carotene, chlorogenic acid, *p*-coumaric acid, histidine, hydrogen peroxide (30% w/w), hydroxylamine hydrochloride, kaempferol, *N*,*N*-dimethyl-*p*-nitrosoaniline, α-naphthylamine, R-phycoerythrin (R-PE) from *Porphydium cruentum*, quercetin, sodium nitrite, sodium tungstate dihydrate, sulfanilic acid, xanthine, and xanthine oxide were purchased from Sigma (St. Louis, MO). Ether, sodium hypochlorite, α-tocopherol, titanium (IV) chloride, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), *N*-ethylmaleimide, and trichloroacetic acid were obtained from Aldrich Chemical Co. (Milwaukee, WI). Salicylic acid was purchased from Fisher (Pittsburgh, PA). 2′, 2′ azobis (2-amidinopropane) dihydrochloride (AAPH) was purchased from Wako Chemicals USA, Inc. (Richmond, VA). EDTA (ethylenediaminetetracetic acid, disodium salt, dihydrate-Na_2 EDTA·$2H_2O$) was obtained from Life Technologies (Rockville, MD). All anthocyanins and aglycons were purchased from Indofine Chemical Co., Inc. (Somerville, NJ).

Plant Materials and Experimental Plans. Uniform-sized plants of approximately one-year-old Earliglow and Kent cultivars were used. The plants were propagated by runner-tip cuttings in June and plants were grown in 2-L plastic pots

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(15.0 × 12.0 cm, E. C. Geiger, Inc., Harleysville, PA) containing Pro Mix BX (Premier Brands Inc., Stamford, CT) in a greenhouse. Radiation sources in the greenhouse consisted of natural daylight and Watt-Miser incandescent lamps (Nela Park, Cleveland, OH) that provided a PAR around 700 μmol $m^{-2}s^{-1}$ for 14 h/d (6:00 a.m. − 8:00 p.m.). Temperatures were set around 25 °C during the day and 20 °C at night. During the growing season, all plants were watered daily and fertilized biweekly with 150 mL/plant of Peters fertilize (20:20:20, N/P/K). Prior to initiation of temperature treatments, plants were exposed to the ambient winter temperatures of Beltsville, Maryland, in an unheated greenhouse from October to February. Plants were then moved to a heated greenhouse (25 °C during the day and 20 °C at night) for approximately 1.5 months to force flowering. Blossoms were self-pollinated by hand using a small brush. Plants with the most fruit (at least 10 fruits per plant) at their green fruit stage were selected for the growth chamber experiments. Forty plants each of Earliglow and Kent cultivars were removed from the greenhouse in March and divided into lots of 10 plants. One lot of each cultivar was randomly placed in each of four growth chambers set at day/night temperatures of 18/12, 25/12, 25/22, and 30/22 °C. The plants were in these growth chambers for 1.5 months. The photoperiod for each growth chamber was 14 h (6:00 a.m. − 8:00 p.m.) with a PAR around 700 μmol $m^{-2}s^{-1}$ at plant height. The ripeness of fruit was determined by color. Firm red-ripe fruits free from defects or decay were harvested from each growth chamber for each cultivar during the fruiting stage. The berries were cut into small slices, mixed, and then used for analyses.

Fruit Sample Preparation. To prepare the juice samples, three100-g samples of berries from three replicates of each cultivar of each treatment were pulverized and then centrifuged at 14000*g* for 20 min at 4 °C. The supernatants were transferred to vials, stored at −80 °C, and then used for analyses.

Peroxyl radicals (ROO•) − ORAC Assay. The procedures for the ORAC assay on strawberries were modified from a previously described method by Cao et al. (*11*). This assay measures the effect of antioxidant components in fruit juices of strawberries on the decline in R-phycoerythrin (R-PE) fluorescence induced by a peroxyl radical generator, 2′,2′ azobis (2-amidinopropane) dihydrochloride (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 of Trolox (a water-soluble α-tocopherol analogue) was used 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 (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 on a fresh weight basis (*11*):

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

where K = sample dilution factor and S = the area under the fluorescence decay curve of the sample, Trolox, or blank. S is calculated as follows:

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

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

Superoxide Radical ($O_2^{\bullet-}$) Assay. The assay for superoxide radical ($O_2^{\bullet-}$) was determined using the methods of Elstner and Heupel (*12*) with slight modifications. The $O_2^{\bullet-}$ was generated by xanthine/xanthine−oxidase systems (*13*). Nitrite formation from hydroxylammonium chloride was determined at 530 nm in the spectrophotometer. The reaction mixture contained 1.0 mL of 65 mM sodium 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 addition of 0.3 mL of xanthine oxidase (containing 60 μg of protein). The total reaction volume was 2.0 mL and was incubated at 25 °C for 20 min. Then 0.5 mL was removed from the above reaction mixture, then 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 the mixture was allowed to stand at room temperature for 20 min, its optical density 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 the $O_2^{\bullet-}$ value was expressed as μmol of α-tocopherol equivalent per gram fresh weight.

Hydrogen Peroxide (H_2O_2) Assay. The assay for hydrogen peroxide in strawberry juice was carried out by following procedures previously described by Patterson et al. (*14*) for fruit juices of blackberry, raspberry, cranberry, blueberry, and strawberry. This assay measures the direct reaction of hydrogen peroxide and Ti(IV). Titanium reagent (135 μL of 20% $TiCl_4$ in concd HCl) was added to 100 μL of fruit juice, 0.815 mL of sodium phosphate buffer (0.17 M, pH 7.4), 200 μL of NH_4OH (17.0 M), and 100 μL of H_2O_2 (1.35 mM) 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 gram fresh weight.

Hydroxyl Radical (OH•) Assay. The assay for hydroxyl radical (OH•) was determined using the methods of Richmond et al. (*13*) with slight modifications. The OH• in aqueous media is generated through the Fenton reaction. The reaction mixture contained 0.24 M potassium 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 was incubated at 25 °C for 90 min. Then 120 μL of 6 M HCl was added, followed by extraction in 4 mL of chilled ether. 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 the mixture was allowed to stand 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•) was measured and used for determining the OH• scavenging capacity of fruit juice. The antioxidant capacity of fruit juice against OH• value was expressed as μmol of chlorogenic acid equivalent per gram fresh weight.

Singlet Oxygen ($^1O_2$) Assay. The production of singlet oxygen ($^1O_2$) by sodium hypochloride and H_2O_2 was determined by using a spectrophotometric method according to Chakraborty

Table 1. Effect of Plant Growth Temperature on Antioxidant Activity Against Peroxyl Radicals (ROO•), Superoxide Radicals ($O_2^{\bullet-}$), Hydrogen Peroxide (H_2O_2), Hydroxyl Radicals (OH•), and Singlet Oxygen ($^1O_2$) in Fruit Juice of Two Strawberry Cultivars[a]

temperature (day/night °C)	cultivar	ROO• ORAC (μmol TE/g)[b]	$O_2^{\bullet-}$ (μmol α-tocopherol/g)[c]	H_2O_2 (μmol ascorbate/g)[d]	OH• (μmol chlorogenic acid/g)[e]	$^1O_2$ (μmol β-carotene/g)[f]
18/12	Earliglow	12.6 ± 0.3	4.24 ± 0.13	2.16 ± 0.04	4.19 ± 0.23	0.55 ± 0.02
	Kent	14.2 ± 0.2	4.38 ± 0.09	2.21 ± 0.03	4.76 ± 0.16	0.58 ± 0.03
25/12	Earliglow	15.4 ± 0.3	4.39 ± 0.11	2.51 ± 0.08	4.38 ± 0.25	0.63 ± 0.04
	Kent	16.4 ± 0.2	4.51 ± 0.08	2.62 ± 0.07	5.13 ± 0.29	0.68 ± 0.02
25/22	Earliglow	16.8 ± 0.1	4.42 ± 0.26	2.80 ± 0.11	4.61 ± 0.32	0.69 ± 0.01
	Kent	17.6 ± 0.3	4.73 ± 0.15	2.98 ± 0.15	5.34 ± 0.18	0.70 ± 0.02
30/22	Earliglow	18.9 ± 0.2	4.67 ± 0.18	3.36 ± 0.09	5.73 ± 0.42	0.70 ± 0.03
	Kent	19.8 ± 0.3	4.95 ± 0.21	3.48 ± 0.12	6.32 ± 0.27	0.77 ± 0.04
$LSD_{0.05}$		0.75	0.13	0.11	0.18	0.04
significance[g]						
temperature (*T*)		**	**	**	**	**
cultivar (*C*)		**	**	**	**	ns
(*T* × *C*)		**	**	**	**	ns

[a] Data expressed as mean ± SEM. [b] Data expressed as micromoles of Trolox equivalents per gram of fresh weight. [c] Data expressed as micromoles of α-tocopherol equivalents per gram of fresh weight. [d] Data expressed as micromoles of ascorbate equivalents per gram of fresh weight. [e] Data expressed as micromoles of chlorogenic acid equivalents per gram of fresh weight. [f] Data expressed as micromoles of β-carotene equivalents per gram of fresh weight. [g] ns,**, nonsignificant or significant, respectively, at $p \leq 0.05$.

and Tripathy (*15*) with minor modifications in which *N*,*N* dimethyl-*p*-nitrosoaniline was used as a selective scavenger of $^1O_2$ and histidine was used as a trap for $^1O_2$ acceptor. The bleaching of *N*, *N* dimethyl-*p*- nitrosoaniline as induced by the reaction of $^1O_2$ with histidine was monitored spectrophotometrically at 440 nm. The assay mixture contained 45 mM sodium phosphate buffer (pH 7.1), 10 mM histidine, 10 mM NaOCl, 10 mM H_2O_2, 50 mM *N*, *N* dimethyl-*p*-nitrosoaniline, and 0.1 mL of fruit juice. The total reaction volume was 2.0 mL and was incubated at 30 °C for 40 min. The extent of $^1O_2$ production was determined by measuring the decrease in absorbance of *N*, *N* dimethyl-*p*-nitrosoaniline at 440 nm. Relative scavenging efficiency (% inhibition production of $^1O_2$) 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 ($^1O_2$) was measured and used for determining the $^1O_2$ scavenging capacity of fruit juice. The antioxidant capacity of fruit juice against $^1O_2$ value was expressed as μmol of β-carotene equivalent per gram fresh weight.

HPLC Analysis of Strawberry Anthocyanins and Phenolic Compounds. High-performance liquid chromatography (HPLC) was used to separate and determine individual anthocyanins and phenolic compounds in strawberry tissue samples. Fruit samples of 5 g were extracted twice with 15 mL of acetone using a Polytron (Brinkmann Instruments, Inc., Westbury, NY) for 1 min. Extracts (30 mL) were combined and concentrated to 1 mL using a Buchler Evapomix (Fort Lee, NJ) in a water bath at 35 °C. The concentrated sample was dissolved in 10 mL of acidified water (3% formic acid) and then passed through a C_{18} Sep-Pak cartridge (Waters), which was previously activated with methanol followed by water and then 3% aqueous formic acid. Anthocyanins and other phenolics were adsorbed onto the column while sugars, acids, and other water-soluble compounds were eluted with 10 mL of 3% aqueous formic acid. The anthocyanins and other phenolics were then recovered with 2.0 mL of acidified methanol containing 3% formic acid. The methanolic extract was passed through a 0.45-μm membrane filter (Millipore, MSI, Westboro, MA) and 20 μL was analyzed by HPLC. The samples were analyzed using a Waters (Waters Associated, Millipore, Milford, MA) HPLC system equipped with two pumps (600 E system controller) coupled with a photodiode array detector (Waters 990 series). Samples were injected at ambient temperature (20 °C) onto a reverse-phase NOVA-PAK C_{18} column (150 × 3.9 mm, particle size 4 μm) with a guard column (NOVA-PAK C_{18}, 20 × 3.9 mm, particle size 4 μm) (Sentry guard holder universal). The mobile phase was acidified water containing 2.5% formic acid (A) and acetonitrile (B) in a linear gradient from 5% to 20% B in the first 15 min, followed by a linear gradient from 20 to 30% B for 5 min, then an isocratic mixture for 5 min, followed by a linear gradient from 30 to 90% B for 5 min, and an isocratic mixture for 2 min before returning to the initial conditions. The flow rate was 1.0 mL/min and the wavelengths of detection were set at 320, 350, and 510 nm. Scanning between 240 and 550 nm was performed, and data were collected by the Waters 990 3-D chromatography data system. Retention times and spectra were compared to those of pure standards.

Statistical Analysis. Data were subjected to analysis of variance using NCSS (*16*), and the effect of cultivars and growing temperatures on the values of flavonoid concentrations in strawberry fruit juice and their antioxidant capacity to ROO•, $O_2^{\bullet-}$, H_2O_2, OH•, and $^1O_2$ radicals were evaluated by the Tukey−Kramer multiple-comparison test used in NCSS. Differences at $P < 0.05$ were considered significant.

RESULTS

The effects of growing temperatures during day and night on antioxidant activity against ROO•, $O_2^{\bullet-}$, H_2O_2, OH•, and $^1O_2$ radicals in the juice of two strawberry cultivars (Earliglow and Kent) were significant (Table 1). Strawberry growth under high temperature conditions significantly enhanced fruit ROO• absorbance capacity, as well as $O_2^{\bullet-}$, H_2O_2, OH•, and $^1O_2$. Plants grown in the cool day and night temperatures (18/12 °C) generally had the lowest antioxidant capacity. In strawberry, the antioxidant capacity values against ROO• ranged from 12.6 to 19.8 μmol Trolox/g fresh wt. and $O_2^{\bullet-}$ values ranged from 4.24 to 4.95 μmol α-tocopherol/g fresh wt. The antioxidant capacity values against H_2O_2, OH•, and $^1O_2$ values ranged from 2.16 to 3.48 μmol ascorbate/g fresh wt., 4.19 to 6.32 μmol chlorogenic acid/g fresh wt., and 0.55 to 0.77 μmol β-carotene/g fresh wt., respectively (Table 1). The Kent cv. strawberry had higher values of antioxidant capacity compared to that of Earliglow cv. In both cultivars, an increase in night temperature from 12 to 22 °C, with the day temperature kept constant at 25 °C, resulted in a significant increase in values of antioxidant capacity. The highest day/night temperature (30/22 °C) yielded fruit with the most ROO• absorbance capacity, as well as $O_2^{\bullet-}$, H_2O_2, OH•, and $^1O_2$ absorbance capacity (Table 1).

The HPLC analysis of strawberry fruit juice showed that, in addition to anthocyanins, other phenolic compounds were present in significant amounts (Tables 2 and 3). Compounds such as *p*-coumaroylglucose, dihy-

Table 2. Effect of Plant Growth Temperature on *p*-Coumaroylglucose, Dihydroflavonol, Quercetin 3-Glucoside, Quercetin 3-Glucuronide, Kaempferol 3-Glucoside, and Kaempferol 3-Glucuronide Content (μg/g) in Fruit Juice of Two Strawberry Cultivars[a]

temperature (day/night °C)	cultivar	*p*-coumaroyl glucose[b]	dihydroflavonol[b]	quercetin 3-glucoside and quercetin 3-glucuronide[c]	kaempferol 3-glucoside[c]	kaempferol 3-glucuronide[c]
18/12	Earliglow	28.7 ± 1.4	2.8 ± 0.2	–	1.0 ± 0.04	1.1 ± 0.05
	Kent	30.8 ± 1.2	–	2.2 ± 1.3	1.1 ± 0.02	1.3 ± 0.04
25/12	Earliglow	32.5 ± 1.8	3.2 ± 0.1	–	1.3 ± 0.01	1.8 ± 0.07
	Kent	46.7 ± 2.0	–	3.6 ± 1.2	1.4 ± 0.03	2.0 ± 0.13
25/22	Earliglow	56.0 ± 1.9	3.7 ± 0.3	–	1.5 ± 0.01	2.7 ± 0.25
	Kent	61.5 ± 2.6	–	15.7 ± 2.1	1.6 ± 0.04	2.4 ± 0.16
30/22	Earliglow	65.9 ± 2.4	5.0 ± 0.4	–	1.7 ± 0.02	4.6 ± 0.11
	Kent	73.4 ± 2.7	–	21.4 ± 1.8	1.7 ± 0.01	4.5 ± 0.34
$LSD_{0.05}$		1.78	0.45	1.35	0.07	0.08
significance[d]						
temperature (*T*)		**	**	**	**	**
cultivar (*C*)		**	**	**	ns	ns
(*T* × *C*)		**	**	**	**	**

[a] Data expressed as mean ± SEM. [b] Data expressed as micrograms of *p*-coumaric acid equivalents per gram of fresh weight. [c] Data expressed as micrograms of quercetin 3-glucoside equivalents per gram of fresh weight. [d] ns,**, nonsignificant or significant, respectively, at $p \leq 0.05$.

Table 3. Effect of Plant Growth Temperature on Cyanidin 3-Glucoside, Pelargonidin 3-Glucoside, Pelargonidin 3-Rutinoside, Cyanidin 3-Glucoside-Succinate, and Pelargonidin 3-Glucoside-Succinate Content (μg/g) in Fruit Juice of Two Strawberry Cultivars[a,b]

temperature (day/night °C)	cultivar	cyanidin 3-glucoside	pelargonidin 3-glucoside	pelargonidin 3-rutinoside	cyanidin 3-glucoside-succinate	pelargonidin 3-glucoside-succinate
18/12	Earliglow	18.5 ± 1.3	291.3 ± 18.5	–	6.1 ± 0.9	62.2 ± 1.2
	Kent	28.1 ± 2.4	363.6 ± 13.1	24.7 ± 1.5	8.4 ± 0.3	60.8 ± 0.9
25/12	Earliglow	25.5 ± 2.1	445.3 ± 16.2	–	8.9 ± 0.4	97.7 ± 2.3
	Kent	30.2 ± 1.9	478.4 ± 15.8	41.3 ± 2.1	12.2 ± 1.5	103.4 ± 1.8
25/22	Earliglow	27.6 ± 1.7	506.8 ± 23.2	–	9.9 ± 0.8	134.8 ± 2.1
	Kent	31.4 ± 2.3	670.2 ± 32.1	43.8 ± 1.2	13.7 ± 1.1	166.5 ± 5.7
30/22	Earliglow	40.8 ± 2.2	782.7 ± 27.4	–	12.8 ± 0.7	244.0 ± 8.5
	Kent	45.8 ± 2.4	945.1 ± 31.7	50.9 ± 2.3	19.8 ± 1.3	224.5 ± 4.2
$LSD_{0.05}$		2.84	31.2	2.21	2.19	19.8
significance[c]						
temperature (*T*)		**	**	**	**	**
cultivar (*C*)		**	**	**	**	**
(*T* × *C*)		**	**	**	**	**

[a] Data expressed as mean ± SEM. [b] Data expressed as micrograms of cyanidin 3-glucoside equivalents per gram of fresh weight. [c] **, Significant at $p \leq 0.05$.

droflavonol, quercetin 3-glucoside, 3-glucuronide, kaempferol 3-glucoside, and kaempferol 3-glucuronide were detected (Table 2). The presence of *p*-coumaroylglucose in Kent and Earliglow fruit juice ranged from 28.7 to 73.4 μg/g fresh wt. Dihydroflavonol (2.8−5.0 μg/g fresh wt.) was found only in Earliglow fruit, whereas quercetin 3-glucoside and quercetin 3-glucuronide (2.2−21.4 μg/g fresh wt.) occurred only in Kent fruit. The content of flavonols such as kaempferol 3-glucoside and kaempferol 3-glucuronide in fruit of both cultivars ranged from 1.0 to 1.7, and 1.1 to 4.6 μg/g fresh wt., respectively. Earliglow and Kent fruit contained four anthocyanins: cyanidin 3-glucoside (18.5 to 45.8 μg/g fresh wt.), pelargonidin 3-glucoside (291.3 to 945.1 μg/g fresh wt.), cyanidin 3-glucoside-succinate (6.1 to 19.8 μg/g fresh wt.), and pelargonidin 3-glucoside-succinate (60.8 to 244.0 μg/g fresh wt.) (Table 3). In addition, pelargonidin 3-rutinoside (24.7 to 50.9 μg/g fresh wt.) was found only in Kent strawberry juice. Pelargonidin-based anthocyanins such as pelargonidin 3-glucoside and pelargonidin 3-glucoside-succinate, were predominant anthocyanins in the strawberry juice of Kent and Earliglow. The content of cyanidin-based anthocyanins, cyanidin 3-glucoside and cyanidin 3-glucoside-succinate, in fruit was much lower than that of pelargonidin-based anthocyanins (Table 3).

High growing temperatures (25 and 30 °C) significantly enhanced the content of flavonoids. Fruit from plants grown in the cool day and night temperature (18/12 °C) treatment generally had the lowest phenolic acid, flavonols, and anthocyanins. An increase in night temperature from 12 to 22 °C, with the day temperature kept constant at 25 °C, resulted in a significant increase in the content of flavonoids in the fruit of Earliglow and Kent. The highest day/night temperature (25 and 30 °C) yielded fruit with the highest amounts of phenolic acid, flavonols, and anthocyanins (Tables 2 and 3). Kent strawberry had higher values of these components compared to those of Earliglow.

DISCUSSION

Growth temperatures affected strawberry fruit antioxidant capacity. The changes of color in strawberries have been attributed to many factors, including maturity, genotype or cultivar, methods of harvesting and handling, cultural practices, and environmental factors (*17*). Higher growth temperatures caused development of fruit color more rapid than that at lower growth temperatures. When the day/night temperature became warmer, the fruit surface and flesh colors became redder and darker (*18*), and their antioxidant content signifi-

cantly increased. Fruit of Kent grown in day/night temperature 30/22 °C had the highest percent inhibition of the free radicals $O_2^{\bullet -}$, H_2O_2, $OH^{\bullet}$, and $^1O_2$, whereas Earliglow fruit grown at 18/12 °C had the lowest capacity for each of these activities. This indicated that the strawberry juice had a remarkably high scavenging activity for chemically generated active oxygen species. For example, with 100 g of juice from fruit of Earliglow grown in 25/12 °C (day/night), the antioxidant capacity against $ROO^{\bullet}$, $O_2^{\bullet -}$, H_2O_2, $OH^{\bullet}$, and $^1O_2$ was equal to 375.6 mg of Trolox, 188.9 mg of α-tocopherol, 44.2 mg of ascorbic acid, 155.7 mg of chlorogenic acid, and 33.8 mg of *β*-carotene, respectively.

Acidic compounds incorporating phenolic groups have been repeatedly implicated as active antioxidants. The activity of cinnamic acid derivatives with two hydroxyl groups is superior to that of other phenolic acids with only one free OH group (*25, 26*). *p*-Coumaroylglucose occurs naturally in strawberry and also had antioxidant activity.

The most important single group of phenolics in plants is the flavonoids, which consist mainly of catechins, proanthocyanidins, anthocyanidins and flavons, and flavonols and their glycosides. Although catechins seem to be widely distributed among plants, they are abundant only in tea leaves. Proanthocyanidins are polyflavonoid in nature, consisting of chains of flavan-3-ol units. They are widely distributed in plants such as apple, grape, strawberry, and plum (*19*). Proanthocyanidins have relatively high molecular weights and have the ability to complex strongly with carbohydrates and proteins. The occurrence of *p*-coumaroylglucose, dihydroflavonol, quercetin 3-glucoside, 3-glucuronide, kaempferol 3-glucoside, and kaempferol 3-glucuronide has been detected in strawberries (*19*−*24*). Dihydroflavonol was found in Earliglow but not in Kent fruit. These flavonols are effective antioxidants (*25*). Kaempferol and quercetin are potent quenchers of $ROO^{\bullet}$, O_2, and $^1O_2$ (*26*). Quercetin and other polyphenols have been shown to play a protective role in carcinogenesis by reducing bioavailability of carcinogens (*27*). Quercetin, with 3′,4′-dihydroxy substitution in the B-ring and conjugation between the A- and B-rings, has high antioxidant potential (*28*). Kaempferol has low antioxidant capacity against peroxyl radicals. The antioxidant capacities for quercetin and kaempferol are 3.29 and 2.67, respectively (*29*). Higher growth temperatures significantly enhanced flavonol content in strawberry fruit and juices, and with high flavonol contents also had high antioxidant activities.

Anthocyanins are plant colorants that occur almost universally, and they are largely responsible for the brilliant orange, pink, scarlet, red, mauve, violet, and blue colors of flower petals and fruits of higher plants. The bright, red color of strawberry fruit is due to their anthocyanin contents. Two anthocyanidin glycosides, pelargonidin 3-glucoside and cyanidin 3-glucoside, are almost exclusively responsible for the red color of strawberries (*30*). The antioxidant capacity of anthocyanidin may be one of their most significant biological properties in humans (*7*). It has been shown that anthocyanidins are strong antioxidants with free radical scavenging properties attributed to the phenolic hydroxyl groups attached to ring structures (*31*−*34*). The hydroxyl radical scavenging activities of flavonoids increase with the number of hydroxyl groups substituted on the B-ring, especially at C-3′ (*28*, *35*). A single hydroxy substituent generates little or no antioxidant. All flavonoids, such as cyanidin, with 3′,4′-dihydroxy substitution in the B ring and conjugation between the A- and B-rings, possess antioxidant activity (*36*) and have antioxidant potentials four times that of Trolox (*32*). Flavonoids have been shown to protect against free-radical damage and low-density lipoprotein oxidation, platelet aggregation, and endothelium-dependent vasodilatation of arteries. Epidemiologic studies have shown a correlation between an increased consumption of antioxidants and a reduced risk of cardiovascular disease and certain types of cancer (*29*, *31*, *34*, *37*, *38*). In this study, we found that growth temperatures affected strawberry fruit juice with regard to its scavenging capacity of active oxygen species. Higher temperatures (25 and 30 °C) significantly enhanced $ROO^{\bullet}$ absorbance capacity of strawberry fruit juice, as well as $O_2^{\bullet -}$, H_2O_2, $OH^{\bullet}$, and $^1O_2$ absorbance capacity. Plants grown in cool day and night temperatures (18/12 °C) generally had the lowest antioxidant capacity. One explanation for this difference could be related to different flavonoid concentrations. Fruit of Earliglow and Ken contained phenolic acid (*p*-coumaroylglucose), flavones (dihydroflavonol, quercetin 3-glucoside, quercetin 3-glucuronide, kaempferol 3-glucoside, and kaempferol 3-glucuronide), and anthocyanins (cyanidin 3-glucoside, pelargonidin 3-glucoside, pelargonidin 3-rutinoside, cyanidin 3-glucoside-succinate and pelargonidin 3-glucoside-succinate). These flavonoids are antioxidants with free radical scavenging properties against active oxygen species ($ROO^{\bullet}$, $O_2^{\bullet -}$, H_2O_2, $OH^{\bullet}$, and $^1O_2$). Strawberry growth under high temperature conditions resulted in significantly increased flavonoids content. However, even though antioxidant activity was the highest at 30/22 °C (day/night), this elevated temperature may not be optimal for obtaining the best quality of strawberry fruit (*18*). It has been shown that *p*-coumaroylglucose has lower antioxidant activities against peroxyl radicals than do flavonoids that contain multiple free hydroxyl groups, and that glycosidic flavonoids usually have low antioxidant activities against peroxyl radicals (*25*, *26*). The antioxidant capacities for cyanidin 3-glucoside and pelargonidin 3-glucoside were found to be 2.24 and 1.54, respectively (*29*, *34*).

In summary, strawberry fruit juice contains flavonoids with potent antioxidant properties. Different hydroxylation and glycosylation may modulate their antioxidative properties. Growth temperatures affect their phenolic acid, flavonol, and anthocyanin content and antioxidant capacity. The concentration of flavonoids in strawberry juice was related to antioxidant activity against $ROO^{\bullet}$, $O_2^{\bullet -}$, H_2O_2, $OH^{\bullet}$, and $^1O_2$ radicals. Strawberry growth under high temperature conditions significantly enhanced the content of flavonoids and the antioxidant capacities in the fruit.

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.

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