JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Compost as a Soil Supplement Increases the Level of Antioxidant Compounds and Oxygen Radical Absorbance Capacity in Strawberries

SHIOW Y. WANG* AND HSIN-SHAN LIN†

Fruit Laboratory, Beltsville Agricultural Research Center, Agricultural Research Service,
U.S. Department of Agriculture, Beltsville, Maryland 20705-2350

Compost as a soil supplement significantly enhanced levels of ascorbic acid (AsA) and glutathione (GSH) and ratios of AsA/dehydroascorbic acid (DHAsA) and GSH/oxidized glutathione (GSSG) in fruit of two strawberry (*Fragaria* × *ananassa* Duch.) cultivars, Allstar and Honeoye. The peroxyl radical ($ROO^•$) as well as the superoxide radical ($O_2^{•-}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^•$), and singlet oxygen ($^1O_2$) absorbance capacity in strawberries increased significantly with increasing fertilizer strength and compost use. The planting medium (compost) × fertilizer interaction for phenolics and flavonoids was significant. Fruit from plants grown in full-strength fertilizer with 50% soil plus 50% compost and 100% compost yielded fruit with the highest levels of phenolics, flavonol, and anthocyanin content. A positive relationship between antioxidant activities and contents of AsA and GSH and ratios of AsA/DHAsA and GSH/GSSG existed in fruit of both strawberry cultivars. Correlation coefficients for the content of antioxidant components versus antioxidant activity [against $ROO^•$, $O_2^{•-}$, H_2O_2, $OH^•$, or $^1O_2$] ranged from $r = 0.7706$ for H_2O_2 versus GSH/GSSH in cv. Allstar to $r = 0.9832$ for $O_2^{•-}$ versus total flavonoids in cv. Allstar.

KEYWORDS: Antioxidant; compost; flavonoids; free radical; fruit; strawberry

INTRODUCTION

Strawberries contain significant levels of biologically active components that impart health benefits beyond basic nutrition (*1*). Strawberries are a major source of dietary antioxidants, thus making them effective in inhibiting the oxidation of human low-density lipoproteins and preventing or alleviating various human diseases caused as a result of oxidative stress (*2*).

Composts have been utilized in agriculture as a significant source of organic matter. When mature composts made from feedstock were used, improvements in plant growth were seen, due to higher levels of nutrients and organic matter (*3*, *4*). Composts also hinder growth of plant pathogens (*5*). Composts have been used as transplant media, as mulch, and for the suppression of weed growth (*4*, *5*). Composts have been shown to be beneficial in fruit, vegetable, and ornamental crop production (*4*, *6*). Our previous study (*7*) has shown that compost enhances strawberry (*Fragaria* × *ananassa* Duch.) plant growth (plant dry weight, fruit yield, fruit size), fruit quality, leaf nitrate reductase activity, and chlorophyll content. In addition, use of compost increased levels of organic acids (malic and citric acid), sugars (fructose, glucose, and total sugars), soluble solids content, and titratable acids content in strawberry fruit (*7*). However, no information is available on the effect of composts as soil amendments on flavonoid content and scavenging capacity against active oxygen species of strawberry fruit. The objective of the present study was to evaluate the effect of compost as a soil supplement on antioxidant activity and flavonoid content in strawberry fruit.

MATERIALS AND METHODS

Chemicals. Ascorbate, β-carotene, chlorogenic acid, *p*-coumaric acid, 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB), histidine, hydrogen peroxide (30% w/w), glutathione (oxidized form), glutathione (GSH, reduced form), glutathione reductase (GR), guaiacol, 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). Ethylenediaminetetraacetic acid (EDTA; 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 Treatments. One hundred and twenty plants each of cvs. Allstar and Honeoye strawberry were grown on four soil

---

* Author to whom correspondence should be addressed [telphone (301) 504-5776; fax (301) 504-5062; E-mail wangs@ba.ars.usda.gov].

† Visiting Scientist; current address: TransWorld Institute of Technology, Taiwan.

10.1021/jf030196x

treatments: (a) 100% soil, (b) 50% soil plus 50% sand, (c) 50% soil plus 50% compost (obtained from the USDA Compost Center, Beltsville Agricultural Research Center), and (d) 100% compost. They were planted in pots (15.0 × 12.0 cm, E. C. Geiger, Inc., Harleysville, PA) and grown in the greenhouse. Each soil treatment was subdivided into three groups: (i) control (no fertilizer, water only); (ii) fertilized biweekly with a half strength of Peter's nutrition solution (20/20/20, N/P/K) (50% fertilizer); and (iii) fertilized biweekly with a full strength of Peter's nutrition solution (100% fertilizer). All plants were watered daily, and each group of each cultivar contained 40 plants randomly placed in the greenhouse. Radiation sources in the greenhouse consisted of natural daylight and incandescent lamps, which provided a level of photosynthetically active radiation (PAR) of ∼700−800 μmol m^{-2} s^{-1} for a 14-h photoperiod (6:00 a.m.−8:00 p.m.). Day and night temperatures were approximately 25 and 20 °C, respectively. The plants were grown for six months as described above and then exposed to ambient winter temperatures in Beltsville, MD, in an unheated greenhouse from October to February. Plants were then moved back to a heated greenhouse to force flowering. The firm-ripe fruits were harvested from each cultivar in each group for each treatment during the fruiting stage for chemical analyses.

Fruit Sample Preparation. To prepare the juice samples, three 100-g samples of berries from 10 plants of each cultivar of each treatment were pulverized with a chilled mortar and pestle 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.

Determination of Ascorbic Acid (AsA) and Dehydroascorbic acid (DHAsA). AsA and DHAsA were determined using the methods of Arakawa et al. (*8*) and Nakagawara and Sagisaka (*9*). DHAsA concentrations were estimated from the difference of "total AsA" and "AsA" concentrations. A standard curve in the range 0−10 μmol of AsA or DHAsA was used. The result was expressed as micromoles of AsA or DHAsA per gram of fresh weight.

Measurement of Glutathione (GSH) and Oxidized Glutathione (GSSG). GSH and GSSG were assayed using the method described by Castillo and Greppin (*10*). Total glutathione content was calculated from a standard curve. GSSG was determined by subtraction of GSH from total glutathione in fruit juice. The result was expressed as nanomoles of GSH or GSSG per gram of fresh weight.

Oxygen Radical Absorbance Capacity (ORAC) Assay. ORAC assays for fruit juice were carried out following procedures modified from a method previously described by Cao et al. (*11*). 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 (Shimadzu Scientific Instruments, Columbia, MD) until the fluorescence of the last reading declined to <5% of the first reading. This usually took ∼70 min. 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 on a Trolox equivalents per gram of fresh weight basis (*11*).

Superoxide Radical ($O_2^{\bullet -}$) Assay. The assay for $O_2^{\bullet -}$ was performed 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 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 $O_2^{\bullet -}$ 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 micromoles of α-tocopherol equivalent per gram of fresh weight. The assay for hydrogen peroxide in fruit extract of strawberry was carried out following procedures previously described by Patterson et al. (*14*). This assay measures the direct reaction of hydrogen peroxide and Ti(IV). 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 was expressed as micromoles of ascorbate equivalent per gram of fresh weight.

Hydroxyl Radical ($OH^{\bullet}$) Assay. The assay for $OH^{\bullet}$ was performed using the methods of Richmond et al. (*13*). The $OH^{\bullet}$ in aqueous media is generated through the Fenton reaction. Relative scavenging efficiency (percent inhibition of hydroxylation) of fruit juice was estimated from the difference in absorbance (OD) with and without addition of 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 extract against $OH^{\bullet}$ value was expressed as micromoles of chlorogenic acid equivalent per gram of fresh weight.

Singlet Oxygen ($^1O_2$) Assay. The production of $^1O_2$ by sodium hypochloride and H_2O_2 was quantified by using a spectrophotometric method according to that of Chakraborty and Tripathy (*15*) with minor modifications in which *N*,*N*-dimethyl-*p*-nitrosoaniline was used as a selective scavenger of $^1O_2$ and histidine 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 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 (percent 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 extract. The scavenging capacity of β-carotene at various concentrations (1−10 μg) on $^1O_2$ was measured and used for determining the $^1O_2$ scavenging capacity of fruit extract. The antioxidant capacity of fruit juice against $^1O_2$ was expressed as micromoles of β-carotene equivalent per gram of fresh weight.

High-Performance Liquid Chromatography (HPLC) Analysis of Strawberry Anthocyanins and Phenolic Compounds. HPLC was used to separate and quantify individual anthocyanins and phenolic compounds in strawberry tissue samples. Fruit samples of 5 g were extracted twice with 15 mL of 80% acetone. The mixtures were shaken at 400 rpm at room temperature on an orbital shaker for 30 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, whereas 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 Associates, 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) (Waters Associates). 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 by the Tukey−Kramer multiple-comparison test used in NCSS (*16*). Differences at $P \leq 0.05$ were considered to be significant. Correlation and regression analyses of AsA, GSH, total flavonoid contents, or ratios of AsA/DHAsA and GSH/GSSG versus antioxidant activity [against $ROO^{\bullet}$, $O_2^{\bullet -}$, H_2O_2, $OH^{\bullet}$, or $^1O_2$] in fruit juice were also performed using NCSS (*16*).

Table 1. Levels of Ascorbic Acid, Dehydroascorbic Acid, Reduced Glutathione, and Oxidized Glutathione and Ratios of AsA/DHAsA and GSH/GSSG in Fruit of Two Strawberry Cultivars, Allstar and Honeoye, from Plants Grown in Plots Amended with Compost as a Soil Supplement

fertilizer	plant medium	AsA (μmol/g of fresh wt)	DHAsA (μmol/g of fresh wt)	GSH (nmol/g of fresh wt)	GSSG (nmol/g of fresh wt)	AsA/DHAsA ratio	GSH/GSSG ratio
			Cv. Allstar				
none	100% soil	2.74 ± 0.13	0.23 ± 0.02	71.9 ± 1.2	11.2 ± 2.4	11.9 ± 0.55	6.42 ± 0.11
	50% soil + 50% sand	2.67 ± 0.10	0.21 ± 0.03	70.6 ± 1.4	10.8 ± 1.8	12.7 ± 0.42	6.54 ± 0.12
	50% soil + 50% compost	3.01 ± 0.09	0.21 ± 0.01	81.7 ± 2.3	9.4 ± 1.9	14.3 ± 0.57	8.69 ± 0.15
	100% compost	3.07 ± 0.04	0.20 ± 0.02	80.3 ± 1.9	9.5 ± 1.6	15.4 ± 0.23	8.45 ± 0.22
50% (half strength)	100% soil	2.93 ± 0.08	0.22 ± 0.04	76.5 ± 2.2	11.6 ± 1.8	13.3 ± 0.49	6.59 ± 0.07
	50% soil + 50% sand	2.82 ± 0.12	0.20 ± 0.03	77.6 ± 1.7	12.0 ± 2.3	14.1 ± 0.38	6.47 ± 0.12
	50% soil + 50% compost	3.19 ± 0.07	0.18 ± 0.02	86.2 ± 1.8	9.8 ± 1.7	17.2 ± 0.21	8.92 ± 0.38
	100% compost	3.11 ± 0.08	0.19 ± 0.01	87.4 ± 2.4	9.6 ± 1.5	16.4 ± 0.61	9.10 ± 0.45
100% (full strength)	100% soil	3.16 ± 0.11	0.20 ± 0.02	82.6 ± 1.7	11.4 ± 1.9	15.8 ± 0.34	7.25 ± 0.09
	50% soil + 50% sand	3.01 ± 0.06	0.18 ± 0.01	81.9 ± 2.3	11.2 ± 2.5	16.7 ± 0.19	7.31 ± 0.42
	50% soil + 50% compost	3.39 ± 0.13	0.18 ± 0.03	90.8 ± 2.6	9.1 ± 1.4	18.8 ± 0.21	9.98 ± 0.49
	100% compost	3.38 ± 0.06	0.19 ± 0.01	89.6 ± 2.2	9.4 ± 1.2	17.8 ± 0.32	9.53 ± 0.21
			Cv. Honeoye				
none	100% soil	3.26 ± 0.10	0.28 ± 0.04	67.7 ± 1.6	11.2 ± 2.1	6.79 ± 0.17	6.04 ± 0.11
	50% soil + 50% sand	3.16 ± 0.09	0.22 ± 0.02	62.8 ± 1.3	9.9 ± 1.9	6.08 ± 0.13	6.34 ± 0.06
	50% soil + 50% compost	3.75 ± 0.13	0.18 ± 0.01	70.9 ± 1.2	10.2 ± 1.4	7.81 ± 0.12	6.95 ± 0.04
	100% compost	3.91 ± 0.12	0.21 ± 0.02	72.1 ± 0.8	10.6 ± 0.9	7.52 ± 0.11	6.80 ± 0.08
50% (half strength)	100% soil	3.44 ± 0.07	0.24 ± 0.01	70.8 ± 2.5	10.6 ± 0.8	7.82 ± 0.09	6.68 ± 0.12
	50% soil + 50% sand	3.24 ± 0.12	0.17 ± 0.02	67.7 ± 1.2	10.0 ± 1.2	6.89 ± 0.08	6.77 ± 0.08
	50% soil + 50% compost	3.90 ± 0.09	0.21 ± 0.03	77.4 ± 1.6	10.6 ± 1.5	9.51 ± 0.21	7.30 ± 0.23
	100% compost	3.98 ± 0.11	0.22 ± 0.01	79.4 ± 1.2	11.0 ± 1.4	9.45 ± 0.18	7.22 ± 0.19
100% (full strength)	100% soil	3.65 ± 0.10	0.20 ± 0.02	75.3 ± 1.5	10.5 ± 1.2	9.13 ± 0.15	7.17 ± 0.24
	50% soil + 50% sand	3.60 ± 0.09	0.25 ± 0.01	70.8 ± 1.1	10.0 ± 1.1	8.00 ± 0.12	7.08 ± 0.15
	50% soil + 50% compost	4.03 ± 0.12	0.16 ± 0.03	84.4 ± 2.7	10.4 ± 0.9	11.19 ± 0.25	8.12 ± 0.16
	100% compost	4.10 ± 0.14	0.15 ± 0.01	84.9 ± 2.4	10.0 ± 1.6	11.71 ± 0.32	8.49 ± 0.12
$LSD_{0.05}$		0.32	0.12	8.26	3.1	0.87	0.52
significance[b]							
fertilizer (F)		*	ns	*	ns	*	*
plant medium (M)		*	ns	*	ns	*	*
cultivar (C)		*	ns	*	ns	*	*
F × M		*	ns	*	ns	*	*

[a] Data expressed as mean SEM ($n = 3$). [b] *, ns, significant or nonsignificant, respectively, at $p \leq 0.05$.

RESULTS

Compost as a soil supplement significantly enhanced the levels of AsA and GSH and the ratios of AsA/DHAsA and GSH/GSSG in fruit of two strawberry cultivars, Allstar and Honeoye (**Table 1**). Plants grown in 100% soil and 50% soil plus 50% sand generally had the lowest levels of AsA and GSH and lowest ratios of AsA/DHAsA and GSH/GSSG compared to the other treatments. Cv. Honeoye strawberries had higher values of AsA and GSH and higher ratios of AsA/DHAsA and GSH/GSSG compared to cv. Allstar. In both cultivars, fruit grown in 50% soil plus 50% compost or in 100% compost had higher concentrations of AsA and GSH and higher ratios of AsA/DHAsA and GSH/GSSG (**Table 1**). The planting medium × fertilizer interaction for AsA and GSH contents and ratios of AsA/DHAsA and GSH/GSSG were significant (**Table 1**). Without compost, the addition of fertilizer resulted in an increase of these values. The effects of compost and fertilizer on DHAsA and GSSG concentrations were not significantly different in the fruit of both cultivars.

The effects of compost and fertilizer on oxygen radical absorbance activity against $ROO^{\bullet}$, $O_2^{\bullet -}$, H_2O_2, $OH^{\bullet}$, and $^1O_2$ radicals in the juice of two strawberry cultivars were significant (**Table 2**). Strawberry plants grown with compost and fertilizer had significantly higher fruit $ROO^{\bullet}$ absorbance capacity, as well as $O_2^{\bullet -}$, H_2O_2, $OH^{\bullet}$, and $^1O_2$. Lowest oxygen radical absorbance capacities were observed in 100% soil and in 50% soil plus 50% sand treatments, whereas the higher oxygen radical absorbance capacities were in 50% soil plus 50% compost and in 100% compost. In cv. Allstar strawberries, the antioxidant capacities against $ROO^{\bullet}$, $O_2^{\bullet -}$, H_2O_2, $OH^{\bullet}$, and $^1O_2$ in the fruit grown in 100% of soil without fertilizer were 9.8 μmol of Trolox/g of fresh weight, 2.29 μmol of α-tocopherol/g of fresh weight, 2.36 μmol of ascorbate/g of fresh weight, 3.66 μmol of chlorogenic acid/g of fresh weight, and 0.441 μmol of β-carotene/g of fresh weight, respectively (**Table 2**). Overall, cv. Honeoye had higher values of antioxidant capacity than cv. Allstar. The planting medium × fertilizer interaction for ORAC in both cultivars was significant (**Table 2**). The $ROO^{\bullet}$ absorbance capacity, as well as $O_2^{\bullet -}$, H_2O_2, $OH^{\bullet}$, and $^1O_2$ absorbance capacities in strawberries, increased significantly with increasing strength of fertilizer, regardless of compost use (**Table 2**).

The planting medium × fertilizer interaction for phenolic acids and flavonoids was significant in both strawberry cultivars (**Tables 3** and **4**). Ellagic acid, *p*-coumaroylglucose, dihydroflavonol, and kaempferol 3-glucoside, as well as cyanidin 3-glucoside, pelargonidin 3-glucoside, cyanidin 3-glucoside-succinate, and pelargonidin 3-glucoside-succinate, in strawberry fruit were increased significantly with the addition of compost and fertilizer. Dihydroflavonol was found only in Honeoye fruit (**Tables 3** and **4**). Cv. Honeoye generally had higher values of these components compared to fruit of cv. Allstar. Pelargonidin-based anthocyanins, such as pelargonidin 3-glucoside and pelargonidin 3-glucoside-succinate, were the predominant anthocyanins in the strawberry juice. The content of cyanidin-

Table 2. Antioxidant Activity against Peroxyl Radicals, Superoxide Radicals, Hydrogen Peroxide, Hydroxyl Radicals, and Singlet Oxygen in Fruit of Two Strawberry Cultivars, Allstar and Honeoye, from Plants Grown in Plots Amended with Compost as a Soil Supplement[a]

fertilizer	plant medium	ROO$^\bullet$ ORAC (μmol of TE/g)	$O_2^{\bullet-}$ (μmol of α-tocopherol/g)	H_2O_2 (μmol of ascorbate/g)	OH$^\bullet$ (μmol of chlorogenic acid/g)	$^1O_2$ (μmol of β-carotene/g)[f]
			Cv. Allstar			
none	100% soil	9.8 ± 0.2	2.29 ± 0.12	2.36 ± 0.05	3.66 ± 0.13	0.44 ± 0.01
	50% soil + 50% sand	9.2 ± 0.1	2.14 ± 0.13	2.26 ± 0.12	3.53 ± 0.14	0.38 ± 0.02
	50% soil + 50% compost	12.2 ± 0.2	2.85 ± 0.11	2.78 ± 0.13	4.12 ± 0.12	0.52 ± 0.02
	100% compost	12.4 ± 0.3	2.93 ± 0.09	2.82 ± 0.08	4.25 ± 0.11	0.53 ± 0.03
50% (half strength)	100% soil	11.3 ± 0.2	2.64 ± 0.04	2.80 ± 0.07	3.37 ± 0.09	0.51 ± 0.02
	50% soil + 50% sand	11.7 ± 0.1	2.73 ± 0.07	2.89 ± 0.09	3.66 ± 0.10	0.49 ± 0.01
	50% soil + 50% compost	14.0 ± 0.3	3.28 ± 0.14	3.21 ± 0.05	4.53 ± 0.13	0.65 ± 0.03
	100% compost	13.6 ± 0.2	3.18 ± 0.10	3.24 ± 0.11	4.52 ± 0.19	0.60 ± 0.04
100% (full strength)	100% soil	13.7 ± 0.3	3.21 ± 0.08	3.22 ± 0.12	4.49 ± 0.12	0.62 ± 0.02
	50% soil + 50% sand	13.4 ± 0.2	3.12 ± 0.06	3.19 ± 0.08	4.44 ± 0.08	0.56 ± 0.01
	50% soil + 50% compost	15.1 ± 0.3	3.53 ± 0.11	3.97 ± 0.15	5.02 ± 0.18	0.69 ± 0.03
	100% compost	15.2 ± 0.1	3.61 ± 0.09	3.77 ± 0.09	4.98 ± 0.17	0.71 ± 0.04
			Cv. Honeoye			
none	100% soil	10.9 ± 0.1	2.73 ± 0.06	2.89 ± 0.03	3.39 ± 0.11	0.24 ± 0.02
	50% soil + 50% sand	12.4 ± 0.2	3.12 ± 0.09	2.97 ± 0.01	3.37 ± 0.07	0.21 ± 0.01
	50% soil + 50% compost	13.3 ± 0.3	3.33 ± 0.12	3.10 ± 0.12	3.89 ± 0.10	0.35 ± 0.02
	100% compost	13.9 ± 0.2	3.48 ± 0.13	3.22 ± 0.11	3.91 ± 0.12	0.43 ± 0.03
50% (half strength)	100% soil	12.5 ± 0.1	3.13 ± 0.08	2.89 ± 0.10	3.74 ± 0.09	0.33 ± 0.02
	50% soil + 50% sand	13.1 ± 0.2	3.29 ± 0.04	3.11 ± 0.07	3.85 ± 0.08	0.30 ± 0.01
	50% soil + 50% compost	15.7 ± 0.3	3.93 ± 0.07	3.45 ± 0.13	4.92 ± 0.17	0.49 ± 0.03
	100% compost	15.8 ± 0.1	3.96 ± 0.10	3.28 ± 0.09	4.89 ± 0.12	0.51 ± 0.02
100% (full strength)	100% soil	14.2 ± 0.2	3.56 ± 0.09	3.14 ± 0.07	4.87 ± 0.08	0.41 ± 0.01
	50% soil + 50% sand	14.6 ± 0.2	3.65 ± 0.07	3.09 ± 0.02	5.03 ± 0.09	0.43 ± 0.02
	50% soil + 50% compost	17.3 ± 0.3	4.35 ± 0.11	3.55 ± 0.16	5.56 ± 0.13	0.55 ± 0.01
	100% compost	17.4 ± 0.2	4.37 ± 0.14	3.48 ± 0.14	5.69 ± 0.10	0.56 ± 0.02
$LSD_{0.05}$		0.75	0.15	0.37	0.21	0.05
significance[b]						
	fertilizer (F)	*	*	*	*	*
	plant medium (M)	*	*	*	*	*
	cultivar (C)	*	*	*	ns	*
	F × M	*	*	*	*	*

[a] Data expressed as mean SEM ($n = 3$). [b] *, ns, significant or nonsignificant, respectively, at $p \leq 0.05$.

based anthocyanins, cyanidin 3-glucoside and cyanidin 3-glucoside-succinate, in fruit was much lower than that of pelargonidin-based anthocyanins (**Tables 3** and **4**). Fruit from plants grown in 100% soil and in 50% soil plus 50% sand without the addition of fertilizer had the lowest phenolic acids, flavonols, and anthocyanins. The addition of compost as a soil supplement resulted in an increase in phenolic acid, flavonol, and anthocyanin contents of fruit. Fruit from plants grown in full-strength fertilizer with 50% soil plus 50% compost and with 100% compost yielded fruit with the highest levels of these compounds (**Tables 3** and **4**).

A positive relationship between antioxidant activities and contents of AsA and GSH and ratios of AsA/DHAsA and GSH/GSSG existed in both cultivars of strawberry grown in different mixtures of fertilizer and compost (**Table 5**). Correlation coefficients for the level of antioxidant components versus antioxidant activity (against ROO$^\bullet$, $O_2^{\bullet-}$, H_2O_2, OH$^\bullet$, or $^1O_2$) ranged from $r = 0.7706$ for H_2O_2 versus GSH/GSSH in cv. Allstar to $r = 0.9832$ for $O_2^{\bullet-}$ versus total flavonoids in cv. Allstar (**Table 5**). The addition of compost and/or fertilizer significantly enhanced antioxidant components and flavonoids in strawberry fruit, and high antioxidants and flavonoid content were associated with high antioxidant activity (**Tables 1−5**).

It is important to note that adding compost as a soil supplement had similar effects on antioxidant components as increasing the strength of fertilizer. For example, plants grown in 50% compost and 50% soil with 50% fertilizer in most cases had equivalent or higher levels of antioxidant activity and flavonoid content compared to plants grown in 100% soil with 100% fertilizer alone.

DISCUSSION

Composting is a key technology for recycling and building soil organic matter as part of sustainable agriculture. Composting offers a number of benefits to farmers, including flexible manure management, potential added income, increased odor control, weed control, and reduced pollutants and diseases (*17*). The addition of compost as a soil supplement significantly enhanced contents of AsA and GSH and ratios of AsA/DHAsA and GSH/GSSG in fruit of two strawberry cultivars, Allstar and Honeoye. It is possible that compost causes changes in soil chemical and physical characteristics, increases beneficial microorganisms (*18*), and increases nutrient availability and uptake (*19*), thus favoring plant and fruit growth. Woese et al. (*20*) reported that the optimal conditions for plant growth generally result in the highest levels of antioxidants. Wang and Lin (*7*) also showed that strawberry plants grown with compost had significantly increased contents of N, K, organic acids (malic and citric acid), sugars (fructose, glucose, and total sugars), and soluble solids and increased titratable acidity in fruit of strawberry.

AsA is a water-soluble antioxidant formed from a six-carbon compound derived from glucose and plays a major role in protecting cells and cell components against free radicals and oxidant damage. The antioxidant activity of AsA results from the ease of its loss of electrons, which serves as a reducing agent

Table 3. Ellagic Acid, *p*-Coumaroylglucose, Dihydroflavonol, Kaempferol 3-Glucoside, and Kaempferol 3-Glucuronide Concentrations in Fruit of Two Strawberry Cultivars, Allstar and Honeoye, from Plants Grown in Plots Amended with Compost as a Soil Supplement[a]

fertilizer	plant medium	ellagic acid (μg/g of fresh wt)	*p*-coumaroylglucose (μg of *p*-coumaric acid equiv/ g of fresh wt)	dihydroflavonol (μg of *p*-coumaric acid equiv/g of fresh wt)	kaempferol 3-glucoside (μg of quercetin 3-glucoside equiv/g of fresh wt)	kaempferol 3-glucuronide (μg of quercetin 3-glucoside equiv/ g of fresh wt)
			Cv. Allstar			
none	100% soil	4.72 ± 0.2	12.0 ± 2.3		1.14 ± 0.02	1.31 ± 0.02
	50% soil + 50% sand	4.53 ± 0.1	11.9 ± 1.7		1.11 ± 0.03	1.14 ± 0.03
	50% soil + 50% compost	5.84 ± 0.3	19.9 ± 2.2		1.41 ± 0.01	1.60 ± 0.01
	100% compost	5.58 ± 0.3	21.6 ± 2.0		1.56 ± 0.04	1.89 ± 0.04
50% (half strength)	100% soil	5.36 ± 0.2	24.3 ± 1.9		1.32 ± 0.02	1.50 ± 0.02
	50% soil + 50% sand	4.96 ± 0.3	23.1 ± 1.7		1.26 ± 0.03	1.75 ± 0.01
	50% soil + 50% compost	6.88 ± 0.4	30.1 ± 2.1		1.68 ± 0.01	1.89 ± 0.03
	100% compost	7.16 ± 0.2	29.6 ± 1.3		1.80 ± 0.05	2.06 ± 0.05
100% (full strength)	100% soil	7.04 ± 0.2	25.2 ± 1.2		1.97 ± 0.04	1.84 ± 0.02
	50% soil + 50% sand	7.12 ± 0.1	26.1 ± 1.1		1.89 ± 0.03	1.86 ± 0.02
	50% soil + 50% compost	8.17 ± 0.5	36.3 ± 2.0		2.24 ± 0.03	2.62 ± 0.01
	100% compost	8.25 ± 0.4	38.1 ± 1.5		2.31 ± 0.02	2.32 ± 0.04
			Cv. Honeoye			
none	100% soil	0.80 ± 0.3	30.0 ± 2.3	1.69 ± 0.1	5.64 ± 0.03	2.96 ± 0.02
	50% soil + 50% sand	0.64 ± 0.2	26.7 ± 1.8	1.61 ± 0.1	4.84 ± 0.04	2.65 ± 0.01
	50% soil + 50% compost	1.92 ± 0.4	49.5 ± 2.7	3.65 ± 0.3	7.48 ± 0.05	4.47 ± 0.03
	100% compost	2.15 ± 0.5	47.0 ± 2.5	3.59 ± 0.2	7.53 ± 0.06	4.61 ± 0.04
50% (half strength)	100% soil	2.08 ± 0.2	37.7 ± 1.9	2.15 ± 0.2	6.69 ± 0.04	3.30 ± 0.02
	50% soil + 50% sand	2.24 ± 0.1	39.1 ± 1.7	2.81 ± 0.1	6.84 ± 0.02	3.35 ± 0.01
	50% soil + 50% compost	4.32 ± 0.3	50.1 ± 2.6	4.68 ± 0.3	8.21 ± 0.05	5.74 ± 0.04
	100% compost	3.98 ± 0.2	49.3 ± 2.5	4.74 ± 0.5	8.18 ± 0.06	5.65 ± 0.03
100% (full strength)	100% soil	4.04 ± 0.5	44.7 ± 1.5	3.97 ± 0.6	7.86 ± 0.03	5.21 ± 0.03
	50% soil + 50% sand	4.16 ± 0.2	41.8 ± 1.3	3.88 ± 0.2	7.98 ± 0.02	5.32 ± 0.02
	50% soil + 50% compost	5.80 ± 0.3	68.4 ± 2.6	5.74 ± 0.7	8.84 ± 0.05	6.64 ± 0.05
	100% compost	6.26 ± 0.4	70.5 ± 3.2	5.86 ± 0.6	8.63 ± 0.06	6.49 ± 0.04
$LSD_{0.05}$		0.67	3.27	1.15	0.07	0.08
significance[b]						
	fertilizer (F)	*	*	*	*	*
	plant medium (M)	*	*	*	*	*
	cultivar (C)	*	*	*	*	*
	F × M	*	*	*	*	*

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

for many reactive oxidant species, thus making it very effective in biological systems (*21*). The antioxidant activities against $ROO^{\bullet}$, $O_2^{\bullet -}$, H_2O_2, $OH^{\bullet}$, and $^1O_2$ radicals in strawberries positively correlated with ascorbic contents ($r = 0.7898-0.9765$). The close correlations between antioxidant activities and the ratio of AsA/DHAsA ($r = 0.8621-0.9616$) were also evident. Therefore, increased content of AsA and ratio of AsA/DHAsA in fruit of strawberry from plants grown in compost-amended soil may increase the removal of free radicals. Foyer (*21*) reported that the reducing power of AsA is capable of neutralizing the physiologically reactive oxidants and nitrogen species.

Compost as a soil supplement significantly increased GSH concentration and the GSH/GSSG ratio in strawberry fruit. The use of compost can also reduce the amount of fertilizer required for enhancing AsA and GSH contents and ratios of AsA/DHAsA and GSH/GSSG (**Table 1**). GSH is found in very high concentrations in many cells and plays an important role in the stabilization of several enzymes. It converts many oxidants such as H_2O_2 to their oxidized form, a disulfide known as GSSG (*22*). GSH also directly reduces $OH^{\bullet}$ radicals (*23*) and scavenges $^1O_2$ (*24*). It was reported that a high GSH content and high GSH/GSSG ratio are necessary for several physiological functions. These include activation and inactivation of redox-dependent enzyme systems (*25*) and regeneration of cellular antioxidant AsA under oxidative conditions (*21*). Increased GSH and the ratio of GSH/GSSG were associated with increased antioxidant activity in fruit from plants grown from compost-amended soil. The antioxidant activities against $ROO^{\bullet}$, $O_2^{\bullet -}$, H_2O_2, $OH^{\bullet}$, and $^1O_2$ radicals in strawberries positively correlated with GSH contents ($r = 0.8749-0.9745$). Correlations between antioxidant activities and the ratio of GSH/GSSG ($r = 0.7706-0.9527$) were also found.

Strawberry plants grown with composts as soil supplements had significantly increased inhibition of the free radicals $ROO^{\bullet}$, $O_2^{\bullet -}$, H_2O_2, $OH^{\bullet}$, and $^1O_2$ in fruit. This indicated that strawberry fruit grown with compost had high scavenging activity for chemically generated active oxygen species. The $ROO^{\bullet}$ absorbance capacity, as well as $O_2^{\bullet -}$, H_2O_2, $OH^{\bullet}$, and $^1O_2$, in strawberries also increased significantly with increasing strength of fertilizer. Our earlier work showed that fertilizer strength affects strawberry plant growth (*7*) and that the optimal conditions for plant growth generally result in the highest antioxidant capacity (*20*).

Compost and fertilizer significantly enhanced flavonoid content in strawberry fruit. The total flavonoid content positively correlated with antioxidant activities against $ROO^{\bullet}$, $O_2^{\bullet -}$, H_2O_2, $OH^{\bullet}$, and $^1O_2$ radicals in strawberries ($r = 0.8966-0.9832$). Flavonoids consist of flavonols, flavones, flavanones, anthocyanins, catechins, and biflavans. The antioxidant activity of

Table 4. Cyanidin 3-Glucoside, Pelargonidin 3-Glucoside, Cyanidin 3-Glucoside-succinate, and Pelargonidin 3-Glucoside-succinate Concentrations in Fruit of Two Strawberry Cultivars, Allstar and Honeoye, from Plants Grown in Plots Amended with Compost as a Soil Supplement[a]

fertilizer	plant medium	cyanidin 3-glucoside (μg of cyanidin 3-glucoside equiv/g of fresh wt)	pelargonidin 3-glucoside (μg of cyanidin 3-glucoside equiv/g of fresh wt)	cyanidin 3-glucoside-succinate (μg of cyanidin 3-glucoside equiv/g of fresh wt)	pelargonidin 3-glucoside-succinate (μg of cyanidin 3-glucoside equiv/g of fresh wt)
			Cv. Allstar		
none	100% soil	5.6 ± 0.2	281.1 ± 7.5	2.49 ± 0.5	46.7 ± 2.1
	50% soil + 50% sand	4.5 ± 0.1	275.6 ± 13.1	2.10 ± 0.7	45.6 ± 1.9
	50% soil + 50% compost	8.9 ± 0.4	336.5 ± 11.2	3.97 ± 0.8	60.0 ± 1.5
	100% compost	8.8 ± 0.3	339.6 ± 14.1	3.24 ± 0.6	67.4 ± 2.3
50% (half strength)	100% soil	7.6 ± 0.2	321.2 ± 10.0	3.63 ± 0.5	62.8 ± 2.7
	50% soil + 50% sand	8.2 ± 0.1	318.3 ± 12.8	3.58 ± 0.3	62.7 ± 3.1
	50% soil + 50% compost	11.5 ± 0.3	383.2 ± 15.6	4.69 ± 0.8	71.8 ± 2.9
	100% compost	12.1 ± 0.2	375.4 ± 13.7	4.57 ± 0.6	74.2 ± 4.2
100% (full strength)	100% soil	11.9 ± 0.1	355.3 ± 11.3	4.29 ± 0.4	68.7 ± 1.5
	50% soil + 50% sand	12.1 ± 0.2	367.0 ± 15.1	4.14 ± 0.2	70.2 ± 2.2
	50% soil + 50% compost	16.6 ± 0.3	418.8 ± 14.8	5.39 ± 0.3	81.3 ± 5.7
	100% compost	15.9 ± 0.4	425.7 ± 16.2	5.62 ± 0.5	83.9 ± 3.2
			Cv. Honeoye		
none	100% soil	11.0 ± 0.3	570.4 ± 15.4	1.42 ± 0.1	70.4 ± 2.6
	50% soil + 50% sand	12.6 ± 0.5	580.6 ± 16.6	1.31 ± 0.2	70.8 ± 1.3
	50% soil + 50% compost	16.1 ± 0.4	718.0 ± 17.5	2.44 ± 0.2	82.9 ± 1.4
	100% compost	15.7 ± 0.2	723.3 ± 16.8	2.69 ± 0.3	83.7 ± 2.5
50% (half strength)	100% soil	12.8 ± 0.5	658.1 ± 18.1	2.58 ± 0.1	77.4 ± 2.2
	50% soil + 50% sand	14.9 ± 0.9	599.7 ± 17.5	2.35 ± 0.3	75.0 ± 1.3
	50% soil + 50% compost	22.6 ± 5.1	771.4 ± 16.4	3.38 ± 0.2	88.9 ± 6.2
	100% compost	21.1 ± 4.5	768.3 ± 15.6	3.21 ± 0.1	86.8 ± 5.7
100% (full strength)	100% soil	21.2 ± 0.8	713.1 ± 13.2	2.57 ± 0.2	83.1 ± 3.2
	50% soil + 50% sand	20.4 ± 0.5	732.3 ± 15.4	2.49 ± 0.1	80.6 ± 1.5
	50% soil + 50% compost	31.6 ± 4.8	847.1 ± 12.3	3.84 ± 0.6	95.9 ± 4.2
	100% compost	34.1 ± 4.3	833.2 ± 16.7	3.79 ± 0.5	91.3 ± 2.9
$LSD_{0.05}$		4.08	21.1	0.65	7.43
	significance[b]				
	fertilizer (F)	*	*	*	*
	plant medium (M)	*	*	*	*
	cultivar (C)	*	*	*	*
	F × M	*	*	*	*

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

Table 5. Correlation Coefficients (r) between Ascorbic Acid, Reduced Glutathione, and Total Flavonoid Contents or Ratios of AsA/DHAsA and GSH/GSSG and Antioxidant Activity [against Peroxyl Radicals, Superoxide Radicals, Hydrogen Peroxide, Hydroxyl Radicals, or Singlet Oxygen] in Fruit of Two Strawberry Cultivars, Allstar and Honeoye, from Plants Grown in Different Strengths of Fertilizer and Compost

	$ROO^{\bullet-}$ ORAC	$O_2^{\bullet-}$	H_2O_2	$OH^{\bullet}$	$^1O_2$
		Cv. Allstar			
AsA	0.9628	0.9673	0.9388	0.9216	0.9765
GSH	0.9745	0.9731	0.9415	0.9247	0.9574
AsA/DHAsA	0.9619	0.9588	0.9427	0.9418	0.9271
GSH/GSSG	0.8072	0.8118	0.7706	0.8641	0.8033
total flavonoids	0.9827	0.9832	0.9782	0.9121	0.9733
		Cv. Honeoye			
AsA	0.8680	0.8640	0.8519	0.7898	0.9458
GSH	0.9150	0.9144	0.8749	0.8988	0.9442
AsA/DHAsA	0.9219	0.9219	0.8621	0.9273	0.9188
GSH/GSSG	0.9515	0.9527	0.8806	0.9226	0.8937
total flavonoids	0.9476	0.9450	0.8966	0.9082	0.9715

flavonoids may be attributed to the phenolic hydroxy groups attached to ring structures (*26*, *27*). The flavonoids have the ability to scavenge superoxide ($O_2^{\bullet-}$), singlet oxygen ($^1O_2$), and alkyl peroxyl radicals (*28*). Flavonols such as *p*-coumaroylglucose, dihydroflavonol, quercetin 3-glucoside, quercetin 3-glucuronide, kaempferol 3-glucoside, and kaempferol 3-glucuronide found in strawberries are all effective antioxidants (*29*). Kaempferol and quercetin are potent quenchers of $ROO^{\bullet}$, $O_2^{\bullet-}$, and $^1O_2$ (*30*). Quercetin and other polyphenols have been shown to play a protective role in carcinogenesis by reducing bioavailability of carcinogens (*31*). The antioxidant capacities measured by the ORAC assay for quercetin and kaempferol are 3.29 and 2.67, respectively (*32*). In general, flavones have higher antioxidant activities compared to anthocyanins with the same hydroxylation patterns (*33*).

Strawberry plants grown in soil supplemented with fertilizer and compost had significantly enhanced anthocyanin content in their fruit. The increased anthocyanins in strawberry fruit were associated with increased antioxidant capacities, which may allow for quenching of the excited state of active oxygen species. The anthocyanins have high antioxidant capacities, and natural anthocyanins are glycosides that release aglycon forms (anthocyanidins) by hydrolysis (*34*). Some common anthocyanidins have varying hydroxyl or methyl substitutions in their basic structure, flavylium ion, and the common anthocyanins are either 3- or 3,5-glycosylated. In anthocyanins, free radical scavenging properties of the phenolic hydroxyl groups attached to ring structures are responsible for their strong antioxidant properties (*2*, *34*). Anthocyanins have been reported to help reduce damage caused by free radical activity such as low-density lipoprotein oxidation, platelet aggregation, and endothelium-dependent vasodilation of arteries (*1*, *2*).

Collectively, the data presented here suggest that compost supplements can increase the AsA, GSH, flavonol, and anthocyanin concentrations as well as the ratios of AsA/DHAsA and GSH/GSSG in fruit. All of these components had potent antioxidant properties against $ROO^\bullet$, $O_2^{\bullet -}$, H_2O_2, $OH^\bullet$, and $^1O_2$ radicals. Furthermore, use of compost can reduce the need for fertilizer application, because adding compost as a soil supplement had nearly equivalent effects as increasing the strength of fertilizer.

ABBREVIATIONS USED

AAPH, 2′,2′-Azobis(2-amidinopropane) dihydrochloride; AsA, ascorbic acid; DHAsA, dehydroascorbic acid; GR, glutathione reductase; GSH, reduced glutathione; GSSG, oxidized glutathione; ORAC, oxygen radical absorbance capacity; PAR, photosynthetically active radiation; R-PE, (*R*)-phycoerythrin; $ROO^\bullet$, peroxyl radical; $O_2^{\bullet -}$, superoxide radical; H_2O_2, hydrogen peroxide; $OH^\bullet$, hydroxyl radical; $^1O_2$, singlet oxygen; Trolox, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; TE, Trolox equivalents.

LITERATURE CITED

(1) 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.

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

(3) Maynard, A. A. Cumulative effect of annual additions of MSW compost on the yield of field-grown tomatoes. *Compost Sci. Utilization* **1995**, *3* (2), 47−54.

(4) Ozores-Hampton, M. Compost as an alternative weed control method. *HortScience* **1998**, *33*, 938−940.

(5) Roe, N. E. Compost utilization for vegetable and fruit crops. *HortScience* **1998**, *33*, 934−937.

(6) Fitzpatrick, G. E.; Duke, E. R.; Klock-Moore, K. A. Use compost products for ornamental crop production: research and grower experience. *HortScience* **1998**, *33*, 941−944.

(7) Wang, S. Y.; Lin, H. S. Composts as soil supplement enhanced plant growth and fruit quality of strawberry. *J. Plant Nutr.* **2002**, *25*, 2243−2259.

(8) Arakawa, N.; Tsutsumi, K.; Sanceda, N. G.; Kurata, T.; Inagaki, C. A rapid and sensitive method for the determination of ascorbic acid using 4,7-diphenyl-1,10-phenanthroline. *Agric. Biol. Chem.* **1981**, *45*, 1289−1290.

(9) Nakagawara, S.; Sagisaka, S. Increase in enzyme activities related to ascorbate metabolism during cold acclimation of poplar twigs. *Plant Cell Physiol.* **1984**, *25*, 899−906.

(10) Castillo, F. J.; Greppin, H. Extracellular ascorbic acid and enzyme activities related to ascorbic acid metabolism in *Sedum album* L. leaves after ozone exposure. *Environ. Exp. Bot.* **1988**, *28*, 232−233.

(11) Cao, G.; Alessio, H. M.; Culter, R. G. Oxygen-radical absorbance capacity assay for antioxidants. *Free Radical Biol. Med.* **1993**, *14*, 303−311.

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

(13) 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.

(14) 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.

(15) 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.

(16) NCSS 97. *Statistical System for Windows*; NCSS Press: Kaysville, UT, 1997.

(17) Northeast Regional Agricultural Engineering Service. *On-farm Composting Handbook*; NRAES-54; Rynk, R., Ed.; Riley Robb Hall, Cooperative Extension: Ithaca, NY, 1992.

(18) Chung, Y. H. A.; Hoitink, J.; Lipps, P. E. Interactions between organic matter decomposition level and soilborne disease severity. *Agric. Ecosyst. Environ.* **1988**, *24*, 183−193.

(19) Fontes, R. R. The effects of amendments on properties of mulch sandy soil and on growth of tomato and cucumber. Ph.D. Dissertation, University of Florida, Gainesville, FL, 1985.

(20) Woese, K.; Lange, D.; Boess, C.; Bögl, K. W. A comparison of organically and conventionally grown foods—results of a review of the relevant literature. *J. Sci. Food Agric.* **1997**, *74*, 281−293.

(21) 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.

(22) 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.

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

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

(25) Ziegler, D. M. Role of reversible oxidation−reduction of enzyme thiols−disulfides in metabolic regulation. *Annu. Rev. Biochem.* **1985**, *54*, 305−329.

(26) Rice-Evans, C. A.; Miller, N. J. Structure-antioxidant activity relationships of flavonoids and isoflavonoids. In *Flavonoids in Health and Disease*; Rice-Evans, C. A., Packer, C., Eds.; Dekker: New York, 1998; pp 199−219.

(27) Shahidi, F.; Wanasundara, P. K. J. Phenolic antioxidants. *Crit. Rev. Food Sci. Nutr.* **1992**, *32*, 67−103.

(28) Jovanovic, S. V.; Steenken, S.; Simic, M. G.; Hara, Y. Antioxidant properties of flavonoids: reduction potentials and electron-transfer reactions of flavonoid radicals. In *Flavonoids in Health and Disease*; Rice-Evans, C. A., Packer, C., Eds.; Dekker: New York, 1998; pp 137−161.

(29) Pratt, D. E. Natural antioxidants from plant material. *ACS Symp. Ser.* **1992**, *507*, 54−71.

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

(31) Starvic, B.; Matula, T. I.; Klassen, R.; Downie, R. H.; Wood, R. J. Effect of flavonoids on mutagenicity and bioavailability of xenobiotics in food. In *Phenolic Compounds in Food and Their Effects on Health II. Antioxidants and Cancer Prevention*; Huang, M. T., Ho, C. T., Lee, C. Y., Eds.; ACS Symposium Series 507; American Chemical Society: Washington, DC, 1992; pp 239−249.

(32) Cao, G.; Sofic, E.; Prior, R. L. Antioxidant and prooxidant behavior of flavonoids: Structure−activity relationships. *Free Radical Biol. Med.* **1997**, *22*, 749−760.

(33) Bors, W.; Heller, W.; Michel, S.; Saran, M. Flavonoids as antioxidants: determination of radical scavenging efficiencies. *Methods Enzymol.* **1990**, *186*, 343−355.

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

Received for review March 27, 2003. Revised manuscript received August 25, 2003. Accepted August 27, 2003.

JF030196X