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Effect of cultural system and storage temperature on antioxidant capacity and phenolic compounds in strawberries

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ABSTRACT

The effects of cultural systems and storage temperatures on antioxidant enzyme activities and non-enzyme antioxidant components in two cultivars ('Earliglow' and 'Allstar') of strawberries were investigated. Fruit samples were hand-harvested from organic and conventional farms in Maryland, USA, and were stored at 10, 5 and 0 °C. The results from this study showed that strawberries grown from organic culture exhibited generally higher activities in antioxidant enzymes. Moreover, the organic culture also produced fruits with higher level of antioxidant contents. Strawberries stored at higher temperature (10 °C) had higher activities of antioxidant enzymes and antioxidant capacities than those stored at lower temperatures (0 or 5 °C), in both organic and conventional cultural systems. In conclusion, strawberries produced from organic culture contained significantly higher antioxidant capacities and flavonoid contents than those produced from conventional culture, and even though low storage temperatures retarded decay, they also reduced the increase in antioxidant activities.

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1. Introduction

Organic products are becoming increasingly popular because of the concerns over environmental contamination and health benefits (Bourn & Prescott, 2002). Previous research has reported that organic fruits and vegetables have higher levels of flavonoids and ascorbic acid (Asami, Hong, Barrett, & Mitchell, 2005; Mitchell et al., 2007). In addition, higher levels of anthocyanins and phenolic compounds, and higher antioxidant capacities were found in organically cultivated blueberries (Wang, Chen, Sciarappa, Wang, & Camp, 2008). Moreover, a higher antiproliferative activity towards cancer cells was found in extracts from organically grown strawberries than conventionally grown (Olsson, Andersson, Oredsson, Berglund, & Gustavsson, 2006). However, other studies have reported that there is not sufficient evidence to make valid conclusions, as the scientific research has not proved that organic foods are superior in nutritional quality and safety (Lairon, 2009; Magkos, Arvaniti, & Zampelas, 2003). Therefore, additional research comparing organic foods with conventional products is needed to aid in further evaluations.

Strawberries are good sources of natural antioxidants, such as flavonoids, vitamins and glutathione (Wang, Cao, & Prior, 1996). Previous research showed that strawberry extracts exhibited a high level of antioxidant capacity against free radical species (Velioglu, Mazza, Gao, & Oomah, 1998). There is strong evidence in the literature linking increased stress with high levels of free radicals, resulting in adverse effects on human health including cancer (Heinonen, Meyer, & Frankel, 1998). Therefore, strawberries may be effective in decreasing the risk of cancer and in preventing various human diseases caused by oxidative stress (Block, Patterson, & Subar, 1992). However, strawberries are highly susceptible to infection by pathogens and spoil rapidly after harvest. Low temperature storage was effective to reduce decay and maintain the acceptable overall quality of strawberries. However, it has been reported that the levels of anthocyanin and aroma compounds in strawberries were lower at lower temperatures than at higher temperature storage (Ayala-Zavalaa, Wang, Wang, & Gonzalez-Aguilarc, 2004). It is not known whether fruit grown from organic and conventional cultures react similarly.

The objectives of this study were to evaluate the effect of the cultural system, organic or conventional, and the storage temperature (0, 5 or 10 °C) on the antioxidant capacity, the antioxidant enzyme and the non-enzyme component in strawberries.

2. Materials and methods

2.1. Source of materials and fruit sample preparation

Strawberries (*Fragaria* × *ananssa* cvs. Earliglow or Allstar) were hand-harvested at commercially mature stage from organic or conventional orchards in Maryland, USA. The organically grown

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strawberries were obtained from an USDA Certified Organic Farm. No synthetic herbicides or insecticides were used. Four weeks before planting, aged composted horse manure, along with granite dust, were applied to the soil. The plants were fertilised in early spring with McGeary Organics general purpose fertilizer (N–P–K, 5–3–4), which was formulated to meet the National Organic Program standards (containing steamed bone meal, feather meal, soybean meal, langbeinite and compost). The water management was done by drip irrigation. Weed control was performed by hand weeding and rototilling. Wood chips and dried straws were applied as mulch to conserve moisture and help control weeds. The conventionally grown strawberries were fertilised with 84 kg of actual nitrogen/hectare, 56 kg of actual phosphorus/hectare, and 56 kg of actual potassium/hectare in early April. An additional 28 kg of nitrogen/hectare were applied later. Herbicides Poast and Herbimax were applied at 2.4 L/hectare, each in mid-April for weed control. Fungicides Switch (0.9 L/hectare) and Captec (0.1 L/hectare) were applied in late April for disease prevention. An insecticide, Lorsban, was applied at 2.4 L/hectare in late April to control root weevils and strawberry clippers. Captec (4.8 L/hectare) along with another fungicide, Elevate, were applied again in mid-May for disease control. An herbicide, Stinger, was used for spot treatment of Canadian thistle throughout the summer, whenever necessary. Weed killers, Select (0.5 L/hectare) and Herbimax (2.4 L/hectare), were applied in mid-May to control weeds. In late May, an insecticide, Brigade (1.8 L//hectare) was applied to control sap beetles and a fungicide, Pristine (1.3 L/hectare) was used to control the fungus diseases. Drip irrigation was applied throughout the season to maintain proper soil moisture.

Fruit samples were selected for uniform size, colour and absence of mechanical damage. Strawberries were placed in plastic trays and stored at 0, 5 or 10 °C. Composite 5-g samples were collected from 20 berries on the 7th day after harvest. Samples were frozen immediately in liquid nitrogen, then stored at −80 °C until analysis.

2.2. Antioxidant enzyme measurements

Five grams of fruit tissue were homogenised in 5 ml 0.1 M Tris-HCl buffer (pH 7.8) containing 2 mM EDTA-Na, and 2 mM dithiothreitol. The homogenate was centrifuged at 25,000*g* for 20 min at 4 °C, and the supernatant was used for the enzymes assays. The glutathione reductase (GR) activity was assayed according to Smith, Vierheller, and Thorne (1988). One unit of GR was defined as a decrease of 0.001 in absorbance per min, at 340 nm, under the assay conditions. The glutathione peroxidase (GSH-POD) activity was determined using the method of Tappel (1978). One unit of GSH-POD was defined as a decrease of 0.001 in absorbance per min, at 340 nm, under the assay conditions.

The ascorbate peroxidase (AsA-POD) was determined according to the method of Amako, Chen, and Asada (1994). One unit of AsA-POD was defined as the amount of enzyme that oxidised 1 μmol ascorbate/min at room temperature. The guaiacol peroxidase (G-POD) activity was assayed using guaiacol as a donor and H_2O_2 as a substrate, according the method of Kochba, Lavee, and Spiege (1997). One unit of POD activity was defined as an increase of 0.001 in absorbance per min, at 470 nm, under the assay conditions. The catalase (CAT) activity was determined by the method of Change and Maehly (1955). One unit of CAT was defined as the amount of enzyme that decomposes 1 μmol of H_2O_2/min at 30 °C. The superoxide dismutase (SOD) activity was determined by the method of Rao, Paliyath, and Ormrod (1996). One unit of SOD activity was defined as the amount of enzyme that caused a 50% inhibition of nitro blue tetrazolium.

The monodehydroascorbate reductase (MDAR) activity was assayed by measuring the rate of NADH oxidation at 340 nm (Nakagawara & Sagisaka, 1984). The reaction mixture contained 50 mM potassium phosphate buffer (pH 7.3), 0.2 mM NADH, 1.0 mM ascorbate, 1.0 unit of ascorbate oxidase, and 0.1 ml crude enzyme extract, in a total volume of 1.0 ml. The reaction was started by adding ascorbate oxidase (from Cucurbita, EC 1.10.3.3). One unit of MDAR was defined as a decrease of 0.001 in absorbance per min, at 340 nm, under the assay conditions.

The dehydroascorbate reductase (DHAR) activity was assayed according to the method of Shigeoka, Nakano, and Kitaoka (1980). One unit of DHAR was defined as a decrease of 0.001 in absorbance per min, at 340 nm, under the assay conditions.

The protein content in the enzyme extracts was determined by the Bradford (1976) method, using bovine serum albumin as a standard. The specific activity of all the enzymes was expressed as units per milligram protein.

2.3. Non-enzyme component measurements

Triplicate strawberry fruit samples of 5 g were homogenised in 8.0 ml ice-cold, degassed 7.5 mM sodium ascorbate solution, with chilled mortar. The homogenate was centrifuged at 30,000*g* for 15 min at 4 °C. The supernatants were used for the glutathione (GSH) and ascorbic acid (AsA) assays. GSH was assayed using the method described by Castillo and Greppin (1988). AsA was determined using the methods of Arakawa, Tsutsumi, Sanceda, Kurata, and Inagaki (1981).

The total anthocyanin contents in fruit juice were determined using the pH differential method (Cheng & Breen, 1991). The absorbance was measured in a Shimadzu spectrophotometer (Shimadzu UV-160) at 510 and 700 nm in buffers at pH 1.0 and 4.5, using $A = [(A_{510} - A_{700})\ \text{pH}\ 1.0 - (A_{510} - A_{700})\ \text{pH}\ 4.5]$. The results were expressed as milligrams of pelargonidin 3-glucoside equivalents per 100 g of fresh weight.

The total phenolic contents in fruit juice extracts were determined with the Folin–Ciocalteu reagent according to the method of Slinkard and Singleton (1977), using gallic acid as a standard. The results were expressed as milligrams of gallic acid equivalent per 100 g of fresh weight.

2.4. Oxygen radical absorbance capacity (ORAC) assay

Three 5-gram composite samples from 30 berries were extracted twice with 25 ml 80% acetone containing 0.2% formic acid. The ORAC assay was carried out according to Huang, Ou, Hampsch-Woodill, Flanagan, and Prior (2002), using a high-throughput instrument platform consisting of a robotic eight-channel liquid handling system.

2.5. Hydroxyl radical scavenging capacity (·OH; HOSC) assay

The HOSC assay was conducted with acetone solutions according to a previously published protocol with some modifications (Richmond, Halliwell, Chauhan, & Darbre, 1981). The assay was carried out using a high-throughput instrument platform consisting of a robotic eight-channel liquid handling system and a 96-well microplate with a FL800 microplate fluorescence reader (Bio-Tek Instruments, Inc., Winooski, VT, USA).

2.6. 2,2-Di (4-tert-octylphenyl)-1-picrylhydrazyl (DPPH) scavenging capacity assay

The method described by Hatano, Kagawa, Yasuhara, and Okuda (1988) was used for determining the antioxidant activity of strawberry extracts on scavenging 2,2-Di (4-tert-octylphenyl)-1-picrylhydrazyl (DPPH) radicals. The decrease in absorbance was measured at 515 nm against a blank without extract, using a spec-

trophotometer. Using a calibration curve with different amounts of DPPH, the ED50 was calculated. The ED50 is the concentration of an antioxidant that is required to quench 50% of the initial DPPH radicals under the experimental conditions given.

2.7. HPLC analysis of strawberry fruit phenolics

High performance liquid chromatography (HPLC) was used to separate and determine individual anthocyanins and phenolic compounds in strawberry fruit tissue. Three 5-gram composite samples from 30 berries were extracted twice with 20 ml 80% acetone containing 0.2% formic acid, using a Polytron homogenizer (Brinkmann Instruments, Inc., Westbury, NY, USA) for 1 min. The extracts (40 ml) were combined and concentrated to 1 ml using a Buchler Evapomix (Fort Lee, NJ, USA) 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 C18 Sep-Pak cartridge (Waters Associated, Millipore, Milford, MA, USA), 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. The anthocyanins and other phenolics were then recovered with 2 ml of acidified methanol containing 3% formic acid. The methanol extract was passed through a 0.45-μm membrane filter (Millipore, MSI, Westboro, MA, USA) and 20 μl were analysed by HPLC. The samples were analysed using a Waters (Waters Associates, Millipore, Milford, MA, USA) 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 C18 column (150 × 3.9 mm, particle size 4 μm) (Waters Associates, Millipore, Milford, MA, USA) equipped with a guard column (NOVA-PAK C18, 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). The flow rate was 1 ml/min, with a gradient profile consisting of A with the following proportions (v/v) of B: 0 min, 3%, 1–10 min, 3–6% B; 10–15 min, 6% B; 15–35 min, 6–18% B; 35–40 min, 18–20% B; 40–45 min, 20–100% B; 45–50 min, 100% B. The phenolic compounds in fruit extracts were identified by their UV spectra, recorded with a photo-diode-array-detector and by chromatographic comparison with authentic markers. The retention times and spectra were compared to those of the pure standards and the results were confirmed by co-injection with authentic standards. Individual flavonols and anthocyanins were quantified by comparison with an external standard of chlorogenic acid, resveratrol, myricetin, quercetin, kaempferol or cyanidin-3-galactoside. Scanning between 250 and 550 nm was performed and data were collected by the Waters 990 3-D chromatography data system.

2.8. Statistical analysis

Experiments were performed using a completely randomized design. All statistical analyses of variance were calculated over two factors, cultural systems and storage temperatures, using the SPSS statistical package (SPSS Inc., Chicago, IL, USA). The main effects were analysed and the means were compared by Duncan's multiple range tests, at a significance level of 0.05.

3. Results and discussion

3.1. Antioxidant enzyme activities

Antioxidant enzymes have the capacity to lower the free radical burden and neutralise excess free radicals created by stress conditions. GR, GSH-POD, SOD, CAT, G-POD, AsA-POD, MDAR, DHAR and the non-enzyme antioxidants, AsA and GSH, were all detected in strawberries.

GR and GSH-POD activities of strawberries grown in organic and conventional culture are shown in Fig. 1A–D. The activities of GR and GSH-POD were generally significantly higher ($p < 0.05$) in organically cultivated strawberries than in conventionally cultivated, for both 'Earliglow' and 'Allstar' cultivars. Higher storage temperature (10 °C) maintained higher activities of GR and GSH-POD than lower storage temperatures (0 and 5 °C) in both cultivars of strawberries. However, no significant difference was found between storage at 0 and 5 °C. GR is a NADPH-dependent enzyme and may be a rate-limiting enzyme for the defense against active O_2 toxicity. GSH-POD may be responsible for scavenging H_2O_2, catalysing the peroxidation of reduced glutathione (GSH), and forming the oxidised disulfide form of glutathione (GSSG) as a product (Gossett, Banks, Millhollon, & Lucas, 1996).

AsA-POD and G-POD activities were higher in 'Earliglow' strawberry than in 'Allstar' strawberry (Fig. 1E–H). Storage temperature played a big role in the changes of both AsA-POD and G-POD activities in both cultivars. The levels of these enzyme activities increased with increasing temperature, and were much higher in the 10 °C storage samples than in the rest of the treatments. The activities of AsA-POD and G-POD were generally significantly higher ($p < 0.05$) in organically cultivated strawberries than in conventionally cultivated. AsA-POD and G-POD are peroxidase enzymes, which can catalyse H_2O_2 oxidoreduction between H_2O_2 and various reductants (Hiraga, Sasaki, Ito, Ohashi, & Matsui, 2001).

As shown in Fig. 2A–D, the activities of CAT and SOD were higher in organically cultivated strawberries than in conventionally cultivated, especially in low temperature storage (5 or 0 °C). In addition, the activities of CAT and SOD in both organically and conventionally cultivated strawberries were higher when stored at 10 °C than at 5 or 0 °C. The SOD is a class of metal-containing proteins, catalysing the dismutation of superoxide radical anions into H_2O_2 and molecular oxygen (Scandalios, 2001).

Apart from the DHAR activity of the Allstar cultivar, there were decreases in the MDAR and DHAR activities for both organic and conventional strawberries after storage for 7 days at 0 and 5 °C. Samples stored at 10 °C had higher levels of MDAR and DHAR activities than those stored at 0 and 5 °C (Fig. 2E–H). The activities of MDAR and DHAR were higher in organically cultivated strawberries than in conventionally cultivated.

Our results showed that strawberries stored at 10 °C had higher antioxidant content and antioxidant enzyme activities than those stored at 0 or 5 °C. However, even though high levels of antioxidant are desirable and beneficial to human health, warm storage temperature is detrimental for the keeping quality of strawberries. Since strawberries are not susceptible to chilling injury, a storage temperature at 0 °C is the optimum storage condition for maintaining the postharvest quality of strawberries (Mitcham, 2002). Therefore, for immediate consumption or short holding period, keeping strawberries at warm temperatures is desirable for obtaining high antioxidants. However, if extending the storage life of the fruit is the goal, keeping the fruit as cold as possible without freezing (e.g. 0 °C) is recommended.

3.2. GSH, AsA, total phenolics and total anthocyanins contents

As shown in Fig. 3, GSH, AsA, total phenolics and total anthocyanins contents, were significantly higher ($p < 0.05$) in organically cultivated strawberries than in conventionally cultivated, for both cultivars. In addition, the strawberries stored at 10 °C had higher level of GSH, AsA, total phenolics and total anthocyanins content than those in strawberries stored at 0 or 5 °C. GSH has an important function in maintaining the cellular redox status (Rennenberg,

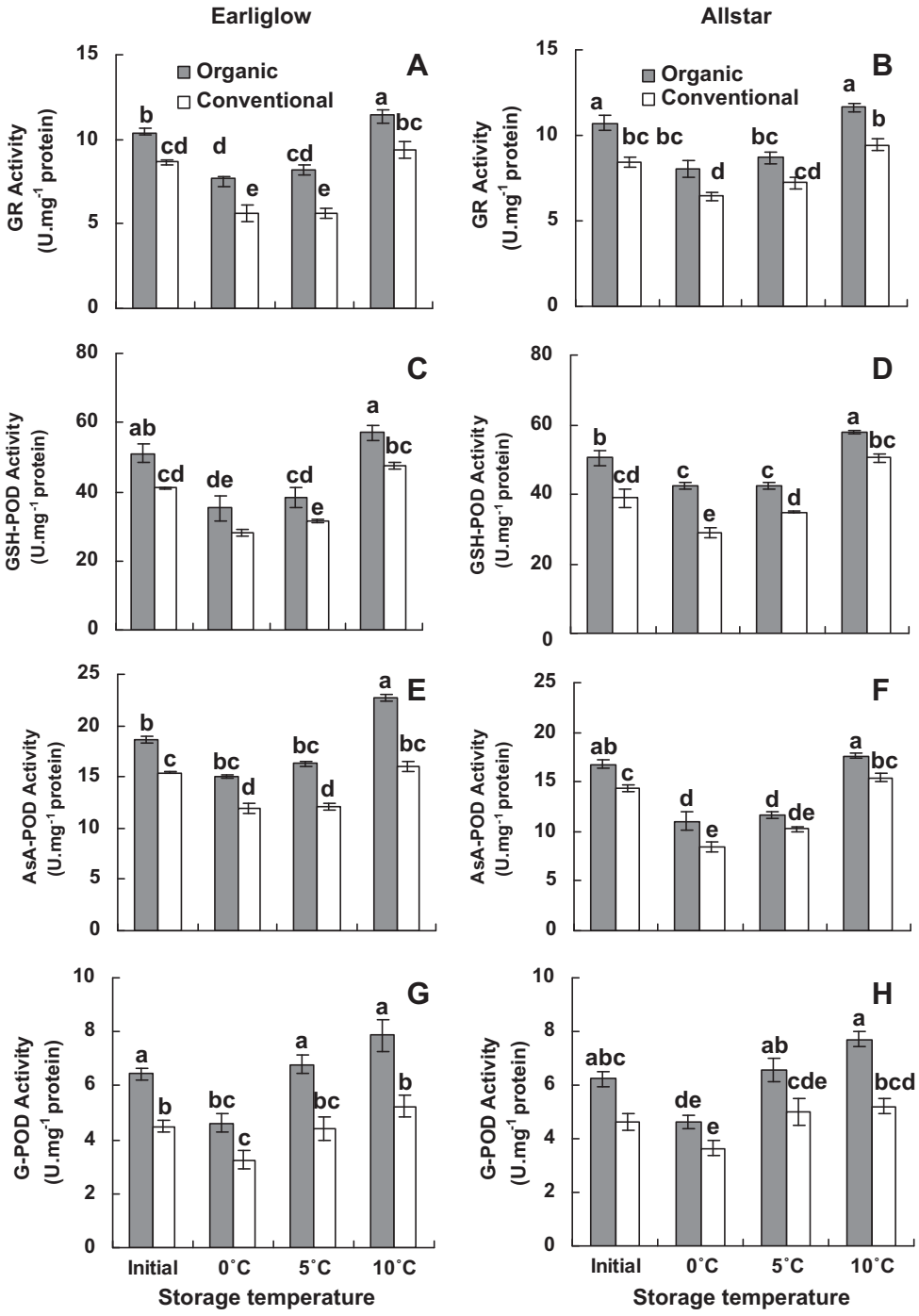

Fig. 1. Effect of the cultivated system and storage temperature on glutathione reductase (GR), glutathione peroxidase (GSH-POD), ascorbate peroxidase (AsA-POD) and guaiacol peroxidase (G-POD) activities of 'Earliglow' or 'Allstar' strawberries, stored for 7 days. Bars represent the standard deviations of triplicate assays. Different letters above the bars indicate the statistically significant difference at $p \leqslant 0.05$.

1980). Ascorbic acid serves as an excellent antioxidant and plays a fundamental role in the removal of hydrogen peroxide and produces DHAsA. Anthocyanins and phenolics are secondary plant metabolites. They protect the plant against damaging photodynamic reactions by quenching the excited state of active oxygen species. Free radical scavenging properties of the phenolic hydroxy groups attached to ring structures, are responsible for the strong antioxidant properties of the anthocyanins (Rice-Evans & Miller, 1996).

3.3. ORAC, .OH, DPPH radicals scavenging capacities

Strawberries not only possess antioxidant activities against DPPH radicals and .OH radicals, but also have the capacity to scavenge oxygen radicals. As shown in Fig. 4, strawberries grown in organic culture had significantly higher ORAC, .OH and DPPH radicals scavenging capacities than those in conventional culture. Similar results were found for blueberry, which indicated that organic cultured blueberry had higher ORAC value (Wang et al., 2008). In

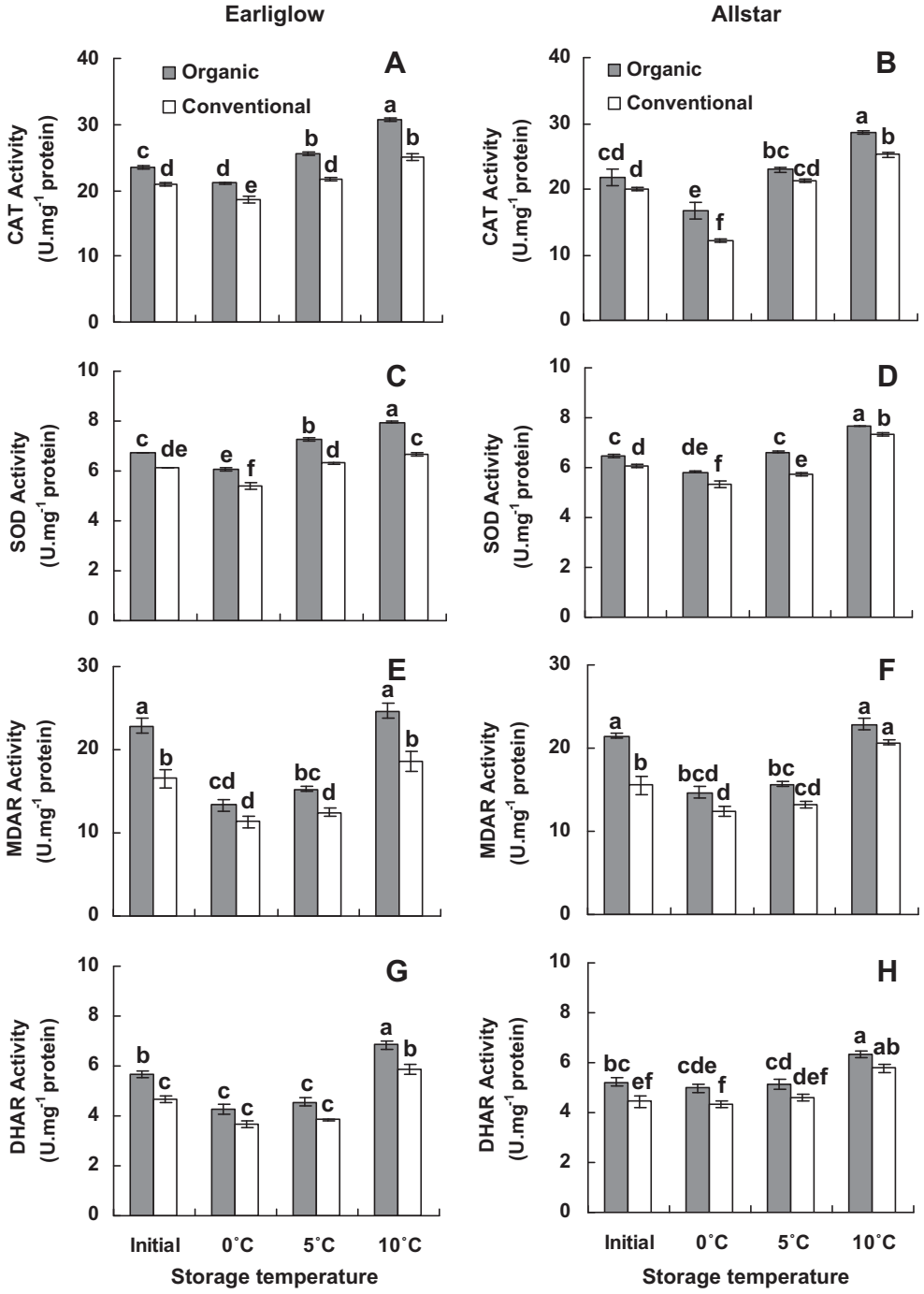

Fig. 2. Effect of the cultivated system and storage temperature on catalase (CAT), superoxide dismutase (SOD), monodehydroascorbate reductase (MDAR) and dehydroascorbate reductase (DHAR) activities of 'Earliglow' or 'Allstar' strawberries, stored for 7 days. Bars represent the standard deviations of triplicate assays. Different letters above the bars indicate the statistically significant difference at $p \leqslant 0.05$.

addition, high temperature storage (10 °C) enhanced the ORAC, **.**OH and DPPH radicals scavenging capacities in both strawberry cultivars.

3.4. HPLC analysis of strawberry phenolics

Phenolic compounds are potent antioxidants that provide health benefits. The flavonoid components and other phenolics in strawberry extracts are shown in Tables 1 and 2. Cyanidin 3-glucoside, petunidin 3-glucoside, cyanidin 3-glucoside-succinate, and petunidin 3-glucoside-succinate, were the predominant anthocyanins, while p-coumaroylglucose, ellagic acid glucoside, quercetin 3-glucoside and kaempferol 3-glucoside, were the predominant phenolics in strawberries. In general, all individual anthocyanins and phenolic compounds were significantly higher in organically cultivated strawberries than in conventionally cultivated, for both 'Allstar' and 'Earliglow' cultivars. In addition, the flavonoid compounds were also affected by storage temperature. The individual anthocyanin contents were significantly higher in strawberries stored at 10 °C than those stored at 0 or 5 °C, and in several cases, for both organic and conventional samples, the anthocyanin contents were higher at 5 °C than at 0 °C. The individual phenolic con-

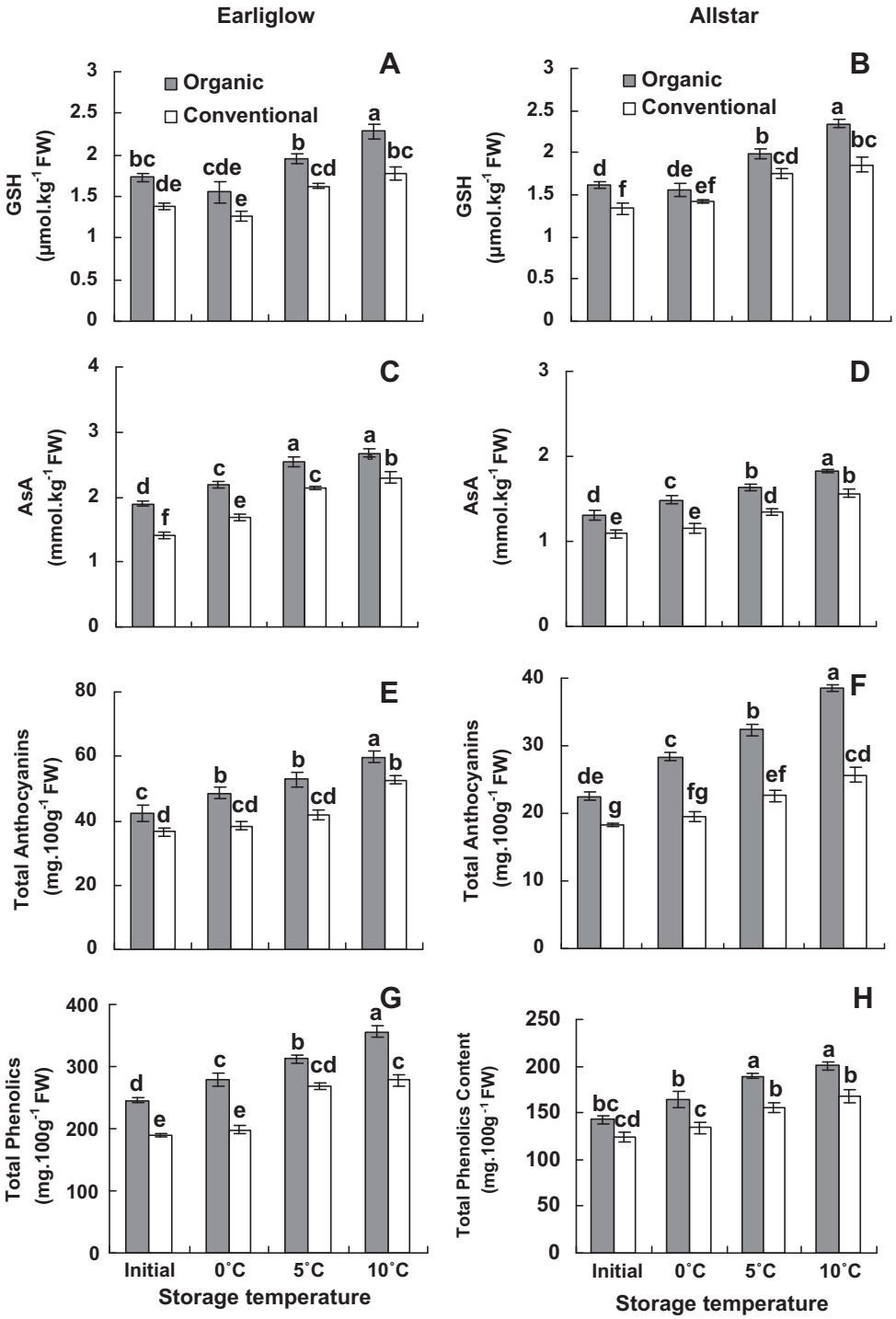

Fig. 3. Effect of the cultivated system and storage temperature on glutathione (GSH), ascorbic acid (AsA), total anthocyanins and total phenolics contents of 'Earliglow' or 'Allstar' strawberries, stored for 7 days. Bars represent the standard deviations of triplicate assays. Different letters above the bars indicate the statistically significant difference at $p \leqslant 0.05$.

tents reacted to the storage temperature in the same way. The antioxidant capacity of flavonoids compounds may be one of their most significant biological properties (Wang et al., 1996). Our data indicated that the cultural systems and storage temperatures significantly affect the antioxidant capacity and the anthocyanin, and phenolic compounds contents of strawberries.

Although there have been reports that no significant differences were found between conventional and organic culture in terms of phytonutrient content, such as vitamin C levels in apple fruits (Weibel, Bickel, Leuthold, & Alfoldi, 2000), many studies have shown that organic products are more nutritious, better tasting, and environmentally friendlier compared to conventionally grown crops (Saba & Messina, 2003). In our study, the results indicated that the organically grown strawberries have a higher antioxidant capacity than the conventional strawberries. In other small fruits, such as blueberries and marionberries, it has been reported that the higher levels of total phenolics were consistently found in organically grown cultivations as compared to those produced by

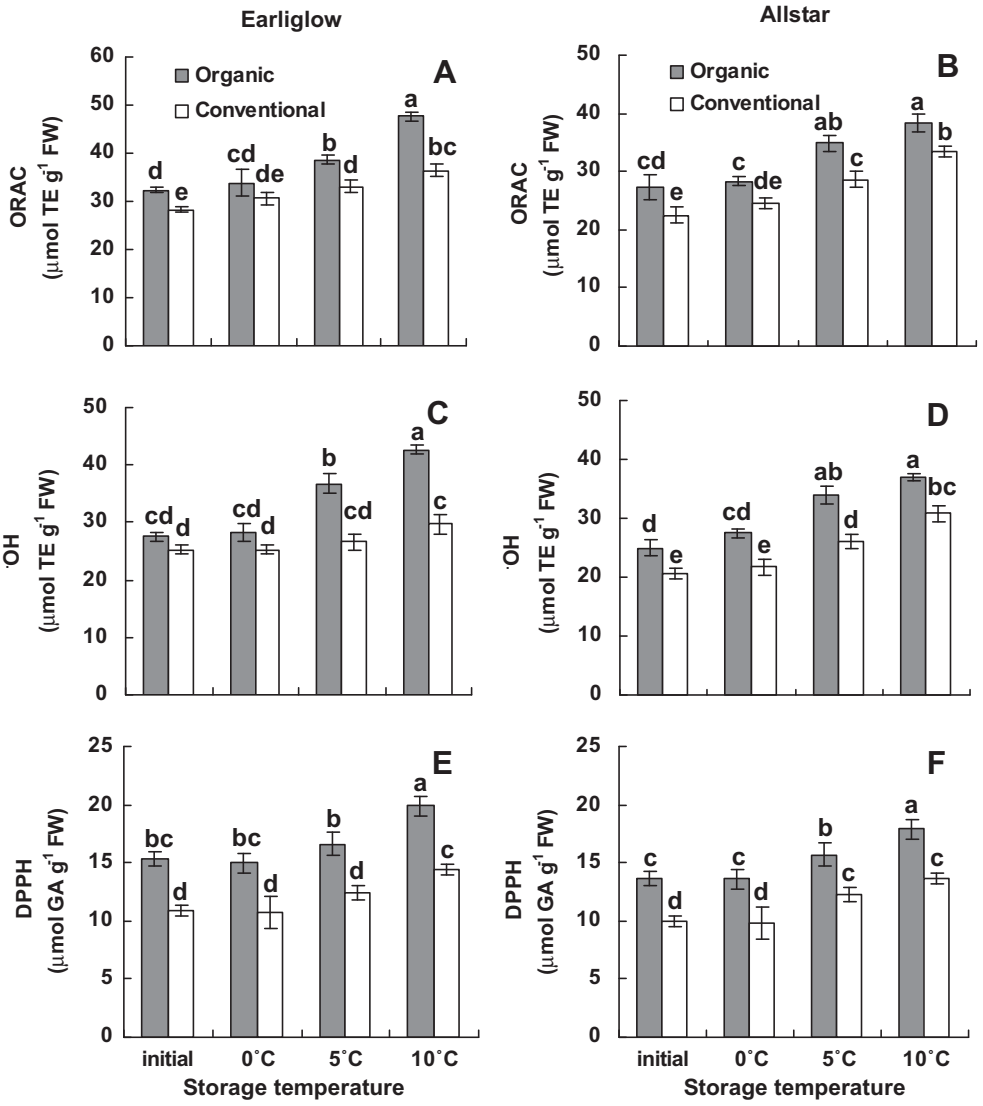

Fig. 4. Effect of the cultivated system and storage temperature on ORAC (A, B), ·OH (C, D) and DPPH (E, F) scavenging capacities of 'Earliglow' or 'Allstar' strawberries, stored for 7 days. Bars represent the standard deviations of triplicate assays. Different letters above the bars indicate the statistically significant difference at $p \leqslant 0.05$.

Table 1
Effect of the cultivation system and storage temperature treatment on anthocyanin contents (μg/g) in strawberries[a].

Strawberry cultivar	Cultural systems	Storage temperature	Cyanidin 3-glucoside	Pelargonidin 3-glucoside	Cyanidin 3-glucoside-succinate	Pelargonidin 3-glucoside-succinate
Allstar	Organic	Initial	21.6 ± 0a	376.5 ± 3.8bc	10.0 ± 0.1bcd	26.3 ± 0.2de
Allstar	Organic	0 °C	14.4 ± 0.9d	363.7 ± 5.0c	9.2 ± 0.4de	26.8 ± 0.2de
Allstar	Organic	5 °C	18.2 ± 0.5bc	392.6 ± 0.1b	10.4 ± 0.2bc	34.1 ± 0.3ab
Allstar	Organic	10 °C	21.6 ± 0.4a	481.6 ± 5.5a	12.6 ± 0.1a	35.7 ± 0.6a
Allstar	Conv.	Initial	16.4 ± 1.1 cd	191.0 ± 7.1f	8.8 ± 0.1e	25.8 ± 1.0ef
Allstar	Conv.	0 °C	9.1 ± 0.6e	176.7 ± 2.4f	7.8 ± 0.5f	23.0 ± 0.3f
Allstar	Conv.	5 °C	15.6 ± 0.6 cd	305.4 ± 3.2e	9.6 ± 0cde	29.1 ± 1.6 cd
Allstar	Conv.	10 °C	19.7 ± 0.9ab	333.0 ± 0d	10.7 ± 0.1b	31.1 ± 1.0bc
Earliglow	Organic	Initial	54.7 ± 1.1b	673.4 ± 5.5bc	19.2 ± 1.2bc	190.6 ± 3.7d
Earliglow	Organic	0 °C	53.7 ± 0.1b	653.4 ± 5.7c	17.6 ± 0.2c	196.8 ± 0.7 cd
Earliglow	Organic	5 °C	58.6 ± 0.3b	697.4 ± 7.7b	18.9 ± 0.1bc	219.9 ± 0.4c
Earliglow	Organic	10 °C	73.9 ± 0.1a	866.9 ± 7.8a	35.0 ± 3.4a	348.3 ± 5.3a
Earliglow	Conv.	Initial	39.1 ± 1.5c	424.5 ± 2.8e	13.6 ± 1.2 cd	136.4 ± 0.4e
Earliglow	Conv.	0 °C	32.4 ± 0.1d	452.8 ± 3.4e	10.6 ± 0.3d	108.3 ± 3.7f
Earliglow	Conv.	5 °C	38.9 ± 2.5c	443.2 ± 12.5e	13.7 ± 0.5 cd	156.9 ± 15.0e
Earliglow	Conv.	10 °C	58.6 ± 1.9b	595.7 ± 9.0d	24.1 ± 1.7b	281.0 ± 10.1b

[a] Data expressed as mean ± SE. Comparisons were made within a cultivar and not between cultivars. Different letters in the same column indicate statistical significant difference at $P \leqslant 0.05$. Data of anthocyanins are expressed as microgram of cyanidin 3-glucoside equivalents per gram of fresh weight.

Table 2
Effect of the cultivation system and storage temperatures treatment on phenolic compounds (μg/g) in strawberries[a].

Strawberry cultivar	Cultural systems	Storage temperature	p-Coumaroyl glucose[b]	Ellagic acid glucoside[b]	Quercetin3-glucoside[c]	Kaempferol 3-glucoside[d]
Allstar	Organic	Initial	27.1 ± 0.8 cd	18.6 ± 0.6ab	12.6 ± 0.3ab	2.4 ± 0.3a
Allstar	Organic	0 °C	26.1 ± 0.3d	16.8 ± 0.3bc	10.4 ± 0bc	1.5 ± 0.2b
Allstar	Organic	5 °C	28.1 ± 0.4b	26.0 ± 0.5a	13.5 ± 1.2a	1.7 ± 0.4b
Allstar	Organic	10 °C	35.2 ± 1.7a	30.9 ± 0.8a	14.7 ± 1.1a	2.7 ± 0.1a
Allstar	Conv.	Initial	16.5 ± 0.3d	14.7 ± 0.4 cd	8.0 ± 0.2 cd	2.1 ± 0ab
Allstar	Conv.	0 °C	15.2 ± 0.2d	14.1 ± 1.6d	7.2 ± 0.1d	1.9 ± 0.1ab
Allstar	Conv.	5 °C	22.2 ± 0d	16.1 ± 2.2 cd	8.6 ± 0.3 cd	1.4 ± 0b
Allstar	Conv.	10 °C	23.9 ± 0.1bc	23.1 ± 1.7c	10.0 ± 0.6c	2.6 ± 0a
Earliglow	Organic	Initial	34.7 ± 1.1bc	18.5 ± 0.5c	33.1 ± 1.6c	2.6 ± 0.1bc
Earliglow	Organic	0 °C	42.9 ± 2.4b	21.4 ± 3.3bc	35.1 ± 2.2bc	2.7 ± 0.4bc
Earliglow	Organic	5 °C	43.8 ± 0.7b	21.4 ± 0.8bc	35.1 ± 2.0bc	3.1 ± 0.1b
Earliglow	Organic	10 °C	59.4 ± 2.8a	28.8 ± 2.4a	48.6 ± 1.5a	3.8 ± 0.1a
Earliglow	Conv.	Initial	25.8 ± 0.2d	12.2 ± 0.3c	31.0 ± 3.2c	1.9 ± 0.1d
Earliglow	Conv.	0 °C	28.2 ± 1.6 cd	13.7 ± 0.7c	28.2 ± 1.0c	2.2 ± 0 cd
Earliglow	Conv.	5 °C	36.7 ± 1.2bcd	17.1 ± 1.0bc	34.2 ± 0.1c	1.9 ± 0.2d
Earliglow	Conv.	10 °C	49.7 ± 0.2b	21.8 ± 0.6b	41.3 ± 0.7b	2.4 ± 0.1 cd

[a] Data expressed as mean ± SE. Comparisons were made within a cultivar and not between cultivars. Different letters in the same column indicate statistical significant difference at $P \leqslant 0.05$.
[b] Data expressed as microgram of ellagic acid equivalents per gram of fresh weight.
[c] Data expressed as microgram of quercetin 3-glucoside equivalents per gram of fresh weight.
[d] Data expressed as microgram of kaempferol 3-glucoside per gram of fresh weight.

conventional agricultural practices (Mitchell et al., 2007; Olsson et al., 2006). It has been hypothesized that because of a tendency to increase the environmental stress on the plant and the activity of phenylalanine ammonialyase (PAL), organic production techniques may cause elevated levels of plant secondary metabolites. The increase of the biologically active phytochemicals in berries, may offer health benefits against several chronic diseases (Young et al., 2005).

It has also been reported that the antioxidant properties of fruits can be influenced by various external factors including the cultural system or the storage temperatures (Ayala-Zavalaa et al., 2004). In our study, the results indicated that strawberries stored at 10 °C had higher antioxidant enzymes activities, higher level of phenolics and anthocyanins contents, and stronger oxygen radical scavenging capacities than those stored at 0 or 5 °C. Similar result was found in a previous study, which indicated that the storage temperature at 10 °C positively enhanced the antioxidant capacity and the production of aroma compounds in strawberries (Ayala-Zavalaa et al., 2004). This suggests that secondary metabolites, such as phenolics or anthocyanins, may be manipulated by the postharvest storage conditions, including the storage temperature.

4. Conclusions

In conclusion, the data presented in this paper indicated that the cultural systems and the storage temperatures significantly affect the antioxidant capacity and flavonoids compounds of strawberries. Strawberries produced from organic culture contained significantly higher level of phytonutrients and antioxidant capacities than those produced from conventional culture. Moreover, a higher storage temperature maintained higher antioxidant capacities and flavonoids compounds than a lower storage temperature.

References

Amako, K., Chen, G. X., & Asada, K. (1994). Separate assay specific for ascorbate peroxidase and guaiacol peroxidase and for the chloroplastic and cytosolic isozymes of ascorbate peroxidase in plants. *Plant Cell Physiology, 35*, 497–504.

Arakawa, N., Tsutsumi, K., Sanceda, N. G., Kurata, T., & Inagaki, C. (1981). A rapid and sensitive method for the determination of ascorbic acid using 4,7-diphenyl-1,10 phenanthroline. *Agricultural and Biological Chemistry, 45*, 1289–1290.

Asami, D. K., Hong, Y. J., Barrett, D. M., & Mitchell, A. E. (2005). Comparison of the total phenolic and ascorbic acid content of freeze-dried and air-dried marionberry, strawberry, and corn grown using conventional, organic and sustainable agricultural practices. *Journal of Agriculture and Food Chemistry, 51*, 1237–1241.

Ayala-Zavalaa, J. F., Wang, S. Y., Wang, C. Y., & Gonzalez-Aguilarc, G. A. (2004). Effect of storage temperatures on antioxidant capacity and aroma compounds in strawberry fruit. *LTW – Food Science and Technology, 37*, 687–695.

Block, G., Patterson, B., & Subar, A. (1992). Fruit, vegetables and cancer prevention: A review of the epidemiological evidence. *Nutrition and Cancer, 18*, 1–29.

Bourn, D., & Prescott, J. A. (2002). Comparison of the nutritional value, sensory qualities, and food safety of organically and conventionally produced foods. *Critical Reviews in Food Science and Nutrition, 42*, 1–34.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle-dye binding. *Analytical Biochemistry, 72*, 248–254.

Castillo, F. J., & Greppin, H. (1988). Extracellular ascorbic acid and enzyme activities related to ascorbic acid metabolism in *Sedum album* L. leaves after ozone exposure. *Environmental and Experimental Botany, 28*, 232–233.

Change, B., & Maehly, A. C. (1955). Assay of catalases and peroxidase. *Methods in Enzymology, 2*, 764–775.

Cheng, G. W., & Breen, P. J. (1991). Activity of phenylalanine ammonialyase (PAL) and concentrations of anthocyanins and phenolics in developing strawberry fruit. *Journal of the American Society for Horticultural Science, 116*, 865–869.

Gossett, D. R., Banks, S. W., Millhollon, E. P., & Lucas, M. C. (1996). Antioxidant response to NaCl-stress in a control and an NaCl-tolerant cotton cell line grown in the presence of paraquat, buthioninsulfoximine, and exogenous glutathione. *Plant Physiology, 112*, 803–806.

Hatano, T., Kagawa, H., Yasuhara, T., & Okuda, T. (1988). Two new flavonoids and other constituents in licorice root: Their relative astringency and radical scavenging effects. *Chemical and Pharmaceutical Bulletin, 36*, 1090–1097.

Heinonen, I. M., Meyer, A. S., & Frankel, E. N. (1998). Antioxidant activity of berry phenolics on human low-density lipoprotein and liposome oxidation. *Journal of Agricultural and Food Chemistry, 46*, 4107–4112.

Hiraga, S., Sasaki, K., Ito, H., Ohashi, Y., & Matsui, H. (2001). A large family of class III plant peroxidases. *Plant Cell Physiology, 42*, 462–468.

Huang, D., Ou, B., Hampsch-Woodill, M., Flanagan, J. A., & Prior, R. L. (2002). High-throughput assay of oxygen radical absorbance capacity (ORAC) using a multichannel liquid handling system coupled with a microplate fluorescence reader in 96-well format. *Journal of Agricultural and Food Chemistry, 50*, 4437–4444.

Kochba, J., Lavee, S., & Spiege, R. P. (1997). Difference in peroxidase activity and isoenzymes in embryogenic and non-embryogenic 'Shamouti' orange ovular callus lines. *Plant Cell Physiology, 18*, 463–467.

Lairon, D. (2009). Nutritional quality and safety of organic food. A review. Agronomy for Sustainable Development doi:10.1051/agro/2009019.

Magkos, F., Arvaniti, F., & Zampelas, A. (2003). Organic food: Nutritious food or food for thought? A review of the evidence. *International Journal of Food Sciences and Nutrition, 54*, 357–371.

Mitcham, E. J. (2002). Strawberry. In: Gross, K. C., Wang, C. Y., & Saltveit, M. (Eds.), The commercial storage of fruits, vegetables, and florist and nursery crops. US Department of Agriculture, Agriculture Handbook 66 on the website of the USDA, Agricultural Research Service, Beltsville Area <http://www.ba.ars.usda.gov/hb66/index.html>.

Mitchell, A. E., Hong, Y. J., Koh, E., Barrett, D. M., Bryant, D. E., Denison, R. F., et al. (2007). Ten-year comparison of the influence of organic and conventional crop management practices on the content of flavonoids in tomatoes. *Journal of Agriculture and Food Chemistry, 55*, 6154–6159.

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

Olsson, M. E., Andersson, C. S., Oredsson, S., Berglund, R. H., & Gustavsson, K. (2006). Antioxidant levels and inhibition of cancer cell proliferation in vitro by extracts from organically and conventionally cultivated strawberries. *Journal of Agriculture and Food Chemistry, 54*, 1248–1255.

Rao, M. V., Paliyath, G., & Ormrod, D. P. (1996). Ultraviolet-B- and ozone-induced biochemical changes in antioxidant enzymes of *Arabidopsis thaliana*. *Plant Physiology, 110*, 125–136.

Rennenberg, H. (1980). Glutathione metabolism and possible biological roles in higher plants. *Phytochemistry, 21*, 2771–2781.

Rice-Evans, C. A., & Miller, N. J. (1996). Antioxidant activities of flavonoids as bioactive components of food. *Biochemical Society Transactions, 24*, 790–795.

Richmond, R., Halliwell, B., Chauhan, J., & Darbre, A. (1981). Superoxide-dependent formation of hydroxyl radicals: Detection of hydroxyl radicals by the hydroxylation of aromatic compound. *Analytical Biochemistry, 118*, 328–330.

Saba, A., & Messina, F. (2003). Attitudes towards organic foods and risk/benefit perception associated with pesticides. *Food Quality Preference, 14*, 637–645.

Scandalios, J. G. (2001). Oxygen stress and superoxide dismutases. *Plant Physiology, 101*, 7–12.

Shigeoka, S., Nakano, Y., & Kitaoka, S. (1980). Metabolism of hydrogen peroxide in *Euglena gracilisz* by L-ascorbic acid peroxidase. *Biochemistry Journal, 186*, 377–380.

Slinkard, K., & Singleton, V. L. (1977). Total phenol analysis: Automation and comparison with manual methods. *American Journal of Enology and Viticulture, 28*, 49–55.

Smith, I. K., Vierheller, T. L., & Thorne, C. A. (1988). Assay of glutathione reductase in crude tissue homogenates using 5,5-dithiobis(2-nitrobenzoic acid). *Analytical Biochemistry, 175*, 408–413.

Tappel, A. L. (1978). Glutathione peroxidase and hydroperoxidase. *Methods in Enzymology, 52c*, 506–513.

Velioglu, Y. S., Mazza, G., Gao, L., & Oomah, B. D. (1998). Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. *Journal of Agricultural and Food Chemistry, 46*, 4113–4117.

Wang, H., Cao, G., & Prior, R. L. (1996). Total antioxidant capacity of fruits. *Journal of Agricultural and Food Chemistry, 44*, 701–705.

Wang, S. Y., Chen, C. T., Sciarappa, W., Wang, C. Y., & Camp, M. J. (2008). Fruit quality, antioxidant capacity, and flavonoid content of organically and conventionally grown blueberries. *Journal of Agriculture and Food Chemistry, 56*, 5788–5794.

Weibel, F. P., Bickel, R., Leuthold, S., & Alfoldi, T. (2000). Are organically grown apples tastier and healthier? A comparative field study using conventional and alternative methods to measure fruit quality. *Acta Horticulturae, 517*, 417–426.

Young, J. E., Zhao, X., Carey, E. E., Welti, R., Yang, S. S., & Wang, W. Q. (2005). Phytochemical phenolics in organically grown vegetables. *Molecular Nutrition and Food Research, 49*, 1136–1142.