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Effect of cultural system and essential oil treatment on antioxidant capacity in raspberries

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ABSTRACT

The effects of cultural system and essential oil treatment on antioxidant capacities in raspberries were evaluated. Raspberries were hand-harvested from organic and conventional farms in Maryland, USA, and were treated with essential oil including carvacrol, anethole, cinnamic acid, perillaldehyde, cinnamaldehyde, and linalool. Results from this study showed that raspberries grown from organic culture exhibited higher value of antioxidant capacities and individual flavonoids contents. Moreover, the organic culture also enhanced the activities of antioxidant enzymes. In addition, essential oil treatments promoted the antioxidant enzymes activities and antioxidant capacities of raspberries, and the most effective compound was perillaldehyde. In conclusion, raspberries produced from organic culture contained significantly higher antioxidant capacities than those produce from conventional culture. Postharvest essential oil treatments have positive effect on enhancing antioxidant capacities in raspberries from both organic and conventional cultures.

1. Introduction

Red raspberry (*Rubus ideaus* L.), a member of the *Rosaceae* family grown as a perennial crop, is popular for its flavour and attractive red colour. The fruit consists of many drupelets and a hollow centre, and is very susceptible to pathogen attack, limiting its postharvest life to 3–5 d under ambient temperature (Chanjirakul, Wang, Wang, & Siriphanich, 2006). Raspberry contains high levels of anthocyanins, flavonoids, and phenolic acids, and is considered a good source of natural antioxidants, which may provide protection against various human diseases caused by oxidative stress (Kahkonen, Hopia, & Heinonen, 2001). Previous studies have reported that the fruit quality and antioxidant capacity in raspberries were affected by pre-harvest or post-harvest factor including light, maturity, or chemical treatments (Wang, 2003; Wang, Chen, & Wang, 2009).

As the increasing concerns over chemical residues and health benefits, organic products are becoming popular, and natural volatile compounds have been tried to replace fungicides. Higher levels of anthocyanins and phenolic compounds, and higher antioxidant capacities were found in organically cultivated strawberries and blueberries (Jin, Wang, Wang, & Zheng, 2011; Olsson, Andersson, Oredsson, Berglund, & Gustavsson, 2006). However, other researchers argued that no sufficient evidence has been obtained to claim that organic foods are superior in nutritional quality and safety (Lairon, 2010). Thus, more study need to be carried out to compare organic foods with conventional products.

Essential oils are aromatic oily extracts obtained from plant materials such as flowers, seeds, leaves, roots, fruits, and other plants parts (Burt, 2004). Various components of essential oils have been identified to be effective in inhibiting microbial growth (Sharma & Tripathi, 2006). Regnier, du Plooy, Combrinck, and Botha (2008) reported that limonene, carvone, and 1,8-cineole were effective against *Botryosphaeria parva* and *Colletotrichum gloeosporioides* in mango fruit. Mari, Leoni, and Cembali (2002) showed that allyl isothiocyanate vapour treatment could control blue mould caused by *Penicillium expansum* in pears. In addition, carvacrol and cinnamic acid have been shown to reduce decay on fresh-cut melon and kiwifruit (Roller & Seedhar, 2002). Moreover, previous studies have shown that treatment with some essential oils increased antioxidant capacities of berry fruits. Strawberries treated with thymol or eugenol maintained higher levels of anthocyanins, flavonoids, and oxygen radical absorbance capacity than untreated fruits (Wang, Wang, Yin, Parry, & Yu, 2007). Linalool, carvacrol, anethole, and perillaldehyde increased anthocyanins, phenolic compounds, and antioxidant activity in blueberries (Wang, Wang, & Chen, 2008). However, it is not known if raspberries would react the same way whether the berries were grown in organic or conventional culture.

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The purpose of this study was to evaluate the effect of cultural system, organic or conventional culture, and essential oil (including carvacrol, anethole, cinnamic acid, perillaldehyde, cinnamaldehyde, and linalool) treatment on the antioxidant enzyme activities, antioxidant capacities, and flavonoids contents in raspberries.

2. Materials and methods

2.1. Source of fruit materials

Red raspberries (*R. ideaus* L., cv. Estes) were hand-harvested at commercially mature stage from organic or conventional orchards near Beltsville, MD. The organically grown raspberries were harvested from an USDA Certified Organic Farm. No synthetic herbicides or insecticides were used. One month before planting, aged composted horse manure along with granite dust were applied to the soil. The plants were fertilized in spring with McGeary Organics general purpose fertilizer (N–P–K, 5–3–4) which was formulated to meet National Organic Program standards (containing steamed bone meal, feather meal, soybean meal, langbeinite, and compost). In summer, approximately six inches of wood chips were applied as a mulch to conserve moisture and help control weeds. Water management was done by drip irrigation. Weed control was performed by hand weeding and rototilling. For insect control, Japanese beetles and other insects were hand-picked from raspberry plants throughout the growing season. In fall, a mineral rock powder known as Planter's II, containing significant quantities of phosphate, calcium, sulphur, magnesium, potassium, and numerous trace minerals including boron, was applied at the rate of 200 lbs/acres. This mineral rock powder was mined in Colorado and pelletized with brewer's yeast and was certified by OMRI for use by organic growers. A light mulch and dried straw were applied in late fall. Each winter, canes were cut and removed or burned. This cane removal helps reduce incidence of cane borer and minimize disease activity. The conventionally grown raspberries were fertilized 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 in late May. Herbicide Poast was applied at 2.4 L hectare^{-1} in mid-May for weed control. Lime sulphur (1 L hectare^{-1}) was applied in early April as fungicide. Additional fungicides Switch (0.9 L hectare^{-1}) and Captan (0.1 L hectare^{-1}) were applied in early July for disease prevention. An insecticides, Capture, was applied at 2.4 L hectare^{-1} in the summer to control insects. An herbicide, Stinger, was used for spot treatment of Canadian thistle throughout the summer whenever necessary. Weed killers, Select (0.5 L hectare^{-1}) and Herbimax (2.4 L hectare^{-1}), were applied in mid-May to control weeds. Drip irrigation was applied throughout the season to maintain proper soil moisture.

2.2. Essential oil treatment and fruit sample preparation

Raspberries sample were selected for uniform size, colour and absence of mechanical damage, and were placed into 1 L polystyrene containers with snap-on lids. Two hundred milligrams of each essential oil including carvacrol, anethole, cinnamic acid, perillaldehyde, cinnamaldehyde, and linalool was put into each of a small beaker and placed in the sealed fruit container. These containers were kept at 10 °C and the essential oils were allowed to vapourise within the containers. Composite five grams of samples were collected from 20 berries on the 7th day after harvest. Samples were frozen immediately in liquid nitrogen and then stored at −80 °C until analysis. Each treatment was replicated three times and the experiment was conducted twice.

2.3. Antioxidant enzyme measurements

Five grams of raspberries tissue were homogenized in 5 ml 0.1 M Tris–HCl buffer (pH 7.8) containing 0.002 M EDTA–Na, and 0.002 M dithiothreitol. The homogenate was centrifuged at 25,000*g* for 20 min at 4 °C, and the supernatant was used for the enzymes assays.

Superoxide dismutase (SOD, EC 1.15.1.1) 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. Catalase (CAT, EC 1.11.1.6) activity was determined by the method of Chance and Maehly (1955). One unit of CAT was defined as the amount of enzyme that decomposes 1 μmol of H_2O_2 min^{-1} at 30 °C. The guaiacol peroxidase (G-POD, EC 1.11.1.7) activity was assayed using guaiacol as a donor and H_2O_2 as a substrate according to the method of Kochba, Lavee, and Spiege-Roy (1977). One unit of G-POD activity was defined as an increase of 0.001 in absorbance per min at 470 nm under the assay conditions. Ascorbate peroxidase (AsA-POD, EC 1.11.1.11) was measured according to the method of Amako, Chen, and Asada (1994). One unit of AsA-POD was defined as the amount of enzyme that oxidized 1 μmol ascorbate min^{-1} at 30 °C. The glutathione reductase (GR, EC1.6.4.2) 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, EC 1.11.1.9) 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 monodehydroascorbate reductase (MDAR, EC 1.6.5.4) activity was assayed by measuring the rate of NADH oxidation at 340 nm (Nakagawara & Sagisaka, 1984). 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, EC 1.8.5.1) activity was assayed according by 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 with Bradford (1976) method, using bovine serum albumin as a standard. Specific activity of all enzymes was expressed as units per milligram protein.

2.4. Total phenolics and total anthocyanins contents measurements

The total phenolics content in raspberry extracts were measured 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 gram of gallic acid equivalent per 100 g of fresh weight. Total anthocyanins contents in raspberry extracts were measured 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 gram of cyanidin-3-glucoside equivalents per 100 g of fresh weight.

2.5. Oxygen radical absorbance capacity (ORAC) assay

Three 5-gram composite samples from 30 raspberries were extracted twice with 25 ml 80% acetone containing 0.2% formic acid. The ORAC assay was used with a high-throughput instrument platform consisting of a robotic eight-channel liquid handling system according to the method of Huang, Ou, Hampsch-Woodill, Flanagan, and Prior (2002). The results were expressed as micromole Trolox equivalents per gram of fresh weight.

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

The HOSC assay was carried out according to the method of Richmond, Halliwell, Chauhan, and Darbre (1981) with some modifications. The assay used 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). The results were expressed as μmol Trolox equivalents per gram of fresh weight.

2.7. 2,2-Di (4-tert-octylphenyl) -1-picrylhydrazyl (DPPH) scavenging capacity assay

The DPPH scavenging capacity assay was carried out according to the method described by Hatano, Kagawa, Yasuhara, and Okuda (1988). The decrease in absorbance was measured at 515 nm against a blank without extract using a FL800 microplate fluorescence reader (Bio-Tek Instruments, Inc., Winooski, VT, USA). The results were expressed as μmol gallic acid equivalents per gram of fresh weight.

2.8. HPLC analysis of raspberry flavonoids

High performance liquid chromatography (HPLC) was used to separate and determine individual phenolic and anthocyanins compounds in raspberry fruit tissue. Three 5-gram composite samples from 20 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 analyzed by HPLC. The samples were analyzed 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) 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% B, 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 photodiode array detector, and by chromatographic comparison with authentic markers. 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 ellagic acid, quercetin, kaempferol, and cyanidin-3-galactoside. Scanning between 250 and 550 nm was performed and data were collected by the Waters 990 3-D chromatography data system.

2.9. Statistical analysis

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

3. Results

3.1. Antioxidant enzymes activities

The antioxidant enzymes activities (including SOD, CAT, G-POD, AsA-POD, GR, GSH-POD, MDAR, and DHAR) of raspberries extracts from 7 days of postharvest storage were shown in Figs. 1 and 2. In general, the activities of all antioxidant enzymes were significantly higher ($p < 0.05$) in organically cultivated raspberries than those in conventionally cultivated. In addition, all essential oil treatments, except for cinnamic acid and linalool treatment, significantly ($p < 0.05$) enhanced the antioxidant enzymes activities of raspberries in both organic and conventional cultivation compared to control. Raspberries extracts from perillaldehyde treatment in organic culture had the highest activities for SOD, GSH-POD, and DHAR, and those from carvacrol, anethole, and perillaldehyde treatment had higher activities for G-POD and MDAR than other treatments. However, little effect in most antioxidant enzymes activities were found between cinnamic acid treatment and control.

3.2. Total phenolics and total anthocyanins contents

As shown in Fig. 3, within each essential oil treatment, the level of total phenolics and total anthocyanins contents were significantly ($p < 0.05$) higher in organically cultivated raspberries than in conventionally cultivated. Essential oil treatments significantly ($p < 0.05$) enhanced the level of total phenolics contents of raspberries in both organic and conventional cultivation compared to control. The raspberry extracts from anethole, perillaldehyde, and cinnamaldehyde treatment had the higher total phenolics contents than those from the control and the other treatments. No significantly differences in the total phenolics contents were found between carvacrol and cinnamic acid treatments. Moreover, the raspberry extracts from anethole and perillaldehyde treatment had the higher total anthocyanins contents than those from the control and the other treatments. No significantly differences in the total anthocyanins contents were found between cinnamic acid and linalool treatments.

3.3. ORAC, ·OH, DPPH radicals scavenging capacities

The antioxidant capacities (including ORAC, ·OH and DPPH radicals scavenging capacities) of raspberry extracts from 7 days of storage are shown in Fig. 4. Raspberries grown in organic culture had significantly ($p < 0.05$) higher ORAC, ·OH and DPPH radicals scavenging capacities than those in conventional culture. All essential oil treatments significantly ($p < 0.05$) enhanced the value of ORAC, ·OH and DPPH radicals scavenging capacities. Raspberry extracts from anethole and perillaldehyde had the higher value of ORAC and ·OH radicals scavenging capacities in organic culture than those from the control and the other treatments. Anethole treatment was the most effective in enhancing the DPPH radicals scavenging capacities than the other treatments.

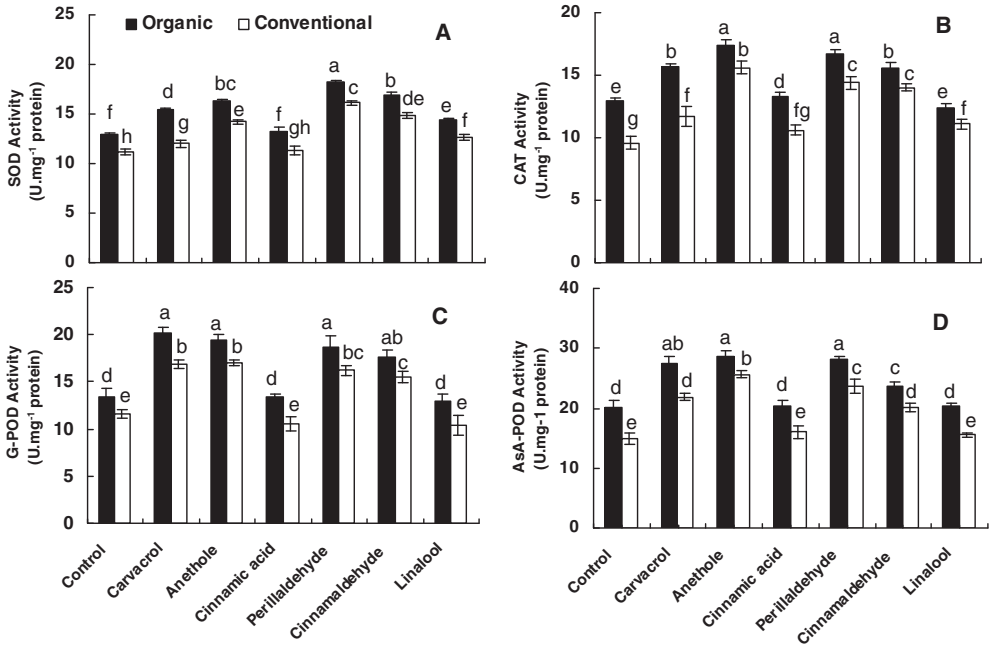

Fig. 1. Effect of the cultural system and essential oil treatment on superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase (G-POD), and ascorbate peroxidase (AsA-POD) activities in raspberries stored for 7 days at 10 °C. Bars represent the standard deviations of triplicate assays. Different letters above the bars indicate the statistically significant difference at $p < 0.05$.

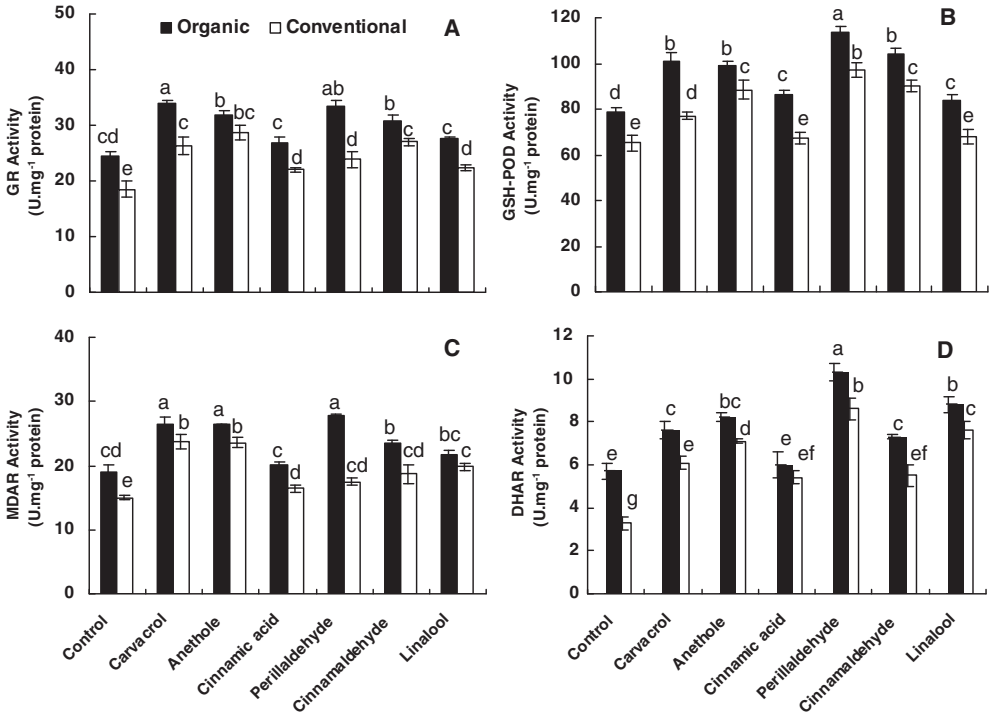

Fig. 2. Effect of the cultural system and essential oil treatment on glutathione reductase (GR), glutathione peroxidase (GSH-POD), monodehydroascorbate reductase (MDAR) and dehydroascorbate reductase (DHAR) activities in raspberries stored for 7 days at 10 °C. Bars represent the standard deviations of triplicate assays. Different letters above the bars indicate the statistically significant difference at $p < 0.05$.

3.4. HPLC analysis of raspberry flavonoids

The individual phenolic and anthocyanin compounds in raspberry extracts were shown in Table 1. Cyanidin 3-sophoroside, cyanidin 3-glucoside, cyanidin 3-glucosylrutinoside, and cyanidin 3-rutinoside were the predominant anthocyanins in raspberries. All individual flavonoids were generally higher in organically cultivated than those in conventionally cultivated raspberries except for quercetin derivative and kaempferol 3-glucoside. Moreover, treatments of anethole and perillaldehyde generally enhanced the level of ellagic acid and most individual anthocyannin compounds in both the organic and conventional raspberries compared

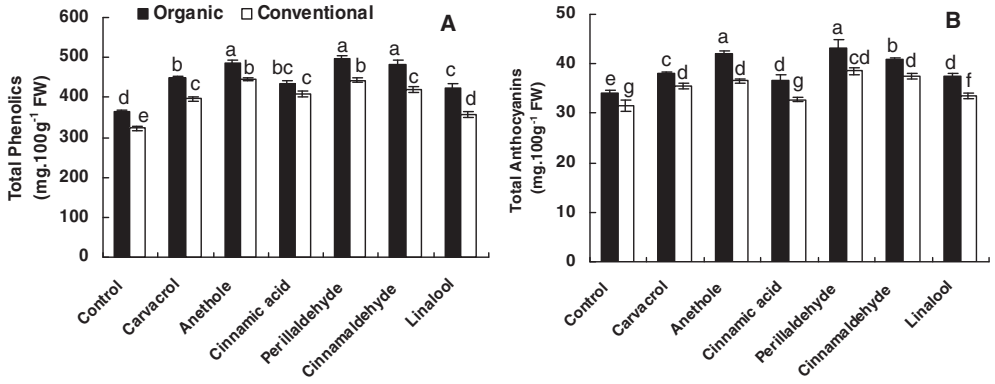

Fig. 3. Effect of the cultural system and essential oil treatment on the total phenolics and total anthocyanins contents in raspberries stored for 7 days at 10 °C. Bars represent the standard deviations of triplicate assays. Different letters above the bars indicate a statistically significant difference at $p < 0.05$.

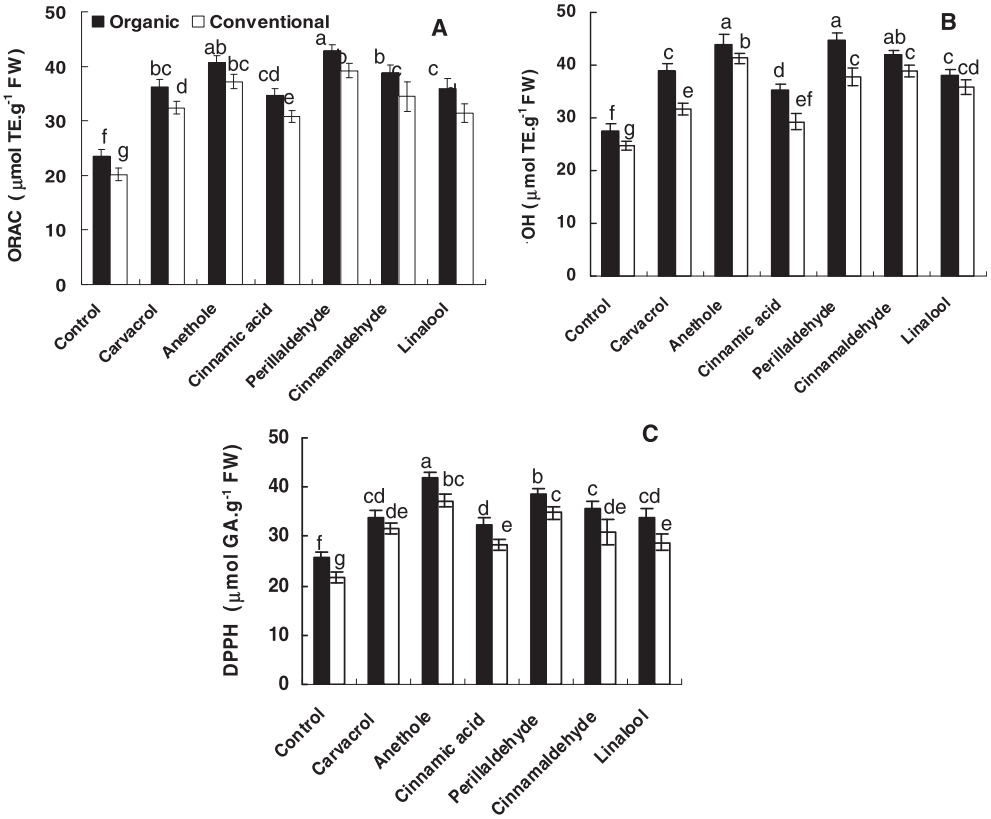

Fig. 4. Effect of the cultural system and essential oil treatment on ORAC, ·OH and DPPH radicals scavenging capacities in raspberries stored for 7 days at 10 °C. Bars represent the standard deviations of triplicate assays. Different letters above the bars indicate a statistically significant difference at $p < 0.05$.

to the control. The raspberry extracts from anethole and perillaldehyde had the higher level of cyanidin 3-sophoroside, cyanidin 3-glucoside, cyanidin 3-glucosylrutinoside, and cyanidin 3-rutinoside in organic culture than those from the control and the other treatments. However, no significant difference was found in the level of quercetin derivative and kaempferol 3-glucoside as affected by the cultural system or the essential oil treatment.

4. Discussion

It is well known that antioxidants, such as anthocyanins, flavonoids, and phenolic acids, can scavenge free radicals, delay lipid oxidation and have health functional properties (Velioglu, Mazza, Gao, & Oomah, 1998). The increase of the biologically active phytochemicals in berries may offer health benefits against several chronic diseases (Young et al., 2005). In the present study, we found that the cultural system significantly affected the antioxidant capacity of raspberries. Previous studies have shown that organic products provide more nutritious, better tasting, and are environmentally friendlier as compared to conventionally grown crops (Saba & Messina, 2003). Our data supported that organically grown raspberries have a higher antioxidant capacity than conventionally grown raspberries. Similar results had been found in other fruits including strawberries, blueberries, and tomatoes (Jin et al., 2011; Mitchell et al., 2007; Wang, Chen, Sciarappa, Wang, & Camp, 2008). It has been hypothesized that because of the tendency to increase environmental stress on the plant organic production techniques, this may induce a higher activity of phenylalanine

Table 1
Effect of the cultural system and essential oil treatment on the anthocyanin and phenolic compounds in raspberries after 7 days of storage at 10 °C.[a]

Essential oil treatment	Cultural system	Ellagic acid[b]	Quercentin 3-glucoside[c]	Quercentin derivative[c]	Kaempferol 3-glucoside[c]	Cyanidin 3-sophoroside[d]	Cyanidin 3-glucoside[d]	Cyanidin 3-glucosylrutinoside[d]	Cyanidin 3-rutinoside[d]
Control	Organic	48.7 ± 0.6d	31.2 ± 1.5bc	8.4 ± 0.4a	9.4 ± 0.8ab	73.4 ± 2.3d	483.6 ± 8.4b	378.3 ± 6.5b	112.8 ± 4.5b
Control	Conv.	42.2 ± 0.5f	28.3 ± 1.2d	7.8 ± 0.6ab	8.6 ± 0.7ab	67.8 ± 3.2e	453.9 ± 9.3c	353.4 ± 7.2d	93.7 ± 3.6d
Carvacrol	Organic	51.3 ± 1.2cd	34.2 ± 1.3ab	8.5 ± 0.5a	10.2 ± 0.8a	84.8 ± 5.1ab	502.3 ± 11.2ab	384.3 ± 8.9ab	118.8 ± 3.5b
Carvacrol	Conv.	46.6 ± 0.8d	30.5 ± 1.8bc	8.1 ± 0.6a	9.6 ± 0.3ab	75.6 ± 2.5cd	480.2 ± 10.3b	363.4 ± 9.1cd	106.3 ± 4.6c
Anethole	Organic	55.7 ± 0.5b	35.2 ± 1.8a	8.8 ± 0.6a	9.8 ± 0.4ab	86.5 ± 3.6a	511.8 ± 8.5a	391.3 ± 7.5a	123.5 ± 5.2ab
Anethole	Conv.	50.3 ± 1.2cd	32.6 ± 1.1b	8.1 ± 0.7a	8.7 ± 0.8a	77.4 ± 2.8c	475.4 ± 10.6bc	375.4 ± 8.8bc	113.8 ± 3.7b
Cinnamic acid	Organic	47.6 ± 1.2d	32.2 ± 1.8b	7.6 ± 0.7ab	9.2 ± 0.6a	75.4 ± 2.2cd	486.5 ± 9.3b	373.5 ± 7.3c	113.3 ± 5.3b
Cinnamic acid	Conv.	44.3 ± 0.8e	29.6 ± 1.5c	7.4 ± 0.6b	8.8 ± 0.7ab	65.8 ± 3.8e	466.4 ± 8.2c	360.8 ± 6.4d	98.4 ± 4.8cd
Perillaldehyde	Organic	58.5 ± 1.4a	35.6 ± 1.7a	9.1 ± 0.8a	9.9 ± 0.7ab	90.3 ± 4.7a	527.5 ± 10.2a	402.4 ± 9.4a	128.6 ± 4.8a
Perillaldehyde	Conv.	54.9 ± 0.9b	31.3 ± 0.8bc	8.3 ± 1.1a	9.3 ± 0.5b	84.7 ± 3.6ab	483.6 ± 9.6b	383.5 ± 8.6ab	115.5 ± 3.5b
Cinnamaldehyde	Organic	52.8 ± 1.0c	36.3 ± 1.3a	8.7 ± 0.8a	9.2 ± 0.6ab	81.6 ± 3.2b	493.5 ± 9.2b	385.8 ± 8.7ab	116.7 ± 5.8b
Cinnamaldehyde	Conv.	48.4 ± 0.8d	32.6 ± 0.8b	8.5 ± 0.6a	8.4 ± 0.7b	73.4 ± 2.7d	472.2 ± 7.8bc	366.3 ± 9.2cd	102.4 ± 4.6c
Linalool	Organic	50.3 ± 1.1c	32.1 ± 1.2b	7.8 ± 0.8ab	9.1 ± 0.5ab	81.3 ± 4.2bc	489.5 ± 8.6b	381.2 ± 7.7b	115.5 ± 3.8b
Linalool	Conv.	46.6 ± 0.8d	29.3 ± 1.3c	7.1 ± 0.6b	8.4 ± 0.6b	72.4 ± 3.5d	468.8 ± 9.4c	364.6 ± 8.3cd	101.6 ± 3.4c

[a] Data expressed as mean ± SD, $n = 3$. Different letters in the same column indicate statistical significant difference at $P \leqslant 0.05$.
[b] Data expressed as micrograms of ellagic acid equivalents per gram of fresh weight.
[c] Data expressed as micrograms of quercentin 3-glucoside equivalents per gram of fresh weight.
[d] Data expressed as micrograms of cyanidin 3-glucoside equivalents per gram of fresh weight.

ammonialyase (PAL) and elevated levels of plant secondary metabolites.

It has also been reported that the antioxidant properties of fruits can be influenced by various external factors including storage temperatures, UV-C illumination and natural volatile compounds vapour (Ayala-Zavala, Wang, Wang, & Gonzalez-Aguilar, 2004; Chanjirakul et al., 2006; Perkins-Veazie, Collins, & Howard, 2008). In our study, the results indicated that raspberries treated with essential oils had higher antioxidant enzymes activities, higher level of phenolics and anthocyanins contents and stronger oxygen radical scavenging capacities than untreated berries for both organically and conventionally grown raspberries. Previous investigations also found similar results suggesting that essential oils, such as eugenol, thymol, and anethole, increased the free radical scavenging capacity and antiproliferative activity in strawberries and blueberries (Wang et al., 2007, 2008). The exact mechanisms for the raspberries treated with essential oils that increase the level of phenol acid and individual flavonoids are not clear. It is hypothesized that essential oils could act as "signaling compounds" that triggers a signal, which resembles a mild stress to the fruit. As a defense response, fruit produce additional phenolic compounds and flavonoids, which increase their antioxidant activities. In our study, we have provided evidence that the essential oils perillaldehyde, anethole, carvacrol, or cinnamaldehyde have a positive effect on enhancing the antioxidant capacities of raspberries.

In general, all essential oils tested in our study delayed the decay development of raspberries compared to control (data not shown). Similar results were also reported by Regnier, Combrinck, du Plooy, and Botha (2010), who found that *Lippia scaberrima* essential oil significantly inhibited the decay of mango and avocado fruit. In addition, essential oil combined with modified atmosphere package (MAP) or edible film coating improved the shelf-life in table grapes and papaya (Bosquez-Molina, Ronquillo-de Jesús, Bautista-Banos, Verde-Calvo, & Morales-López, 2010; Valero et al., 2006). Therefore, techniques combining the use of essential oils and other preservatives or postharvest procedures, such as refrigeration, heat treatment, or high oxygen exposure need to be explored.

The antimicrobial mechanisms or the mode of actions of many essential oils have been well documented; they have been postulated to disrupt the functions of cellular membranes, thus interfering with the active sites of enzymes, and cellular metabolism (Marino, Bersani, & Comi, 2001). Others hypothesized that essential oils may change the permeability of membranes of the microbes for cations and alter the ion gradients that lead to the impairment of vital processes in microbial cells and eventually cell death (Ultee, Kets, & Smid, 1999). In addition, some essential oils, such as carvacrol, anethole, and cinnamic acid could induce constitutive increases in antioxidant titre (enzymatic and nonenzymatic) in plant tissues. Thus, an increase in the antioxidant capacity and free radical scavenging activity would reduce the physiological deterioration and enhance the resistance of tissue against microbial invasion and reduce the spoilage of berry fruit.

In conclusion, this study indicated that cultural systems and essential oil treatment significantly affected the raspberry antioxidant capacity and flavonoids compounds. Red raspberries produced from organic culture contained significantly higher level of phytochemicals and antioxidant capacities than those produced from the conventional culture. Moreover, certain essential oils, such as perillaldehyde, anethole, carvacrol, and cinnamaldehyde have a positive effect on increasing the antioxidant capacities in treated raspberries in both organic and conventional cultures. Thus, these natural products have the potential to preserve the quality of fresh produce.

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