JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Increasing Antioxidant Activity and Reducing Decay of Blueberries by Essential Oils

CHIEN Y. WANG,*,† SHIOW Y. WANG,‡ AND CHITSUN CHEN†

Produce Quality and Safety Laboratory and Genetic Improvement of Fruits and Vegetables Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, Maryland 20705

Several naturally occurring essential oils including carvacrol, anethole, cinnamaldehyde, cinnamic acid, perillaldehyde, linalool, and *p*-cymene were evaluated for their effectiveness in reducing decay and increasing antioxidant levels and activities in 'Duke' blueberries (*Vaccinium corymbosum*). Carvacrol, anethole, and perillaldehyde showed the capability to promote total anthocyanins and total phenolics and enhance antioxidant activity in fruit tissues expressed as oxygen radical absorbance capacity (ORAC) and hydroxyl radical (•OH) scavenging capacity. All of the essential oils tested in this study were able to inhibit fruit decay development to some degree compared to controls. The most effective compound for mold retardation was *p*-cymene, followed by linalool, carvacrol, anethole, and perillaldehyde. Cinnamic acid and cinnamaldehyde also suppressed mold growth, but to a lesser extent. Treatment with carvacrol, anethole, or perillaldehyde also significantly increased the levels of fructose, glucose, and citric acid. Individual flavonoids were variably affected by the essential oils. Levels of chlorogenic acid, which was the major phenolic compound in blueberry fruit, were enhanced by all of the essential oils in this study. Increased amounts of quercetin 3-galactoside and quercetin 3-arabinoside were also found in all treated fruit except samples treated with linalool or *p*-cymene. The major anthocyanin, malvidin 3-galactoside, was enhanced by all essential oils tested except linalool and *p*-cymene. The levels of other individual anthocyanins including petunidin 3-galactoside, delphinidin 3-galactoside, petunidin 3-glucoside, petunidin 3-arabinoside, delphinidin 3-arabinoside, and cyanidin 3-galactoside were higher in treated fruit compared to controls. Those essential oils that have positive effects on enhancing anthocyanins, phenolic compounds, and antioxidant activity of fruit, but inhibitory effects on microbial growth and decay development, deserve further evaluation.

KEYWORDS: Essential oils; antioxidant; anthocyanins; phenolics; blueberries; decay

INTRODUCTION

Essential oils are aromatic oily extracts obtained from plant materials such as buds, flowers, seeds, leaves, barks, roots, fruits, and other parts of the plants (*1*). The antimicrobial properties of essential oils have long been recognized (*2*). Various components of essential oils have been identified to be effective in inhibiting microbial growth (*3*). In our search for natural products to reduce the decay of harvested fruits and vegetables, we have come upon several essential oils that appear to be promising for use in inhibiting the development of pathogenic invasion and spoilage of fresh fruits and vegetables. Treatment of strawberries with thymol, eugenol, or menthol significantly delayed deterioration of the fruit (*4*). The use of these three essential oils to improve fruit quality and safety has also been reported in table grapes and sweet cherries (*5, 6*). In the present study, we further investigated the effects of other essential oil compounds including carvacrol, anethole, cinnamaldehyde, cinnamic acid, perillaldehyde, linalool, and *p*-cymene. The rationale for selecting these compounds for evaluation was because previous findings had indicated that they all had effective antimicrobial actions in fresh produce. Carvacrol and cinnamic acid have been shown to reduce the viable count of natural flora on fresh-cut melon and kiwifruit without causing adverse organoleptic changes (*7*). Carvacrol is a constituent of the ethereal oil of *Origanum hirtum* and is a major component of the essential oils from oregano and thyme. Cinnamic acid is obtained from oils of cinnamon, clove, black pepper, and coriander. Cinnamaldehyde occurs naturally in the bark of cinnamon trees and other species of the genus *Cinnamomum* such as camphor and cassia. Both cinnamaldehyde and cinnamic acid have been used in flavoring, certain pharmaceuticals, and the perfume industry (*8*). Anethole is an aromatic compound from anise, fennel, and star anise. Perillaldehyde is a natural organic compound found most abundantly in the perennial herb

* Author to whom correspondence should be addressed [telephone (301) 504-5981; fax (301) 504-5107; e-mail Chienyi.Wang@ars.usda.gov].

† Produce Quality and Safety Laboratory.

‡ Genetic Improvement of Fruits and Vegetables Laboratory.

10.1021/jf7037696
Published on Web 04/29/2008

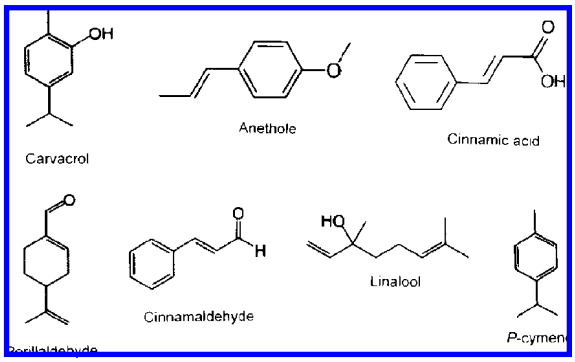

Figure 1. Chemical structures of the active compounds of essential oils used in this study.

perilla, but also in a wide variety of other plants. It is used as a food additive for flavoring and as an ingredient in perfume. Linalool is a naturally occurring terpene alcohol found in over 200 species of flowers and spice plants, mainly from the families Lamiaceae (mints, scented herbs), Lauraceae (laurels, rosewood), and Rutaceae (citrus fruits) (*9, 10*). Because of its pleasant smell, linalool has been used as a scent in products such as soap, detergent, shampoo, and lotion. *p*-Cymene is a constituent of the essential oils from cumin and thyme. It is a biological precursor of carvacrol. When *p*-cymene and carvacrol are used together, synergism can occur against certain microbes (*11*). Carvacrol, anethole, cinnamaldehyde, cinnamic acid, perillaldehyde, linalool, and *p*-cymene are all naturally occurring plant oils, and their effectiveness in inhibiting the decay of blueberry fruit was evaluated in this study.

Increasing evidence has shown that some essential oils also possess antioxidant properties. Ruberto and Baratta (*12*) tested about 100 pure components of essential oils for their antioxidant effectiveness and found that some monoterpene hydrocarbons such as terpinolene, and α- and γ-terpinene exhibited significant antioxidant action, whereas sesquiterpene hydrocarbons and non-isoprenoid components showed low antioxidant effect. Wei and Shibamoto (*13*) studied the antioxidant activities of various main essential oil compounds from several plants and reported that limonene from celery seeds, benzyl acetate from jasmine, α-pinene from juniper berries, myristicin from parsley seeds, patchouli alcohol from patchouli, and citronellol from roses showed high antioxidant activities. Yanishlieva et al. (*14*) examined the antioxidant activity of thymol and carvacrol in two lipid systems and discovered that thymol was a more effective and active antioxidant than carvacrol. Using the electron spin resonance measurement and oxygen radical absorbance capacity (ORAC) assays, we have also detected that thymol had the greatest radical scavenging capacity against DPPH and $^{\bullet}OH$ and had higher levels of antioxidant activity than menthol or eugenol (*4*). Those essential oils that exhibit antioxidant activity could be used to prevent oxidative damage and alleviate any resulting symptoms. However, little information is available on the effect of essential oils on the antioxidant system in blueberries. Blueberries are known to have high antioxidant content (*15, 16*). In this study, we investigated the effect of carvacrol, anethole, cinnamaldehyde, cinnamic acid, perillaldehyde, linalool, and *p*-cymene on blueberry total phenol, total anthocyanins, ORAC, and individual flavonoids as well as fruit quality and decay.

MATERIALS AND METHODS

Fruit Samples and Treatments. Blueberries (*Vaccinium corymbosum* cv. Duke) used in this study were grown at a farm near Beltsville, MD, and were hand-harvested at a commercially mature stage, sorted to eliminate damaged, shriveled, and unripe fruit, and selected for uniform size and color. Selected berries were randomized and used for the experiments. Fifty fruits were placed into 1 L polystyrene containers with snap-on lids. Two hundred milligrams of each essential oil including carvacrol, anethole, cinnamaldehyde, cinnamic acid, perillaldehyde, linalool, and *p*-cymene was put into a small beaker and placed in the sealed fruit container, which was then kept at 10 °C. The chemical structures of the active compounds of these essential oils used in this study are shown in **Figure 1**. Decay was evaluated after 4 weeks of storage at 10 °C. Fruit showing surface mycelial development was considered to be decayed. The severity was expressed as percent of fruit showing fungal symptoms.

Analysis of Sugars and Organic Acids. The berry fruits were homogenized and centrifuged, and the supernatants were dried in vacuo in derivatizing vials. Procedures described by Li and Schuhmann (*17*) were modified for the derivatization of sugars and organic acids. A known amount of β-phenyl-D-glucopyranoside was included in all samples as an internal standard. A Hewlett-Packard 5890 gas chromatograph (Hewlett-Packard, Palo Alto, CA) equipped with a flame ionization detector and a 25 m cross-linked methyl silicon gum capillary column (0.2 mm i.d., 0.33 μm film thickness) was used for the analysis of sugars and organic acids.

Total Anthocyanin and Total Phenolic Content. Five grams of fresh berries was extracted with 25 mL of 80% acetone containing 0.2% formic acid. Total anthocyanin content in fruit extracts was determined using the pH differential method (*18*). Absorbance was measured in a Shimadzu spectrophotometer (Shimadzu UV-160) (Shimadzu Scientific Instruments, Inc., Colmbia, MD) at 510 and 700 nm in buffers at pH 1.0 and 4.5, using $A = [(A_{510} - A_{700})_{pH\ 1.0} - (A_{510} - A_{700})_{pH\ 4.5}]$ with a molar extinction coefficient of cyanidin 3-glucoside of 29600 for blueberries. Results were expressed as milligrams of cyanidin 3-glucoside (CG) equivalent.

Total phenolic content was determined with Folin−Ciocalteu reagent according to the method of Slinkard and Singletonp (*19*) using gallic acid as a standard.

Table 1. Effect of Essential Oil Treatment on Organic Acids, Sugars, and Decay of 'Duke' Blueberries[a]

	organic acids (mg/g of fresh wt)		sugars (mg/g of fresh wt)			
essential oil	citric	malic	fructose	glucose	sucrose	decay (%)
control	6.2 ± 1.6	1.3 ± 0.06	37.4 ± 1.8	26.6 ± 0.8	1.8 ± 0.08	58 ± 3.1
carvacrol	10.4 ± 0.7	2.6 ± 0.09	54.2 ± 2.7	41.3 ± 1.3	3.3 ± 0.14	22 ± 1.6
anethole	11.7 ± 1.3	2.2 ± 0.08	48.7 ± 2.3	35.7 ± 1.6	3.1 ± 0.11	25 ± 0.9
cinnamic acid	5.3 ± 0.8	1.2 ± 0.06	34.6 ± 1.6	28.2 ± 0.7	1.5 ± 0.06	49 ± 2.6
perillaldehyde	7.5 ± 1.2	2.0 ± 0.08	41.6 ± 3.1	33.8 ± 1.6	2.9 ± 0.32	27 ± 1.2
cinnamaldehyde	6.8 ± 0.4	1.6 ± 0.04	38.7 ± 2.2	29.2 ± 1.9	2.4 ± 0.26	46 ± 2.6
linalool	6.0 ± 0.7	1.3 ± 0.05	39.3 ± 1.4	30.7 ± 1.4	2.1 ± 0.45	20 ± 0.6
p-cymene	5.7 ± 0.5	1.4 ± 0.02	32.8 ± 1.3	21.9 ± 0.7	1.6 ± 0.06	16 ± 0.7
significance treatment[b]	*	ns	*	*	ns	*

[a] Data expressed as mean ± SD ($n = 3$). Sugars and organic acids were analyzed after 7 days of storage at 10 °C, and decay was evaluated after 4 weeks of storage at 10 °C. [b] *, ns, significant or nonsignificant, respectively, at $p \leq 0.05$.

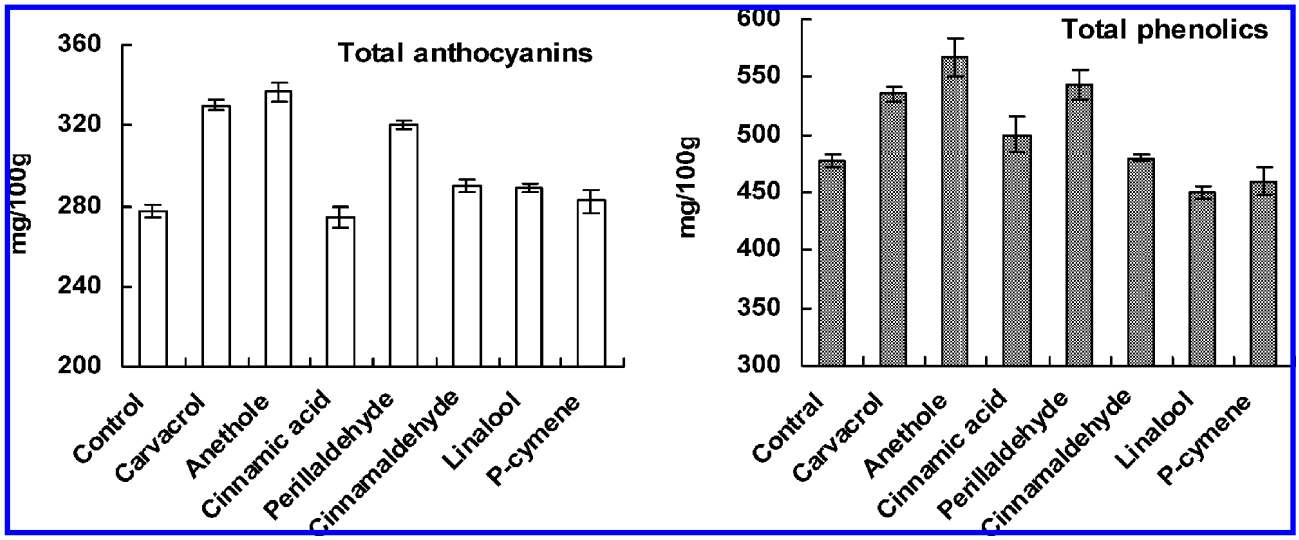

Figure 2. Effect of essential oil treatment on total anthocyanins and total phenolics in 'Duke' blueberries after 7 days of storage at 10 °C. Anthocyanins were expressed as milligrams of cyanidin 3-glucoside (CG) equivalent per 100 g of fresh weight. Total phenolics were expressed as milligrams of gallic acid equivalent per 100 g of fresh weight.

Oxygen Radical Absorbance Capacity (ORAC) Assay. The automated sample preparation was performed using a Precision 2000 instrument. The ORAC assay was carried out using a high-throughput instrument platform consisting of a robotic eight-channel liquid handling system and a microplate fluorescence reader following the protocol previously described (*20*).

Hydroxyl Radical Scavenging Capacity (•OH; HOSC) Assay. Five grams of fresh berries was extracted with 25 mL of 50% acetone. The •OH in aqueous media was generated through the Fenton reaction. The HOSC assay was conducted with acetone solutions according to a previously published protocol (*21*) with some modifications. 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 an FL800 microplate fluorescence reader (Bio-Tek Instruments, Inc., Winooski, VT). Fluorescence was measured every minute for 3 h with an excitation wavelength of 485 nm and emission wavelength of 535 nm. The plate reader was controlled by software KC4 3.0 (revision 29). Sample dilution was accomplished by a Precision 2000 automatic pipetting system managed by precision power software (version 1.0) (Bio-Tek Instruments, Inc.). Reaction mixtures consisted of 115 μL of 3.352×10^{-6} M FL prepared in 75 mM sodium phosphate buffer, 20 μL of standard or sample or blank, 25 μL of 0.1990 M H_2O_2, and 41 μL of 3.43 mM $FeCl_3$. Trolox prepared in 50% acetone at concentrations of 12.5, 25, 50, and 100 μM was used to prepare the standard curve for HOSC quantification. The HOSC values were determined by calculating the net area under the curve (AUC) of the standards and samples. The standard curve was obtained by plotting Trolox concentrations against the average net AUC of the two measurements for each concentration. Final HOSC values were calculated using the regression equation between the Trolox concentration and the net AUC and were expressed as micromoles of Trolox equivalents (TE) per gram of fresh weight.

HPLC Analysis of Blueberry Flavonoids. High-performance liquid chromatography (HPLC) was used to separate and determine individual anthocyanins and phenolic compounds in berry tissue samples. The supernatants (18 mL) from the extractions described above were concentrated to dryness using a Buchler Evapomix (Fort Lee, NJ) in a water bath at 35 °C, dissolved in 4 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% formic acid. Anthocyanins and other phenolics were then recovered with 2.0 mL of acidified methanol containing 3% formic acid. The methanol extract was passed through a 0.45 μm membrane filter (Millipore, MSI, Westboro, MA), and 20 μL was analyzed by HPLC. The samples were determined using a Waters Corp. (Milford, MA) HPLC system coupled with a photodiode array detector (Waters 990 series) and equipped with two pumps (600E system controller). Samples were injected at ambient temperature (20 °C) into a reversed-phase Nova-Pak C_{18} column (150 $\times$ 3.9 mm, particle size = 4 μm) with a guard column (Nova-Pak C_{18}, 20 $\times$ 3.9 mm, particle size = 4 μm) (Waters). The mobile phase consisted of 5% aqueous formic acid (A) and HPLC grade acetonitrile (B). The flow rate was 1 mL/min, with a gradient profile consisting of A with the following proportions (v/v) of B: 0–1 min, 4%; 1–10 min, 4–6% B; 10–15 min, 6% B; 15–35 min, 6–18% B; 35–40 min, 18–20% B; 40–42 min, 20–45% B; 42–45 min, 45–100% B; 45–50 min, 100% B. The phenolic compounds in fruit extracts were identified by their UV spectra, recorded with a diode array detector, and by chromatographic comparison with authentic markers (*22–26*). Individual flavonols and anthocyanins were quantified by comparison with an external standard of myricetin, quercetin, kaempferol, and cyanidin 3-glucoside. Scanning between 250 and 550 nm was performed, and data were collected by using the Waters 990 3D chromatography data system.

Statistical Analysis. The experiment was performed in triplicate. Data were subjected to analysis of variance using the NCSS Statistical Analysis System (Kaysville, UT) (*27*). The values of oxygen radical absorbance capacity (ORAC), total phenolics, and total anthocyanin were evaluated by the Tukey−Kramer multiple-comparison test used in NCSS. Differences at $p \leq 0.05$ were considered to be significant.

RESULTS AND DISCUSSION

All essential oils tested in this study inhibited fruit decay development to some degree compared to controls. The most effective compound in terms of mold retardation was *p*-cymene, followed by linalool, carvacrol, anethole, and perillaldehyde (**Table 1**). Cinnamic acid and cinnamaldehyde also suppressed mold growth, but to a lesser extent. Inhibition of mold growth, which leads to the reduction of fruit decay, can primarily be attributed to the antimicrobial capability of these essential oils. The antimicrobial properties of many essential oils have been well documented (*3*). The antimicrobial mechanism or mode of action of essential oils has been postulated as disruption of cellular membrane functions and interference with active sites of enzymes and cellular metabolism (*28, 29*). Others hypothesized that essential oils may change the permeability of membranes of the microbes for cations and alter the ion gradients that lead to impairment of vital processes in cells and

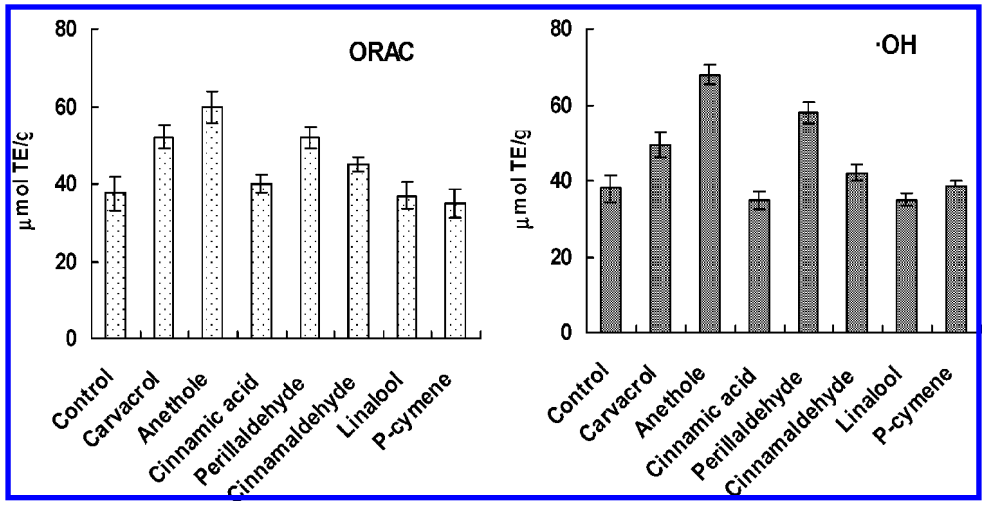

Figure 3. Effect of essential oil treatment on oxygen radical absorbance capacity (ORAC) and hydroxyl radical scavenging capacity (•OH) in 'Duke' blueberries after 7 days of storage at 10 °C. Both ORAC values and hydroxyl radical scavenging capacity are expressed as micromoles of Trolox equivalents (TE) per gram of fresh weight.

Table 2. Effect of Essential Oil Treatment on Chlorogenic Acid, Resveratrol, Myricetin 3-Arabinoside, Quercetin 3-Galactoside, Quercetin3-Arabinoside, Quercetin Derivative, Kaempferol 3-Glucoside, and Kaempferol Derivative Content in 'Duke' Blueberries after 7 Days of Storage at 10 °C[a]

treatment	chlorgenic acid[b]	resveratrol[c]	myricetin 3-arabinoside[d]	quercetin 3-galactoside[e]	quercetin 3-arabinoside[e]	quercetin derivative[e]	kaempferol 3-glucoside[f]	kaempferol derivative[f]
control	124.9 ± 3.4	2.5 ± 0.2	19.9 ± 0.7	108.0 ± 2.3	48.3 ± 0.3	30.9 ± 4.7	16.5 ± 0.1	24.5 ± 0.5
carvacrol	171.9 ± 6.9	5.7 ± 0.5	19.9 ± 0.5	129.7 ± 3.5	63.9 ± 1.5	29.2 ± 0.3	17.9 ± 0.5	25.9 ± 1.0
anethole	174.1 ± 2.3	7.1 ± 1.2	22.7 ± 2.2	162.9 ± 1.3	63.7 ± 1.5	39.7 ± 3.4	16.9 ± 4.1	32.4 ± 1.5
cinnamic acid	169.1 ± 1.8	5.4 ± 0.2	15.7 ± 0.2	146.1 ± 2.1	58.6 ± 0.5	38.0 ± 0.4	15.4 ± 0.6	22.8 ± 1.1
perillaldehyde	151.5 ± 4.3	3.0 ± 0.1	20.5 ± 0.5	130.6 ± 4.6	56.0 ± 2.8	36.4 ± 3.7	17.8 ± 4.1	28.5 ± 0.3
cinnamaldehyde	187.0 ± 5.7	4.0 ± 0.1	16.7 ± 0.6	175.6 ± 5.2	55.2 ± 1.9	37.2 ± 0.4	17.0 ± 0.2	23.6 ± 0.2
linalool	152.1 ± 0.9	3.8 ± 0.1	12.6 ± 0.4	108.3 ± 0.0	48.6 ± 1.0	26.8 ± 4.2	15.0 ± 0.3	24.4 ± 0.6
p-cymene	140.3 ± 2.0	2.5 ± 0.0	19.4 ± 1.0	110.4 ± 3.5	50.1 ± 2.9	26.4 ± 1.1	14.8 ± 1.9	27.0 ± 1.5
significance treatment[g]	*	*	*	*	ns	*	ns	*

[a] Data expressed as mean ± SD ($n = 3$). [b] Data expressed as micrograms of chlorogenic acid equivalents per gram of fresh weight. [c] Data expressed as micrograms of *trans*-resveratrol equivalents per gram of fresh weight. [d] Data expressed as micrograms of myricetin equivalents per gram of fresh weight. [e] Data expressed as micrograms of quercetin equivalents per gram of fresh weight. [f] Data expressed as micrograms of kaempferol equivalents per gram of fresh weight. [g] *, ns, significant or nonsignificant, respectively, at $p \leq 0.05$.

eventually cell death (*30*). Some essential oils such as carvacrol, anethole, and perillaldehyde have also shown the capability to promote total anthocyanins and total phenolics and enhance antioxidant activity in fruit tissues with respect to oxygen radical absorbance capacity and hydroxyl radical scavenging capacity (**Figures 2** and **3**). In this case, an additional mechanism may be involved in maintaining the low incidence of decay. It is possible that essential oils would act as "signaling compounds" that induce constitutive increases in antioxidant titer (enzymatic and nonenzymatic) in tissues. The correlation coefficient (R^2) between the value of ORAC and the percentage of decay in fruit treated with carvacrol, anethole, and perillaldehyde had a negative relationship of 0.9162. An even higher R^2 value (0.9683) was obtained for the negative relationship between decay and scavenging capacity of hydroxyl radicals (•OH). Other essential oils including thymol, menthol, and eugenol have also shown effects on both reducing mold growth and increasing antioxidant activity and free radical scavenging capability (*4*). However, compounds that do not promote antioxidant activity are not necessarily less potent in inhibiting microbial growth. For example, *p*-cymene was the most effective in reducing the spoilage of blueberry fruit in this study, but it did not increase the antioxidant activity of the fruit (**Table 1** and **Figure 2**). Linalool, cinnamic acid, and cinnamaldehyhde also did not increase the antioxidant activity, but had a lower percentage of decay than controls. The correlation coefficients (R^2) between decay and ORAC or •OH scavenging capacity were 0.4143 and 0.0147, respectively, for these essential oils. Allyl isothiocyanate also greatly reduced decay of strawberries, blackberries, and raspberries, but its effects on antioxidant activity and free radical scavenging capacity were inconsistent (*31, 32*). These data suggest that potential antimicrobial activity against pathogens causing spoilage of fresh produce is largely dependent upon the potency of the particular compound in inhibiting the microbes and not as much on its effects on antioxidant promotion.

The effectiveness of essential oil compounds in delaying mold growth seen in this research was different from what has been reported in studies with other fruits. For example, Roller and Seedhar (*7*) showed that no visible spoilage was observed in carvacrol- and cinnamic acid-treated kiwifruit after storage for 5 days at either 4 or 8 °C, whereas we found that blueberries stayed free of decay for 9 and 7 days at 10 °C after being treated with carvacrol and cinnamic acid, respectively. It is possible that different fruits respond differently to the treatments, and various methods of application produce diverse results. Therefore, more research is necessary to ascertain the usefulness of a compound and to determine its optimum concentration, storage temperature, and treatment method for a specific commodity.

Table 3. Effect of Essential Oil Treatment on Delphinidin 3-Galactoside, Petunidin 3-Galactoside, Petunidin 3-Glucoside, Petunidin 3-Arabinoside, Malvidin 3-Galactoside, and Malvidin 3-Arabinoside Content in 'Duke' Blueberries after 7 Days of Storage at 10 °C[a,b]

treatment	delphinidin 3-galactoside	cyanidin 3-galactoside	delphinidin 3-arabinoside	petunidin 3-galactoside	petunidin 3-glucoside	petunidin 3-arabinoside	malvidin 3-galactoside	malvidin 3-arabinoside
control	204.9 ± 5.3	51.3 ± 6.0	77.2 ± 0.9	247.4 ± 1.9	186.1 ± 4.7	161.0 ± 1.4	1001.6 ± 14	513.3 ± 4.9
carvacrol	264.6 ± 3.9	73.1 ± 6.0	100.7 ± 5.2	305.1 ± 5.1	200.1 ± 7.7	202.2 ± 9.9	1101.8 ± 12	604.9 ± 9.4
anethole	340.6 ± 7.4	112.6 ± 3.5	130.4 ± 9.8	330.6 ± 6.6	227.7 ± 7.7	223.3 ± 9.3	1227.4 ± 22	675.4 ± 8.4
cinnamic acid	271.4 ± 7.5	93.5 ± 5.3	100.5 ± 2.2	239.7 ± 0.1	172.4 ± 1.6	170.8 ± 1.6	1031.5 ± 10	533.4 ± 3.4
perillaldehyde	241.6 ± 6.4	71.4 ± 4.9	100.8 ± 1.3	274.8 ± 8.9	207.3 ± 9.1	178.7 ± 1.8	1040.9 ± 11	582.4 ± 6.2
cinnamaldehyde	294.2 ± 9.1	98.3 ± 9.5	120.9 ± 4.3	276.5 ± 9.4	228.6 ± 5.0	210.1 ± 8.4	1076.6 ± 10	569.7 ± 9.2
linalool	216.4 ± 1.1	113.6 ± 9.4	117.8 ± 6.6	291.2 ± 9.9	219.7 ± 8.9	187.1 ± 9.8	1008.2 ± 15	531.1 ± 2.6
p-cymene	228.2 ± 7.1	67.2 ± 2.9	105.4 ± 3.0	288.6 ± 1.8	199.8 ± 5.2	176.1 ± 9.0	1003.4 ± 17	563.1 ± 2.8
significance treatment[c]	*	*	*	*	*	*	*	*

[a] Data expressed as mean ± SD ($n = 3$). [b] Data expressed as micrograms of cyanidin 3-glucoside equivalents per gram of fresh weight. [c] *, ns, significant or nonsignificant at $p \leq 0.05$.

In addition to inhibiting mold growth and retarding decay, certain essential oils also affect the quality of blueberry fruit. Blueberries contain two major sugars, fructose and glucose, with sucrose as a minor constituent (**Table 1**). Citric acid is the predominant organic acid in blueberry fruit, with malic acid, quinic acid, and succinic acid as minor components. Treatment with carvacrol, anethole, or perillaldehyde significantly increased the levels of fructose, glucose, and citric acid (**Table 1**). Higher values of sugar and organic acid content contribute to better quality of fruit. Therefore, those essential oils that have positive effects on sugar and acid content and negative effects on microbial growth deserve further evaluation for their possible use as natural preservative agents for fresh produce.

Further research is also warranted to investigate the synergistic as well as antagonistic effects when different essential oils are mixed in various combinations. Evidence has shown that a synergistic effect can be achieved when *p*-cymene and carvacrol are used together (*33*). *p*-Cymene alone is not a strong antibacterial agent (*34, 35*). However, when *p*-cymene was combined with carvacrol, it resulted in greater microbial inhibition than the sum of the individual effects by each compound. It appears that *p*-cymene can be incorporated into the lipid bilayer and cause the cytoplasmic membrane to swell and therefore allow carvacrol easy access across the membrane into the cell to exert its effect (*33*). Neither cinnamaldehyde nor eugenol retarded the growth of pathogens when applied alone, but their combination was very effective in inhibiting the development of these pathogens (*36*). Thus, it is possible that better results could be obtained if mixtures of essential oils in our study were used. Furthermore, the combination of essential oils and other preservative techniques may yield even more desirable results. Valero and co-workers (*5, 6, 37*) showed that the quality and safety of table grapes and sweet cherries could be improved and storage life could be extended when the use of these natural antifungal compounds was combined with modified atmosphere packaging. Therefore, techniques combining the use of essential oils and other preservatives or other postharvest procedures such as refrigeration, heat treatment, high oxygen exposure, or UV-C illumination need to be explored.

HPLC analysis of blueberry flavonoids revealed that a number of individual anthocyanins and phenolic compounds were present in blueberry fruit (**Tables 2** and **3**). Chlorogenic acid and quercetin 3-galactoside were the two major phenolic compounds. Myricetin 3-arabinoside, quercetin 3-arabinoside, and kaempferol 3-glucoside were also present in moderate amounts. Resveratrol was also detected, but in a relatively smaller amount. All of the essential oils tested enhanced chlorogenic acid levels (**Table 2**). Higher amounts of quercetin 3-galactoside and quercetin 3-arabinoside were also found in all treated fruit except samples treated with linalool or *p*-cymene. Malvidin 3-galactoside was the predominant anthocyanin with values almost twice as high as the second major component, malvidin 3-arabinoside (**Table 3**). Other individual anthocyanins detected in blueberries in this study include (in the order of their abundance) petunidin 3-galactoside, delphinidin 3-galactoside, petunidin 3-glucoside, petunidin 3-arabinoside, delphinidin 3-arabinoside, and cyanidin 3-galactoside. All essential oil treatments enhanced the levels of individual anthocyanins compared to controls, except that the levels of malvidin 3-galactoside from linalool and *p*-cymene treatments were not significantly different from those of the controls. Because malvidin 3-galactoside was the main component, low amounts of this compound in linalool- and *p*-cymene-treated fruit resulted in low total anthocyanin values in these treatments. In a previous study, blueberry fruit exposed to superatmospheric oxygen also maintained higher levels of malvidin 3-galactoside, malvidin 3-arabinoside, petunidin 3-galactoside, delphinidin 3-galactoside, petunidin 3-glucoside, petunidin 3-arabinoside, delphinidin 3-arabinoside, and cyanidin 3-galactoside during storage at 5 °C than untreated fruit (*38*).

In addition to evaluating the effect on inhibiting decay and promoting antioxidant activity, many other factors need to be considered in the determination of the feasibility of essential oils for maintaining the quality of fresh fruits and vegetables. The effects on flavor, taste, texture, ripening behavior, and storage life are equally as important as the inhibition of decay organisms. The extent of these effects on sensory quality might depend on both the kind of essential oil used and the type of fruits or vegetables being treated. Sensory evaluation by a taste panel would need to be performed to establish organoleptic quality and ultimate acceptability.

In summary, this study demonstrated that some essential oils such as carvacrol, anethole, and perillaldehyde not only possessed antimicrobial properties and reduced fruit decay during storage but also exhibited the capability to increase antioxidant activity and levels, as well as sugar and organic acid quality in treated fruit tissues. High antioxidant activity could enhance free radical scavenging capacity and increase the resistance of tissues to oxidative damage. Thus, these natural products have the potential to preserve the quality and safety of fresh produce. They deserve to be evaluated further to determine their effects on flavor, texture, and other postharvest parameters of the fruit.

LITERATURE CITED

(1) Burt, S. Essential oils: their antibacterial properties and potential applications in foods—a review. *Intl. J. Food Microbiol.* **2004**, *94*, 223–253.

(2) Boyle, W. Spices and essential oils as preservatives. *Am. Perfume Essent. Oil Rev.* **1955**, *66*, 25–28.

(3) Nychas, G. J. E. Natural antimicrobials from plants. In *New Methods of Food Preservation*; Gould, G. W., Ed.; Blackie Academic and Professional: London, U.K., 1995; pp 58–89.

(4) Wang, C. Y.; Wang, S. Y.; Yin, J. J.; Parry, J.; Yu, L. L. Enhancing antioxidant, antiproliferation, and free radical scavenging activities in strawberries with essential oils. *J. Agric. Food Chem.* **2007**, *55*, 6527–6532.

(5) Serrano, M.; Martínez-Romero, D.; Castillo, S.; Guillén, F.; Valero, D. The use of antifungal compounds improves the beneficial effect of MAP in sweet cherry storage. *Innovative Food Sci. Emerg. Technol.* **2005**, *6*, 115–123.

(6) Valverde, J. M.; Guillén, F.; Martínez-Romero, D.; Castillo, S.; Serrano, M.; Valero, D. Improvement of table grape quality and safety by the combination of modified atmosphere packaging (MAP) and eugenol, menthol, or thymol. *J. Agric. Food Chem* **2005**, *53*, 7458–7464.

(7) Roller, S.; Seedhar, P. Carvacrol and cinnamic acid inhibit microbial growth in fresh-cut melon and kiwifruit at 4 and 8 °C. *Lett. Appl. Microbiol.* **2002**, *4*, 161–166.

(8) Fenaroli, G. *Fenaroli's Handbook of Flavor Ingredients*, 3rd ed.; CRC Press: Boca Raton, FL, 1995.

(9) Casabianca, H.; Graff, J. B.; Faugier, V.; Fleig, F.; Grenier, C. Enantiomeric distribution studies of linalool and linalyl acetate. A powerful tool for authenticity control of essential oils. *J. High Resolut. Chromatogr.* **1997**, *21*, 107–112.

(10) Lewinshon, E.; Schalechet, F.; Wilkinson, J.; Matsui, K.; Tadmor, Y.; Nam, K,; Amar, O.; lastochkin, E.; Larkov, O.; Ravid, U.; Hiatt, W; Gepstein, S.; Pichersky, E. Enhanced levels of the aroma and flavor compound *S*-linalool by metabolic engineering of the terpenoid pathway in tomato fruit. *Plant Physiol.* **2001**, *127*, 1256–1265.

(11) Ultee, A.; Slump, R. A.; Steging, G.; Smid, E. J. Antimicrobial activity of carvacrol toward *Bacillus cereus* on rice. *J. Food Prot.* **2000**, *63*, 620–624.

(12) Ruberto, G.; Baratta, M. T. Antioxidant activity of selected essential oil components in two lipid model systems. *Food Chem.* **2000**, *69*, 167–174.

(13) Wei, A.; Shibamoto, T. Antioxidant activities and volatile constituents of various essential oils. *J. Agric. Food Chem.* **2007**, *55*, 1737–1742.

(14) Yanishlieva, N. V.; Marinova, E. M.; Gordon, M. H.; Raneva, V. G. Antioxidant activity and mechanism of action of thymol and carvacrol in two lipid systems. *Food Chem.* **1999**, *64*, 59–66.

(15) Kalt, W.; Forney, C. F.; Martin, A.; Prior, R. L. Antioxidant capacity, vitamin C, phenolics, and anthocyanins after fresh storage of small fruits. *J. Agric. Food Chem.* **1999**, *47*, 4638–4644.

(16) Zheng, W.; Wang, S. Y. Oxygen radical absorbing capacity of phenolics in blueberries, cranberries, chokeberries, and lingonberries. *J. Agric. Food Chem.* **2003**, *51*, 501–509.

(17) Li, B. W.; Schuhmann, P. J. Gas−liquid chromatographic analysis of sugars in ready-to-eat breakfast cereals. *J. Food Sci.* **1980**, *45*, 138–141.

(18) Cheng, G. W.; Breen, P. J. Activity of phenylalanine ammonia-lyase (PAL) and concentrations of anthocyanins and phenolics in developing strawberry fruit. *J. Am. Soc. Hortic. Sci.* **1991**, *116*, 865–869.

(19) Slinkard, K.; Singleton, V. L. Total phenol analysis: automation and comparison with manual methods. *Am. J. Enol. Vitic.* **1977**, *28*, 49–55.

(20) Huang, D.; Ou, B.; Hampsch-Woodill, M.; Flanagan, J. A.; Prior, R. L. 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. *J. Agric. Food Chem.* **2002**, *50*, 4437–4444.

(21) Moore, J.; Yin, J.; Yu, L. Novel fluorometric assay for hydroxyl radical scavenging capacity (HOSC) estimation. *J. Agric. Food Chem.* **2006**, *54*, 617–626.

(22) Häkkinen, S.; Heinonen, M.; Kärenlampi, S.; Mykkänen, H.; Ruuskanen, J.; Törrönen, R. Screening of selected flavonoids and phenolic acids in 19 berries. *Food Res. Int.* **1999**, *32*, 345–353.

(23) Mazza, G.; Miniati, E. Small fruits. In *Anthocyanins in Fruits, Vegetables, and Grains*; Mazza, G., Miniati, E., Eds.; CRC Press: Boca Raton, FL, 1993; pp 85–129.

(24) Gao, L.; Mazza, G. Quantitation and distribution of simple and acylated anthocyanins and other phenolics in blueberries. *J. Food Sci.* **1994**, *59*, 1057–1059.

(25) Kader, F.; Rovel, B.; Girardin, M.; Metche, M. Fractionation and identification of the phenolic compounds of highbush blueberries (*Vaccinium corymbosum*, L.). *Food Chem.* **1996**, *55*, 35–40.

(26) Bilyk, A.; Sapers, G. M. Varietal differences in the quercetin, kaempferol, and myricetin contents of highbush blueberry, cranberry, and thornless blackberry fruits. *J. Agric. Food Chem.* **1986**, *34*, 585–588.

(27) *Statistical Analysis and Graphics*; NCSS: Kaysville, UT, 2007.

(28) Farag, R. S.; Daw, Z. Y.; Abo-Raya, S. H. Influence of some spice essential oils on *Aspergillus parasiticus* growth and production of aflatoxins in a synthetic medium. *J. Food Sci.* **1989**, *54*, 74–76.

(29) Marino, M.; Bersani, C.; Comi, G. impedance measurements to study the antimicrobial activity of essential oils from Lamiaceae and Compositae. *Intl. J. Food Microbiol.* **2001**, *67*, 185–187.

(30) Ultee, A.; Kets, E. P. W.; Smid, E. J. Mechanisms of action of cavacrol on the food-borne pathogen *Bacillus cereus*. *Appl. Environ. Microbiol.* **1999**, *65*, 4606–4610.

(31) Chanjirakul, K.; Wang, S. Y.; Wang, C. Y.; Siriphanich, J. Effect of natural volatile compounds on antioxidant capacity and antioxidant enzymes in raspberries. *Postharvest Biol. Technol.* **2006**, *40*, 106–115.

(32) Chanjirakul, K.; Wang, S. Y.; Wang, C. Y.; Siriphanich, J. Natural volatile treatments increase free-radical scavenging capacity of strawberries and blackberries. *J. Sci. Food Agric.* **2007**, *87*, 1463–1472.

(33) Ultee, A.; Bennink, M. H. J.; Moezelaar, R. The phenolic hydroxyl group of carvacrol is essential for action against the food-borne pathogen *Bacillus cereus*. *Appl. Environ. Microbiol.* **2002**, *68*, 1561–1568.

(34) Dorman, H. J. D.; Deans, S. G. Antimicrobial agents from plants: antibacterial activity of plant volatile oils. *J. Appl. Microbiol.* **2000**, *88*, 308–316.

(35) Juven, B. J.; Kanner, J.; Schved, F.; Weisslowicz, H. Factors that interact with the antibacterial action of thyme essential oil and its active constituents. *J. Appl. Bacteriol.* **1994**, *76*, 626–631.

(36) Moleyar, V.; Narasimham, P. Antibacterial activity of essential oil components. *Intl. J. Food Microbiol.* **1992**, *16*, 337–342.

(37) Valero, D.; Valverde, J. M.; Martínez-Romero, D.; Guillén., F.; Castillo, S.; Serrano, M. The combination of modified atmosphere packaging with eugenol or thymol to maintain quality, safety and functional properties of table grapes. *Postharvest Biol. Technol.* **2006**, *41*, 317–327.

(38) Zheng, Y.; Wang, C. Y.; Wang, S. Y.; Zheng, W. Effect of high-oxygen atmospheres on blueberry phenolics, anthocyanins, and antioxidant capacity. *J. Agric. Food Chem.* **2003**, *51*, 7162–7169.

Received for review December 26, 2007. Revised manuscript received March 3, 2008. Accepted March 4, 2008.

JF7037696