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Enhancing Antioxidant Capacity and Reducing Decay of Chinese Bayberries by Essential Oils

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ABSTRACT: The effects of essential oil treatment on fruit decay and antioxidant capacities in Chinese bayberries were evaluated. Chinese bayberries were treated with essential oils, including carvacrol, cinnamaldehyde, perillaldehyde, and linalool. Results from this study indicated that all essential oils significantly reduced fruit decay of Chinese bayberries, and the most effective compound was carvacrol. Treatment with carvacrol, cinnamaldehyde, perillaldehyde, and linalool significantly increased total phenolic, anthocyanin, and individual flavonoid contents. In addition, all essential oils maintained significantly higher antioxidant capacities as measured by scavenging capacity against superoxide, hydroxyl, and 1,1-diphenyl-2-picrylhydrazyl radicals and by the reducing power test compared to the control. Moreover, the activities of antioxidant enzymes were also enhanced by all essential oils. Thus, postharvest essential oil treatment has positive effects on reducing decay and enhancing antioxidant capacities in Chinese bayberries.

KEYWORDS: *Chinese bayberries, decay, antioxidant capacity, essential oil*

INTRODUCTION

Chinese bayberry (*Myrica rubra* Seib and Zucc.), a subtropical fruit native to China, has nutritional and commercial importance in terms of its special functional composition and consumption demand. However, the fruit decays quickly at ambient temperature after harvest, with a shelf life of only 1−2 days, which limits its marketing and results in severe postharvest loss.[1] Chinese bayberry contains high levels of anthocyanins, flavonoids, and phenolic acids and is considered to be a good source of natural antioxidants, which may provide protection against various human diseases caused by oxidative stress.[2] Previous studies have shown that Chinese bayberry has high antioxidant activity against superoxide and hydroxyl radicals, and there is a positive correlation between the antioxidant activity and total phenolic or anthocyanin content.[3]

Essential oils are naturally aromatic compounds obtained from plant materials, including flowers, seeds, leaves, roots, fruits, etc.[4] It has been reported that many essential oils could effectively prevent postharvest diseases of various fruits, such as mango, pear, and kiwifruit.[5−7] In addition, several studies have shown that some essential oils also have the potential fuction of enhancing antioxidant capacities in various kinds of fruits. Wang et al.[8] reported that flavonoid contents and oxygen radical absorbance capacity in blueberries were enhanced by carvacrol, anethole, or perillaldehyde. Methly jasmonate or tea tree oil could increase the antioxidant capacities and antioxidant enzyme activities in Chinese bayberries or raspberries.[9,10] In our previous study, we also found that essential oils, including carvacrol, anethole, cinnamic acid, and perillaldehyde, enhanced antioxidant capacities and flavonoid contents in raspberries.[11] However, little information is available on the effect of essential oils on fruit decay and antioxidant capacity in Chinese bayberry fruit.

The purpose of this study was to investigate the effect of essential oil (including carvacrol, perillaldehyde, cinnamaldehyde, and linalool) treatment on fruit decay, antioxidant enzyme activities, antioxidant capacities, and individual flavonoid contents in Chinese bayberry during storage at 0 °C.

MATERIALS AND METHODS

Fruit Sample and Chemicals. Chinese bayberries (*M. rubra* Seib and Zucc. cv. Wumei) used in this study were hand-harvested at commercially mature stage from an orchard in Suzhou, Jiangsu Province, China. Fruits were transported within 4 h to our laboratory and selected for uniform size, color, and absence of defects. The 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) and essential oils, including carvacrol, cinnamaldehyde, perillaldehyde, and linalool, were purchased from Sigma Chemical Co., Ltd.

Treatment with Essential Oil. In a preliminary experiment, we tested a series of concentrations of each essential oil, including carvacrol, cinnamaldehyde, perillaldehyde, and linalool, namely, 0.5, 1, 5, and 10 μL L^{-1}. All essential oils at the concentration of 0.5 or 1 μL L^{-1} significantly inhibited fruit decay, and 1 μL L^{-1} had the better effect. However, 5 or 10 μL L^{-1} essential oil treatment caused some chemical injuries, including discoloration or smelly flavor, in Chinese bayberry fruit (data not shown). Thus, the concentration of 1 μL L^{-1} was chosen to use in this experiment. A total of 30 Chinese bayberries were placed in 1 L sealed polystyrene containers with a filter paper inside the cover. A total of 1 μL of each essential oil, including carvacrol, cinnamaldehyde, perillaldehyde, and linalool, was spotted onto the filter paper, allowing the essential oil to evaporate within the containers. Afterward, sealed polystyrene containers were placed at 0 °C for storage. Three containers of bayberries from each treatment were taken before essential oil treatment (day 0) and at 3 day intervals during storage for decay evaluation and quality parameter analysis. Tissue samples from berries without defect were mixed and frozen immediately in liquid nitrogen and then stored at −80 °C until analysis. Each treatment was replicated 3 times, and the whole experiment was conducted twice with similar results; therefore, only the result from one experiment was presented in this study.

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Table 1. Effect of Essential Oil Treatment on Fruit Decay, TSS, TA, and AsA in Chinese Bayberry[a]

storage (days)	treatment	decay (%)	TSS (%)	TA (%)	AsA (g/kg of FW)
day 0		0.00 ± 0.00	12.07 ± 0.05	1.04 ± 0.02	0.33 ± 0.01
day 3	control	7.04 ± 0.52 aD	11.77 ± 0.15 aA	1.00 ± 0.01 aA	0.30 ± 0.01 aA
	carvacrol	1.25 ± 0.29 dD	11.93 ± 0.06 aA	0.98 ± 0.03 aA	0.31 ± 0.01 aA
	cinnamaldehyde	1.25 ± 0.24 dD	11.83 ± 0.11 aA	0.99 ± 0.02 aA	0.29 ± 0.02 aA
	perillaldehyde	1.42 ± 0.17 cD	11.97 ± 0.06 aA	0.98 ± 0.05 aA	0.31 ± 0.02 aA
	linalool	2.85 ± 0.26 bD	11.93 ± 0.09 aA	1.00 ± 0.02 aA	0.30 ± 0.02 aA
day 6	control	12.79 ± 0.35 aC	11.07 ± 0.06 aB	0.97 ± 0.03 aA	0.26 ± 0.02 aB
	carvacrol	2.50 ± 0.32 dC	11.23 ± 0.12 aB	0.96 ± 0.02 aA	0.29 ± 0.01 aA
	cinnamaldehyde	4.70 ± 0.29 cC	11.07 ± 0.12 aB	0.97 ± 0.02 aA	0.28 ± 0.02 aA
	perillaldehyde	8.12 ± 0.11 bC	11.20 ± 0.10 aB	0.96 ± 0.01 aA	0.28 ± 0.01 aA
	linalool	4.70 ± 0.41 cC	11.13 ± 0.03 aB	0.98 ± 0.03 aA	0.27 ± 0.03 aA
day 9	control	28.52 ± 0.31 aB	11.07 ± 0.12 aB	0.88 ± 0.01 aB	0.25 ± 0.01 aB
	carvacrol	14.47 ± 0.19 eB	11.17 ± 0.06 aB	0.89 ± 0.00 aB	0.27 ± 0.01 aA
	cinnamaldehyde	16.25 ± 0.23 dB	11.27 ± 0.13 aB	0.91 ± 0.02 aB	0.26 ± 0.02 aA
	perillaldehyde	19.92 ± 0.61 bB	11.00 ± 0.10 aB	0.90 ± 0.02 aB	0.25 ± 0.00 aA
	linalool	17.41 ± 0.14 cB	10.93 ± 0.15 aB	0.89 ± 0.01 aB	0.27 ± 0.02 aA
day 12	control	33.70 ± 1.42 aA	9.97 ± 0.15 aC	0.85 ± 0.03 aB	0.24 ± 0.02 aB
	carvacrol	19.11 ± 0.98 dA	10.23 ± 0.06 aC	0.86 ± 0.02 aB	0.26 ± 0.03 aA
	cinnamaldehyde	23.17 ± 1.12 cA	10.17 ± 0.04 aC	0.87 ± 0.05 aB	0.25 ± 0.02 aA
	perillaldehyde	25.56 ± 0.37 bA	10.13 ± 0.02 aC	0.85 ± 0.02 aC	0.24 ± 0.01 aA
	linalool	26.07 ± 0.49 bA	10.17 ± 0.01 aC	0.84 ± 0.01 aC	0.23 ± 0.03 aA

[a]Data are expressed as the mean ± standard deviation (SD) ($n = 3$). Values in the same column with different letters for each day were significantly different at $p < 0.05$. Lowercase letters represented significant difference among treatment factors, and capital letters represented significant difference among storage time factors.

Fruit Quality Measurements. Fruit decay was visually evaluated using 30 fruits from each replicate during the course of the experiment. Berries showing surface mold growth were considered to be decayed. The severity of fruit decay was expressed as a percentage of fruit showing fungal symptoms.

A total of 10 fruits from each replicate were wrapped in cheesecloth and squeezed with a hand press, and the juice was analyzed for total soluble solids (TSS) and titratable acidity (TA). TSS was determined at 25 °C using a digital refractometer (PR-101, Spectrum Technologies, Plainfield, IL). TA (as the percentage of citric acid) was determined by titrating 20 mL of bayberry juice to pH 8.2 with 0.1 M NaOH. Ascorbic acid (AsA) was determined using the methods by Arakawa et al.[12] The results were expressed as grams of AsA per kilogram of fresh weight.

Total Phenolic, Anthocyanin, and Flavonoid Measurements. The total phenolic content in bayberry juice extracts was determined with a Folin−Ciocalteu reagent according to the method by Slinkard and Singleton,[13] using gallic acid as a standard. Results were expressed as milligrams of gallic acid equivalent per 100 g of fresh weight.

The total anthocyanin content in bayberry juice extracts was determined using the pH differential method.[14] 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 $A = [(A_{510} - A_{700})_{\text{pH 1.0}} - (A_{510} - A_{700})_{\text{pH 4.5}}]$. Results were expressed as milligrams of cyanidin-3-glucoside equivalents per 100 g of fresh weight.

The total flavonoid content in bayberry juice extracts was determined according to the method by Toor and Savage.[15] Absorbance was measured at 510 nm, and results were expressed as milligrams of rutin equivalents per 100 g of fresh weight.

Antioxidant Enzyme Measurements. Superoxide dismutase (SOD) was extracted from 1 g of tissue with 5 mL of 50 mM sodium phosphate buffer (pH 7.8) at 4 °C. The homogenate was centrifuged at 20000*g* for 20 min (4 °C), and the supernatant was used to determine SOD activity from the method by Rao et al.[16] A total of 1 unit of SOD activity was defined as the amount of enzyme that caused a 50% inhibition of nitro blue tetrazolium.

Catalase (CAT) activity was determined from the method by Chance and Maehly.[17] A total of 1 unit of CAT was defined as the amount of enzyme that decomposes 1 μmol of H_2O_2/min at 30 °C.

Guaiacol peroxidase (POD) activity was assayed using guaiacol as a donor and H_2O_2 as a substrate according to the method by Kochba et al.[18] A total of 1 unit of POD activity was defined as an increase of 0.001 in absorbance per minute at 470 nm under the assay conditions.

Ascorbate peroxidase (APX) was determined according to the method by Amako et al.[19] A total of 1 unit of APX was defined as the amount of enzyme that oxidized 1 μmol of ascorbate/min at room temperature.

The protein content in the enzyme extracts was determined with the Bradford[20] method, using bovine serum albumin as a standard. The specific activity of all of the enzymes was expressed as units per milligram of protein.

Scavenging Activities against $O_2\cdot^-$, ·OH, and DPPH Radicals and Reducing Power Measurements. Three 5 g composite samples from 30 bayberries were extracted twice with 25 mL of 80% acetone containing 0.2% formic acid. The extracts were centrifuged at 20000*g* for 20 min (4 °C), and the supernatant was used for the following assay. The assay of superoxide radical ($O_2\cdot^-$) scavenging activity was based on the capacity of bayberry juice extracts to inhibit formazan formation by scavenging the superoxide radicals generated in a riboflavin light/nitro blue tetrazolium system.[21] The percentage inhibition of superoxide anion generation was calculated using the following formula: $O_2\cdot^-$ radical scavenging activity (%) = 100 − (absorbance of the sample/absorbance of the control) × 100.

Hydroxyl radical (·OH) scavenging activity of bayberry juice extracts was determined according to the method by Halliwell et al.[22] The result was calculated according to following formula: ·OH radical scavenging activity (%) = 100 − (absorbance of the sample/absorbance of the control) × 100.

DPPH radical scavenging activity of bayberry juice extracts was determined according to the method of Hatano et al.[23] The result was calculated according to the following formula: DPPH radical scavenging activity (%) = 100 − (absorbance of sample/absorbance of control) × 100.

The reducing power of bayberry juice extracts was determined according to the method by Oyaizu.[24] Bayberry juice extracts were mixed with 2.5 mL of 200 mmol/L sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide. The mixture was incubated at 50 °C for 20 min. The upper layer (5 mL) was mixed with 5 mL of

Table 2. Effect of Essential Oil Treatment on Total Phenolic, Total Anthocyanin, and Total Flavonoid Contents in Chinese Bayberry[a]

storage (days)	treatment	total phenolic (mg/100 g of FW)	total anthocyanin (mg/100 g of FW)	total flavonoid (mg/100 g of FW)
day 0		290.89 ± 8.13	96.62 ± 3.43	126.57 ± 2.35
day 3	control	271.15 ± 9.06 eA	111.24 ± 3.32 dA	106.19 ± 0.92 eA
	carvacrol	358.64 ± 4.01 aA	158.25 ± 4.29 aA	150.60 ± 1.33 aA
	cinnamaldehyde	340.43 ± 3.01 bA	144.45 ± 5.54 bA	139.75 ± 0.21 bA
	perillaldehyde	310.99 ± 2.02 cA	126.42 ± 2.87 cA	117.47 ± 0.39 cA
	linalool	302.15 ± 3.33 dA	125.65 ± 3.26 cA	113.88 ± 0.99 dA
day 6	control	235.62 ± 5.77 eB	103.09 ± 5.85 dA	80.62 ± 2.77 dB
	carvacrol	294.65 ± 3.28 aB	164.30 ± 4.72 aA	110.05 ± 0.72 aB
	cinnamaldehyde	277.55 ± 6.75 bB	144.34 ± 3.49 bA	104.10 ± 0.53 bB
	perillaldehyde	263.31 ± 4.81 cB	133.82 ± 3.81 cA	90.13 ± 0.40 cB
	linalool	253.77 ± 4.93 dB	128.67 ± 1.31 cA	89.34 ± 0.83 cB
day 9	control	236.14 ± 4.53 eB	92.85 ± 4.83 eB	62.98 ± 0.93 dC
	carvacrol	283.20 ± 2.25 aC	144.57 ± 4.29 aB	81.33 ± 0.76 aC
	cinnamaldehyde	270.77 ± 6.03 bB	133.05 ± 2.53 bB	75.69 ± 0.61 bC
	perillaldehyde	255.89 ± 4.25 cB	124.92 ± 0.41 cB	71.87 ± 0.31 cC
	linalool	245.85 ± 2.89 dB	110.61 ± 0.41 dB	70.96 ± 0.53 cC
day 12	control	188.48 ± 4.75 dC	83.50 ± 1.32 eC	55.37 ± 0.21 dD
	carvacrol	254.28 ± 1.80 aD	136.11 ± 5.18 aB	74.32 ± 0.83 aD
	cinnamaldehyde	234.80 ± 3.25 bC	126.67 ± 2.12 bC	68.62 ± 0.83 bD
	perillaldehyde	217.43 ± 8.29 cC	118.66 ± 0.37 cC	66.11 ± 0.31 cD
	linalool	207.46 ± 4.75 cC	110.67 ± 0.40 dB	66.57 ± 0.53 cD

[a]Data are expressed as the mean ± SD (n = 3). Values in the same column with different letters for each day were significantly different at $p < 0.05$. Lowercase letters represented significant difference among treatment factors, and capital letters represented significant difference among storage time factors.

distilled water and 1 mL of 0.1% ferric chloride, and the absorbance was measured at 700 nm. The result was expressed as the absorbance of mixtures measured at 700 nm.

High-Performance Liquid Chromatography (HPLC) Analysis of Anthocyanin and Phenolic Compounds. HPLC was used to separate and determine individual anthocyanin and phenolic compounds in Chinese bayberry fruit tissue. A total of 5 g of composite samples from 30 berries was extracted twice with 20 mL of 80% acetone containing 0.2% formic acid. The supernatants from the extracts described above were concentrated to dryness using a rotavapor in a water bath at 35 °C, dissolved in 5 mL of acidified water (3% formic acid), and then passed through a C18 Sep-Pak cartridge (Supelco Corp., Bellefonte, PA), 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. The anthocyanins and other phenolics were then recovered with 3.0 mL of acidified methanol, containing 3% formic acid. The methanol extract was passed though a 0.45 μm membrane filter (Millipore Corp., Bedford, MA). HPLC analysis was carried out by means of an Agilent HPLC series 1100 equipped with 130 Chemstation software and a model G1315B diode array detector (Agilent Corp., Santa Clara, CA). A total of 20 μL of samples was injected at ambient temperature (20 °C) into a reserved-phase Nova-Pak C18 column (250 × 5 mm, 5 μm, Agilent Corp.). The mobile phase consisted of 3% aqueous formic acid (A) and HPLC-grade methanol (B) with a linear gradient from 0 to 14% B at the first 1 min, followed by a linear gradient from 14 to 60% B for the next 39 min, and then followed by 100% B for 5 min before returning to the initial condition. The flow rate was 1 mL min^{-1}, and the wavelengths of detection were set at 280, 370, and 520 nm. Scanning between 240 and 550 nm was performed. Retention times and spectra were compared to those of the pure standards [gallic acid (purity ≥99%), protocatechuic acid (purity ≥97%), myricetin (purity ≥96%), quercetin-3-*o*-rutinoside (purity ≥97%), and cyanidin-3-glucoside (purity ≥98%) were purchased from Sigma Chemical Co. (St. Louis, MO)], and the results were confirmed by co-injection with authentic standards.

Statistical Analysis. Experiments were performed using a completely randomized design. All statistical analyses were performed using the SPSS 13.0 statistical package (SPSS, Inc., Chicago, IL). The data were analyzed by two-way analysis of variance (ANOVA) with treatment and storage time as factors. The means were separated by Tukey's test, and differences at $p < 0.05$ were considered to be significant.

RESULTS

Fruit Decay and Quality. All essential oil treatments in this study significantly inhibited fruit decay development compared to control (Table 1). The most effective compound in terms of mold retardation was carvacrol, followed by cinnamaldehyde, linalool, and perillaldehyde. At the end of the storage, decay incidence of carvacrol-treated fruit was only 19.11%, whereas 33.70% of fruit decay was shown in control fruit. As shown in Table 1, TSS, TA, and AsA contents of Chinese bayberry decreased gradually during storage. No significant ($p > 0.05$) differences in TSS, TA, and AsA levels were observed among all of the essential oil treatments during storage at 0 °C.

Total Phenolic, Anthocyanin, and Flavonoid Contents. The total phenolic and flavonoid contents in control fruit decreased gradually during storage, whereas the total anthocyanin content in control fruit exhibited a slight increase over the first 3 days and decreased gradually afterward (Table 2). This indicated that the content of anthocyanins could be accumulated in Chinese bayberry fruit during the storage time. All essential oil treatments maintained a significantly ($p < 0.05$) higher total phenolic content during the storage compared to the control. The most effective compound was carvacrol, followed by cinnamaldehyde. However, no significant difference was found in total phenolic and total flavonoid contents between linalool and perillaldehyde treatments.

HPLC Analysis of Chinese Bayberry Flavonoids. Individual phenolic and anthocyanin compounds in Chinese

Table 3. Effect of Essential Oil Treatment on Gallic Acid, Protocatechuic Acid, Myricetin, Quercetin-3-*o*-rutinoside, and Cyanidin-3-glucoside Contents in Chinese Bayberry[a]

storage (days)	treatment	gallic acid[b]	protocatechuic acid[b]	myricetin[b]	quercetin-3-*o*-rutinoside[b]	cyanidin-3-glucoside[b]
day 0		4.76 ± 0.56	3.86 ± 0.43	8.36 ± 0.55	27.94 ± 1.74	50.19 ± 2.00
day 3	control	5.27 ± 0.37 dA	4.37 ± 0.29 dA	16.32 ± 1.12 eA	58.23 ± 1.26 eB	70.20 ± 0.97 eA
	carvacrol	8.44 ± 0.39 aA	7.27 ± 0.25 aA	32.83 ± 1.53 aB	89.31 ± 1.14 aA	109.44 ± 1.80 aB
	cinnamaldehyde	7.28 ± 0.11 bA	6.85 ± 0.15 bA	28.95 ± 1.18 bA	83.21 ± 1.04 bA	103.19 ± 0.95 bA
	perillaldehyde	6.36 ± 0.50 cA	5.13 ± 0.13 cA	22.56 ± 0.79 dB	67.21 ± 1.20 dB	90.26 ± 0.64 dA
	linalool	6.54 ± 0.34 cA	5.48 ± 0.24 cA	25.47 ± 0.8 cB	70.88 ± 0.94 cB	94.22 ± 1.59 cB
day 6	control	3.97 ± 0.31 dB	2.47 ± 0.12 dB	17.41 ± 0.87 dA	64.69 ± 0.80 eA	70.57 ± 0.65 eA
	carvacrol	6.69 ± 0.33 aB	3.84 ± 0.11 aB	35.29 ± 0.95 aA	90.89 ± 0.90 aA	115.44 ± 1.36 aA
	cinnamaldehyde	6.05 ± 0.15 bB	3.43 ± 0.05 bB	29.52 ± 0.40 bA	83.69 ± 2.00 bA	105.08 ± 0.89 bA
	perillaldehyde	5.16 ± 0.43 cC	3.26 ± 0.07 cB	27.78 ± 0.94 cA	72.49 ± 0.39 dA	90.92 ± 0.53 dA
	linalool	5.64 ± 0.09 cB	3.05 ± 0.14 cB	28.03 ± 1.01 cA	74.07 ± 0.95 cA	97.73 ± 0.75 cA
day 9	control	3.85 ± 0.10 cB	2.36 ± 0.07 dB	9.89 ± 0.56 dB	33.76 ± 0.26 dC	68.57 ± 0.91 eA
	carvacrol	6.39 ± 0.15 aB	3.59 ± 0.08 aC	22.03 ± 0.58 aC	70.85 ± 0.95 aB	95.96 ± 0.40 aC
	cinnamaldehyde	6.01 ± 0.27 aB	3.16 ± 0.25 bB	19.49 ± 0.37 bB	67.83 ± 0.88 bB	92.91 ± 0.49 bB
	perillaldehyde	5.50 ± 0.28 bB	2.62 ± 0.23 cC	15.89 ± 0.51 cC	54.70 ± 0.87 cC	81.44 ± 1.33 dB
	linalool	5.04 ± 0.49 bC	2.86 ± 0.38 bcB	16.67 ± 0.52 cC	54.48 ± 0.56 cC	84.33 ± 0.31 cC
day 12	control	3.41 ± 0.05 eC	2.34 ± 0.15 dB	8.20 ± 0.74 eC	31.36 ± 0.9 eC	57.84 ± 1.46 eB
	carvacrol	5.51 ± 0.28 aC	3.21 ± 0.28 aD	20.66 ± 0.29 aC	68.76 ± 1.28 aB	95.43 ± 0.50 aC
	cinnamaldehyde	4.98 ± 0.14 bC	2.74 ± 0.20 bC	18.64 ± 0.78 bB	62.61 ± 0.77 bC	90.72 ± 0.70 bC
	perillaldehyde	4.52 ± 0.08 cC	2.75 ± 0.14 bC	14.19 ± 0.88 dC	51.25 ± 0.68 dC	80.42 ± 0.38 dB
	linalool	4.05 ± 0.22 dC	2.40 ± 0.18 cC	15.88 ± 0.29 cC	54.22 ± 0.97 cC	83.04 ± 1.62 cC

[a]Data are expressed as the mean ± SD (n = 3). Values in the same column with different letters for each day were significantly different at $p < 0.05$. Lowercase letters represented significant difference among treatment factors, and capital letters represented significant difference among storage time factors. [b]Data are expressed as micrograms per gram of fresh weight.

Table 4. Effect of the Essential Oil Treatment on SOD, POD, CAT, and APX Activities in Chinese Bayberry[a]

storage (days)	treatment	SOD activity	POD activity	CAT activity	APX activity
day 0		157.76 ± 6.20	78.65 ± 2.34	55.32 ± 2.12	28.23 ± 0.61
day 3	control	130.33 ± 2.01 dA	80.17 ± 1.13 dA	41.25 ± 1.21 eA	18.50 ± 0.92 eA
	carvacrol	165.26 ± 4.21 aA	99.84 ± 3.13 aA	71.93 ± 2.15 aA	43.66 ± 0.70 aA
	cinnamaldehyde	152.64 ± 2.12 bA	92.68 ± 2.35 bA	63.54 ± 0.58 bA	36.19 ± 1.48 bA
	perillaldehyde	144.90 ± 1.13 cA	85.73 ± 2.07 cA	55.44 ± 1.63 cA	33.39 ± 0.61 cA
	linalool	144.38 ± 2.65 cA	84.24 ± 1.85 cA	51.11 ± 1.51 dA	27.38 ± 0.73 dA
day 6	control	121.60 ± 4.32 dB	51.20 ± 2.12 dB	33.36 ± 2.08 dB	14.57 ± 1.25 dB
	carvacrol	153.35 ± 2.32 aB	79.14 ± 2.35 aB	51.45 ± 1.23 aB	36.76 ± 0.74 aB
	cinnamaldehyde	144.86 ± 1.38 bB	71.77 ± 4.32 bB	47.67 ± 1.53 bB	32.42 ± 1.25 bB
	perillaldehyde	138.49 ± 1.01 cB	63.10 ± 2.65 cB	40.12 ± 1.32 cB	22.47 ± 1.23 cB
	linalool	137.82 ± 1.63 cB	62.68 ± 2.32 cB	39.66 ± 1.15 cB	21.03 ± 1.05 cB
day 9	control	115.71 ± 2.12 dB	39.84 ± 2.25 cC	30.45 ± 1.03 dB	13.05 ± 0.65 dB
	carvacrol	139.46 ± 2.23 aC	59.61 ± 3.25 aC	45.37 ± 1.21 aC	28.08 ± 1.09 aC
	cinnamaldehyde	134.53 ± 1.61 bC	56.39 ± 2.02 aC	42.42 ± 0.63 bC	24.75 ± 0.73 bC
	perillaldehyde	123.65 ± 1.33 cC	47.31 ± 2.12 bC	37.94 ± 1.28 cB	15.85 ± 0.62 cC
	linalool	122.09 ± 0.81 cC	48.35 ± 3.01 bC	37.24 ± 1.15 cB	17.04 ± 1.22 cC
day 12	control	95.29 ± 1.23 dC	38.51 ± 2.32 cC	22.66 ± 0.83 dC	8.95 ± 1.29 cC
	carvacrol	135.25 ± 1.63 aC	56.16 ± 2.63 aC	42.66 ± 2.21 aC	25.16 ± 1.23 aC
	cinnamaldehyde	131.27 ± 1.14 bC	54.13 ± 2.57 aC	37.86 ± 1.23 bD	23.25 ± 1.05 aC
	perillaldehyde	116.92 ± 1.02 cD	45.67 ± 2.02 bC	32.13 ± 1.42 cC	14.25 ± 0.72 bC
	linalool	118.19 ± 1.12 cD	48.06 ± 1.89 bC	33.98 ± 0.63 cC	15.68 ± 0.81 bC

[a]Data are expressed as the mean ± SD (n = 3). Values in the same column with different letters for each day were significantly different at $p < 0.05$. Lowercase letters represented significant difference among treatment factors, and capital letters represented significant difference among storage time factors.

bayberries were shown in Table 3. Gallic acid, protocatechuic acid, myricetin, and quercetin-3-*o*-rutinoside were the four main phenolic compounds, while cyanidin-3-glucoside was the major anthocyanin in Chinese bayberries. In general, all essential oils significantly ($p < 0.05$) maintained higher levels of cyanidin-3-glucoside and other individual phenolic compounds compared to the control. Carvacrol was also the most effective compound in maintaining individual phenolic and anthocyanin compounds in Chinese bayberries among all of the essential oils.

Antioxidant Enzyme Activities. The activities of antioxidant enzymes (including SOD, CAT, POD, and APX) of Chinese bayberries increased slightly on the third day and decreased gradually during the remainder of storage in carvacrol- and cinnamaldehyde-treated fruit, while in control

Table 5. Effect of Essential Oil Treatment on $O_2\cdot^-$ Production, ·OH Scavenging Activity, DPPH Scavenging Activity, and Reducing Power in Chinese Bayberry[a]

storage (days)	treatment	$O_2\cdot^-$ scavenging activity (%)	·OH scavenging activity (%)	DPPH scavenging activity (%)	reducing power (A_{700})
day 0		34.01 ± 0.37	32.19 ± 1.15	88.33 ± 0.12	0.65 ± 0.01
day 3	control	22.93 ± 0.96 dA	25.56 ± 0.40 eA	86.19 ± 0.20 dA	0.50 ± 0.01 dA
	carvacrol	36.77 ± 0.65 aA	31.59 ± 0.06 aA	90.49 ± 0.12 aA	0.71 ± 0.03 aA
	cinnamaldehyde	30.08 ± 0.45 bA	30.36 ± 0.06 bA	89.22 ± 0.35 bA	0.65 ± 0.02 bA
	perillaldehyde	26.15 ± 0.75 cA	29.21 ± 0.25 cA	88.75 ± 0.21 cA	0.59 ± 0.02 cA
	linalool	25.71 ± 0.41 cA	27.48 ± 0.12 dA	88.57 ± 0.12 cA	0.56 ± 0.01 cA
day 6	control	18.48 ± 0.71 dB	15.15 ± 0.61 dB	65.71 ± 0.15 dB	0.41 ± 0.02 dB
	carvacrol	29.88 ± 0.54 aB	25.57 ± 0.29 aB	73.90 ± 0.35 aB	0.69 ± 0.01 aA
	cinnamaldehyde	24.05 ± 0.81 bB	22.85 ± 0.21 bB	71.63 ± 0.25 bB	0.63 ± 0.02 bA
	perillaldehyde	22.73 ± 0.51 cB	18.86 ± 0.15 cB	71.09 ± 0.55 bB	0.58 ± 0.01 cA
	linalool	21.73 ± 0.63 cB	18.58 ± 0.30 cB	68.59 ± 0.06 cB	0.57 ± 0.03 cA
day 9	control	11.41 ± 0.36 dC	13.52 ± 0.40 dC	47.32 ± 0.30 dC	0.35 ± 0.01 cC
	carvacrol	20.77 ± 0.72 aC	18.64 ± 0.15 aC	51.83 ± 0.15 aC	0.57 ± 0.01 aB
	cinnamaldehyde	16.15 ± 0.70 bC	17.33 ± 0.25 bC	50.41 ± 0.10 bC	0.56 ± 0.02 aB
	perillaldehyde	13.92 ± 0.98 cC	12.09 ± 0.26 cC	50.04 ± 0.21 cC	0.52 ± 0.00 bB
	linalool	14.39 ± 0.52 cC	11.91 ± 0.30 cC	49.01 ± 0.12 cC	0.50 ± 0.02 bB
day 12	control	8.09 ± 0.58 dD	7.35 ± 0.39 dD	24.31 ± 0.45 dD	0.25 ± 0.01 cD
	carvacrol	13.77 ± 0.61 aD	12.52 ± 0.12 aD	30.47 ± 0.46 aD	0.47 ± 0.02 aC
	cinnamaldehyde	11.57 ± 0.49 bD	10.36 ± 0.46 bD	28.73 ± 0.15 bD	0.45 ± 0.02 aC
	perillaldehyde	9.69 ± 0.95 cD	9.45 ± 0.23 cD	27.34 ± 0.38 cD	0.41 ± 0.01 bC
	linalool	10.22 ± 0.59 cD	9.24 ± 0.21 cD	27.19 ± 0.12 cD	0.40 ± 0.02 bC

[a]Data are expressed as the mean ± SD ($n = 3$). Values in the same column with different letters for each day were significantly different at $p < 0.05$. Lowercase letters represented significant difference among treatment factors, and capital letters represented significant difference among storage time factors.

bayberries and in those treated with perillaldehyde and linalool, decreased antioxidant enzymes occurred from day 0 (Table 4). All essential oil treatments significantly ($p < 0.05$) enhanced the antioxidant enzyme activities of Chinese bayberries. Chinese bayberries treated with carvacrol had the highest activities for SOD, POD, CAT, and APX. However, little effect in antioxidant enzyme activities was found between the treatment with linalool and perillaldehyde.

$O_2\cdot^-$, ·OH, and DPPH Radical Scavenging Capacities and Reducing Power. The antioxidant capacities measured as $O_2\cdot^-$, ·OH, and DPPH radical scavenging capacities and reducing power in Chinese bayberry extracts were shown in Table 5. $O_2\cdot^-$, ·OH, and DPPH radical scavenging capacities and reducing power in bayberry fruit were decreased gradually during storage time compared to the harvest time (day 0). The scavenging activities of $O_2\cdot^-$ and ·OH radicals decreased rapidly during the storage time, while DPPH scavenging activities and reducing power decreased slowly before day 6. All essential oil treatments significantly ($p < 0.05$) maintained the higher value of $O_2\cdot^-$ and ·OH radical scavenging capacities, whereas carvacrol treatment was the most effective followed by cinnamaldehyde. No significant difference was found in $O_2\cdot^-$ and ·OH radical scavenging capacities between linalool and perillaldehyde treatments. DPPH radical scavenging capacities and reducing power decreased gradually with storage time. All essential oil treatments significantly ($p < 0.05$) inhibited the decrease of DPPH radical scavenging capacity and reducing power.

DISCUSSION

In the present study, we found that all essential oils delayed fungal decay development of Chinese bayberry fruit. Similar results were also observed in blueberries and mango fruit.[5,8] The effective concentration of essential oils on reducing decay ranged from 1 to 200 μL L^{-1} according to various horticultural crops.[5−8] It is possible that different horticultural crops respond differently to the treatments, and various methods of application produce diverse results. In addition, essential oil combined with a modified atmosphere packaging (MAP) or edible film coating improved shelf life in table grapes and papaya.[25,26] This indicated that the techniques of essential oil treatment or combining with other postharvest procedures have protential application in the berry fruit industry. Thus, more studies are needed to explore new treatment methods and to confirm their optimum concentration, combination mode, and application condition for a specific commodity.

It has been postulated that the control of postharvest diseases by many essential oils is due to their direct inhibitory effect on pathogen growth and interference with active sites of enzymes and cellular metabolism.[27] Moreover, essential oils may also change the permeability of membranes of the microbes for cations and alter the ion gradients that lead to impairment of vital processes in microbial cells and eventually cell death.[28] In addition, some essential oils, such as carvacrol, perillaldehyde, and linalool, could induce constitutive increases in the antioxidants of plant tissues, including enzymatic and non-enzymatic systems.[8,11] Therefore, 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 bayberry fruit; however, such action mechanisms need to be further investigation.

Phenolic and anthocyanin compounds in berry fruit play an important role in scavenging free radicals. Thus, these compounds may help protect cells against the oxidative damage caused by free radicals.[2,3] Previous studies have been reported that the level of antioxidants of fruits can be influenced by various postharvest treatments, including heat, UV−C illumi-

nation, high oxygen treatment, and natural volatile compound vapor.[1,10,29,30] In our study, total phenolic, total anthocyanin, and individual flavonoid contents in Chinese bayberry were increased by the different essential oil treatments. This is consistent with the findings by Wang et al.[8] in blueberry. Previous studies also have suggested that methyl jasmonate treatment can enhance total phenolic and total anthocyanin contents in Chinese bayberry and raspberry.[9,10] It is hypothesized that essential oils would act as "signaling compounds" that trigger a signal that resembles a mild stress to the fruit. As a defense response, fruit produces additional phenolic compounds and flavonoids and increases their antioxidant activities.[31] Several studies have shown that the accumulation of phenols and anthocyanins paralleled the increase in phenylalanine ammonialyase (PAL) activity in Chinese bayberry and grape fruits.[9,32] It is possible that essential oils play positive roles in affecting plant secondary metabolites and stimulating biosynthesis of phenolic and anthocyanin compounds by inducing higher activity of PAL. However, the exact mechanisms for enhancing antioxidant capacities of Chinese bayberries by essential oils need to be further studied.

Antioxidant enzymes also have a positive relationship to antioxidant capacities. SOD, a class of metal-containing proteins, catalyze the dismutation reaction of $O_2\cdot^-$ into H_2O_2. POD, CAT, and APX converts H_2O_2 to oxygen and water and, therefore, limits the potential for further free radical production from H_2O_2.[33] Chanjirakul et al.[10] reported that antioxidant enzyme activities in raspberries were increased by methyl jasmonate treatment. Our results showed that Chinese bayberry fruit treated with essential oils had higher antioxidant enzyme activities (SOD, POD, CAT, and APX) than the control (Table 4). This is consistent with our previous findings in raspberry.[11]

It has been well-known that Chinese bayberries are a good source of natural antioxidants, including phenolic compounds and anthocyanins, which have a beneficial effect on scavenging free radicals.[3] Several previous studies have been shown that essential oils increased the antioxidant activity in berry fruits, such as strawberries, blueberries, and raspberries.[8,10,34] In the present study, the scavenging activity against DPPH, superoxide, and hydroxyl radicals and the reducing power in essential-oil-treated berries were significantly higher than those in the control fruit (Table 5). It has been reported that the scavenging activity against free radicals in berry fruit is associated with antioxidant enzyme activities or contents of phenolic and anthocyanin compounds.[10,33] It could be possible that essential oil treatment activated the antioxidant system or secondary metabolites, which was responsible for scavenging free radicals and enhancing antioxidant capacity.

In conclusion, postharvest application of essential oil significantly reduced fruit decay and enhanced antioxidant capacity of Chinese bayberry. Essential oils, such as carvacrol, cinnamaldehyde, perillaldehyde, and linalool, have positive effects on increasing antioxidant capacities, enhancing antioxidant enzyme activities, and maintaining a higher level of total phenolics, anthocyanins, flavonoids, and individual phenolic and anthocyanin compounds. Thus, these natural products have the potential application to reduce decay and preserve the high quality of Chinese bayberry fruit.

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Notes

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