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Effect of Methyl Jasmonate in Combination with Ethanol Treatment on Postharvest Decay and Antioxidant Capacity in Chinese Bayberries

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The effects of methyl jasmonate (MeJA) in combination with ethanol (EtOH) treatment on green mold rot caused by *Penicillium citrinum*, natural decay, and antioxidant capacity in harvested Chinese bayberries were investigated. MeJA at 10 μmol L^{-1} in combination with EtOH at 22.32 μmol L^{-1} was most effective in reducing green mold rot and natural decay. This combined treatment also significantly inhibited spore germination and germ tube elongation of the pathogen in vitro compared with MeJA alone or control. Meanwhile, the combination-treated bayberries exhibited highest reducing power, scavenging activities against superoxide, hydroxyl, and 1,1-diphenyl-2-picrylhydrazyl radicals, and contents of total phenolics, flavonoids, and anthocyanins as well as individual phenolic compounds. Fruit quality parameters were not significantly affected by these treatments. These results suggest that the combination of MeJA and EtOH had an additive effect in reducing postharvest decay and improving antioxidant capacity in Chinese bayberries.

KEYWORDS: Chinese bayberries; methyl jasmonate; ethanol; decay; antioxidant capacity

INTRODUCTION

Chinese bayberry (*Myrica rubra* Sieb. & Zucc.), a subtropical fruit native to China, consists of capsule-like cellules termed flesh segments and is noted for its attractive red to purple color and appealing flavor. Meanwhile, the fruit is also a good source of natural antioxidants, thus making it potentially effective in inhibiting oxidation of human low-density lipoproteins (*1*). However, Chinese bayberry has a very short shelf life because of its high susceptibility to fungal infection. The most common postharvest pathogen observed in Chinese bayberry is *Penicillium citrinum* Thom, which is difficult to control because of its ability to germinate at temperatures between 0 and 35 °C and proliferate by mycelial growth from berry to berry (*2*). The negative impact of fungicides on both the environment and human health has encouraged researchers to explore alternative measures for controlling postharvest diseases (*3*).

Methyl jasmonate (MeJA), as a naturally occurring plant growth regulator, has been shown to play an important role in activating resistance of host against pathogens and, therefore, to effectively suppress some important postharvest diseases in a number of fruits (*4*−*7*). In addition, it has been reported that a postharvest MeJA treatment retained higher levels of sugars and organic acids in radishes and mangoes, thereby maintaining better quality (*8*, *9*). However, the relatively narrow antimicrobial spectrum has made MeJA treatment a less attractive option than the synthetic fungicides (*6*). Therefore, integrated strategies have been investigated to improve the antimicrobial activity of MeJA treatment. For example, combining antagonistic yeast or hot air with MeJA was found to be very effective in enhancing the efficacy of decay control of MeJA treatment (*10*, *11*).

Ethanol (EtOH) vapor treatment has shown promise in preventing postharvest diseases in some harvested commodities including Chinese bayberry (*12*). The effect of EtOH in controlling decay could be associated with its fungicidal properties. However, ethanol is a volatile compound, which does not deposit persistent antifungal residue to protect fruit from recontamination after treatment throughout the storage period (*13*). It is therefore necessary to find a combined treatment strategy for EtOH to overcome this shortcoming.

Antioxidant content is becoming an increasingly important parameter with respect to fruit and vegetable quality. The application of MeJA maintained higher levels of bioactive compounds and enhanced antioxidant capacity in some berry fruits including raspberry (*14*), strawberry (*15*), blackberry (*16*), and Chinese bayberry (*7*). Treatment with low doses of EtOH was also reported to increase anthocyanins level in table grapes, suggesting the potential of using EtOH in enhancing antioxidant capacity in fruit (*17*).

Because both MeJA and EtOH are already classified by the U.S. Food and Drug Administration (FDA) as Generally Recognized As Safe (GRAS) substances, they may have potential commercial applications in postharvest treatments for quality maintenance by reducing decay and enhancing antioxidant activity. Thus, the objectives of this study were to evaluate the effect of the combination of MeJA and EtOH in controlling green mold decay caused by

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P. citrinum on harvested Chinese bayberries and to investigate its influence on total phenolics, total flavonoids, total anthocyanins, and antioxidant activity as well as the main individual phenolic constituents during postharvest storage. In addition, the effect of the combined treatment on fruit natural decay and quality was also investigated.

MATERIALS AND METHODS

Chemicals. Commercial standards of gallic acid (purity ≥ 99%), protocatechuic acid (purity ≥ 97%), quercetin-3-*O*-rutinoside (purity ≥ 97%), myricetin (purity ≥ 96%), and cyanidin-3-glucoside (purity ≥ 98%) were purchased from Sigma Chemical Co. (St. Louis, MO). Methanol, acetone, ethanol, acetaldehyde, formic acid, and water were of HPLC grade and purchased from Scigene Co. (Nanjing, Jiangsu, China). 1,1-Diphenyl-2-picrylhydrazyl (DPPH), Folin−Ciocalteu, deoxyribose, nitroblue tetrazolium (NBT), riboflavin, methionine, ferric chloride, methionine, potassium ferricyanide, and trichloroacetic acid (TCA) reagents were of analytical grade and purchased from Sigma Chemical Co.

Fruit and Pathogen. Chinese bayberries (*M. rubra* Seib & Zucc. cv. Wumei) were hand-harvested at mature stage from an orchard in Suzhou, Jiangsu province, China, and transported within 4 h to our laboratory. At harvest, the firmness of the berry fruit was 4.2 N as determined by a TA-XT2i texture analyzer (UK) with a total soluble solids content of 10.5%. The berries were selected for uniform size and color and the absence of visual defects before use.

The pathogen *P. citrinum* was isolated from infected Chinese bayberries and preserved on potato dextrose agar media (PDA; extract of boiled potatoes, 200 mL; dextrose, 20 g; agar, 20 g in 800 mL of deionized water) at 2 °C. The fresh cultures were incubated on PDA at 25 °C before use. Spore suspensions of *P. citrinum* were prepared by removing the spores from the sporulating edges of the 14-day-old cultures with a bacteriological loop, suspending them in sterile distilled water containing 0.05% (w/v) Tween-80 and filtering through four layers of sterile cheesecloth to remove any adhering mycelia. Spore concentrations were determined with a hemocytometer and adjusted to 1.0×10^6 spores mL^{-1} with sterile distilled water.

Effects of MeJA and EtOH in Controlling Green Mold Decay on Artificially Inoculated Chinese Bayberries. Ten micromoles per liter MeJA and 22.32 μmol L^{-1} EtOH were chosen for treatment in this study on the basis of previous experiments in Chinese bayberries (*7*, *18*). Chinese bayberries were superficially disinfected by immersion for 1 min in 1% (v/v) sodium hypochlorite, rinsed with fresh water, and allowed to dry by air at a room temperature (20 °C). Then the fruits were inoculated by spraying them with the spore suspension of *P. citrinum*. After air-drying, the inoculated fruits were divided into four groups and subjected to the following treatments: (1) CK (control), the inoculated fruits did not receive any treatment and served as the control; (2) MeJA, the inoculated fruits were placed in a 40 L airtight container for 10 μmol L^{-1} MeJA treatment (an appropriate amount of MeJA liquid was spotted onto filter paper inside the container and incubated at 20 °C for 6 h); (3) EtOH, the inoculated fruits were placed in a 40 L airtight container for 22.32 μmol L^{-1} EtOH treatment (an appropriate amount of ethanol liquid was spotted onto filter paper inside the container and incubated at 20 °C for 6 h); (4) MeJA + EtOH, the inoculated fruits were placed in a 40 L airtight container and treated with 10 μmol L^{-1} MeJA plus 22.32 μmol L^{-1} EtOH at 20 °C for 6 h. After treatments, all of the fruits were sealed in polyethylene-lined plastic boxes to retain high relative humidity (approximately 90%) and stored at 1 °C for 8 days. The percentage of decayed fruit (showing visible mold growth) was recorded at 2-day intervals during the storage. There were three replicates of 60 fruit each per treatment, and the experiment was conducted twice.

Effects of MeJA and EtOH on Spore Germination and Germ Tube Elongation of *P. citrinum* in vitro. The tests were conducted using 10 mL glass tubes containing 6 mL of PDB (PDA without agar), to which an appropriate amount of sterile liquid MeJA or EtOH or both was added to achieve the required treatment concentration as described above, with no addition of MeJA or EtOH as control. Then aliquots (100 μL) of the spore suspensions of *P. citrinum* were added into all tubes. After 24 h of incubation at 25 °C on a rotary shaker (100 rpm), at least 100 spores were observed to determine germination rate and germ tube length in three different microscopic fields. Spores were considered to be germinated when germ tube length was equal to or greater than spore length. Each treatment consisted of three replicates with 10 PDB tubes, and the experiment was repeated twice.

Effects of MeJA and EtOH on Antioxidant Capacity in Harvested Chinese Bayberries. Fresh Chinese bayberries without inoculation were respectively treated with 10 μmol L^{-1} MeJA, 22.32 μmol L^{-1} EtOH, or their combination and then stored at 1 °C for 8 days as described above. Tissue samples were collected from fruits without defects before treatment (time 0) and at 2-day intervals during the storage for measurements of antioxidant parameters. The samples were mixed and frozen immediately in liquid nitrogen and then stored at −80 °C until used. There were three replicates of 120 fruits each per treatment, and the experiment was conducted twice.

Extraction Preparation. To prepare the fruit extract, 5 g of frozen tissue from each of three replicates at each time point was extracted twice with 10 mL of precooled 80% (v/v) acetone containing 0.2% (v/v) formic acid and then centrifuged at 10000*g* for 20 min at 4 °C. The supernatants were combined, and the final volume was made up to 25 mL for analysis of total phenolics, total anthocyanins, total flavonoids, and oxygen radical scavenging activities and for HPLC analysis for main individual phenolic constituents.

Total Phenolics, Flavonoids, and Anthocyanins Determinations. Total phenolics content of bayberry extract was determined with Folin−Ciocalteu reagent according to the method of Slinkard and Singleton (*19*) using gallic acid as a standard. Results were expressed as milligrams of gallic acid (GAE) equivalent per 100 g of fresh weight (FW).

Total anthocyanins content of bayberry extract was measured using the pH differential method (*20*). Absorbance was measured at 510 and 700 nm, respectively, in different buffers at pH 1.0 and 4.5, using $A = [(A_{510} - A_{700})_{pH1.0} - (A_{510} - A_{700})_{pH4.5}]$ with a molar extinction coefficient for cyanidin-3-glucoside of 29600. Results were expressed as milligrams of cyanidin-3-glucoside (C3G) equivalents per 100 g of FW.

Total flavonoids content of bayberry extract was measured according to method of Toor and Savage (*21*). Absorbance was measured at 510 nm, and results were expressed as milligrams of quercetin-3-*O*-rutinoside (rutin) equivalents per 100 g of FW.

HPLC Analysis. Individual phenolic compounds in tissue samples were separated and determined by high-performance liquid chromatography (HPLC) according to the method of Bao et al. (*1*) with slight modifications. 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 C_{18} Sep-Pak cartridge (Supelco Corp., Bellefonte, PA), which was previously activated with methanol followed by water and then 3% (v/v) 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 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 1100 series equipped with 130 Chemstation software and a model G1314A diode array detector (Agilent Corp., Santa Clara, CA). Twenty-microliter samples were injected at ambient temperature (20 °C) into a reversed-phase Nova-Pak C_{18} column (250 × 4.6 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 with those of authentication standards.

Radical Scavenging Activities and Reducing Power Determinations. The assay of superoxide scavenging activity was based on the capacity of the extract to inhibit formazan formation by scavenging the superoxide radicals generated in a riboflavin−light−nitroblue tetrazolium system (*22*). The percentage inhibition of superoxide anion generation was calculated using the following formula: superoxide radical scavenging activity (%) = 100 − (absorbance of sample/absorbance of control) × 100.

Hydroxyl radical scavenging activity of the extract was determined according to the deoxyribose method described previously (*23*). The result

was calculated according to the following formula: hydroxyl radical scavenging activity (%) = 100 − (absorbance of sample/absorbance of control) × 100.

The DPPH radical scavenging activity of the extract was estimated following the method of Liu et al. (*24*). 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 the extract was determined according to the method of Ozsoy et al. (*25*). The result was expressed as the absorbance of mixtures measured at 700 nm.

Effects of MeJA and EtOH on Natural Decay and Quality in Chinese Bayberries. Fresh Chinese bayberries without inoculation were respectively treated with 10 μmol L^{-1} MeJA, 22.32 μmol L^{-1} EtOH, or their combination as described above and then stored at 20 °C for 3 days or at 1 °C for 7 days followed by 1 day at 20 °C to investigate the effect of these treatments for reducing natural decay and maintaining the quality of Chinese bayberries under stimulated distribution conditions. Decay incidence and quality parameters were determined afterward. There were three replicates of 120 fruits each per treatment, and the experiment was conducted twice.

Total Soluble Solids (TSS), Titratable Acidity (TA), and pH Value Assays. Twenty fruits from each replicate were wrapped in cheesecloth and squeezed with a hand press, and the juice was analyzed for TSS, TA, and pH value. TSS was determined at 25 °C with a portable refractometer (WYT-4, Quanzhou, China) and expressed as °Brix. TA was determined by titrating 20 mL of bayberry juice to pH 8.2 with 0.1 mol L^{-1} NaOH and expressed as millimoles of H^+ per 100 g of FW. pH value was measured with a pH-meter (PHS-25B, Shanghai, China).

Ethanol and Acetaldehyde Assays. Ethanol and acetaldehyde levels were measured according to the method of Zhang et al. (*12*) with some modifications. Juice from 10 g of 20 fruits from each replicate was incubated in a water bath at 60 °C for 2 h, and a 1 mL gas sample was withdrawn from the headspace of a 20 mL test tube with a rubber cap by a plastic hypodermic syringe and analyzed by a gas chromatograph (model SP-6800A; Lunan Chemical Engineering Instrument Co. Ltd., Shandong, China) with a flame ionization detector (FID) and a GDX-502 activated alumina glass column. The injector, oven, and detector temperatures were 140, 110, and 140 °C, respectively. Nitrogen was the carrier gas. The results were expressed as nanomoles per kilogram of FW.

Sensory Analysis. Ten panelists, aged between 20 and 50 years old, were recruited from the students and personnel of our laboratory and trained before each evaluation. The panelists received 25 fruits from each treatment per evaluation in random order. The samples were presented to the panelists in individual rooms under white lights. The panelists were subsequently asked to score each fruit for firmness, color, juiciness, flavor (including sweetness, acidity, and odor), taste, and visual appearance on a nine-point scale: 1 = extremely poor, 3 = poor, 5 = acceptable (limit of marketability), 7 = good, 9 = excellent.

Statistical Analysis. Experiments were performed using a completely randomized design. Statistical analyses of variance were calculated over two factors: treatment and time in storage using software SPSS 13.0 (SPSS Inc., Chicago, IL). The significant main effects and interactions were chosen and successively analyzed through a Duncan's or Tukey's multiple-range test. Differences at $P < 0.05$ were considered to be significant. For percentage values, statistical analysis was carried out after arc sin transformation.

RESULTS

Effects of MeJA and EtOH in Controlling Green Mold Decay on Artificially Inoculated Chinese Bayberries. Both postharvest treatments resulted in significantly lower incidence of green mold decay caused by *P. citrinum* compared with controls during 8 days of storage at 1 °C (**Figure 1**). No significant difference of decay incidence was found between the two treatments. The combined treatment of MeJA with EtOH further reduced green mold decay in comparison with the treatment of MeJA or EtOH alone.

Effects of MeJA and EtOH on Spore Germination and Germ Tube Elongation of *P. citrinum* in Vitro. EtOH, when applied either alone or in combination with MeJA, was capable of inhibiting the growth of *P. citrinum*; the spore germination rate and germ tube length were significantly lower in the two treated samples than in the controls after 24 h of incubation at 25 °C (**Table 1**). On the contrary, MeJA alone had no effect on *P. citrinum* growth.

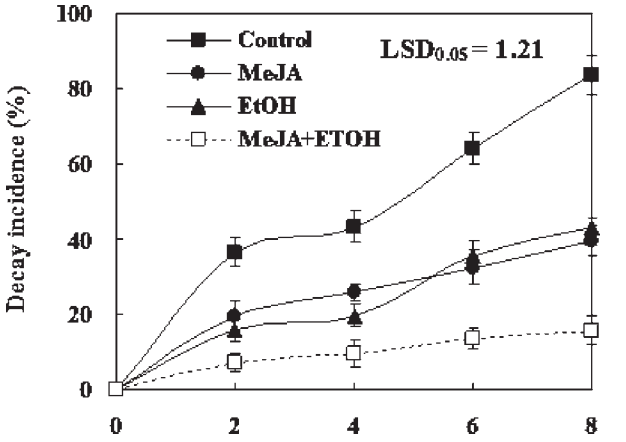

Figure 1. Effects of MeJA at 10 μmol L^{-1} and EtOH at 22.32 μmol L^{-1} on green mold decay on Chinese bayberries artificially inoculated with *P. citrinum* during storage at 1 °C for 8 days. Data are expressed as the mean ± SE of triplicate assays. Vertical bars represent the standard errors of the means.

Table 1. Effects of MeJA at 10 μmol L^{-1} and EtOH at 22.32 μmol L^{-1} on Spore Germination and Germ Tube Elongation of *P. citrinum* in Vitro[a]

treatment	spore germination (%)	germ tube length (μm)
control	52.33 a	12.51 a
MeJA	49.86 a	11.57 a
EtOH	27.12 b	4.25 b
MeJA + EtOH	24.54 b	3.71 b

[a] Spore germination and germ tube length were measured microscopically using approximately 100 spores of the pathogen after 24 h of incubation at 25 °C in PDB tubes. Data are expressed as means of triplicate assays. Values in a column followed by different letters are significantly different according to Duncan's multiple-range test at the $P < 0.05$ level.

Effects of MeJA and EtOH on Total Phenolics, Anthocyanins, and Flavonoids Contents in Harvested Chinese Bayberries. The total phenolics and flavoniods contents in control fruit decreased gradually during storage at 1 °C for 8 days. However, the total anthocyanin content increased over the first 2 days and then decreased gradually during the remainder of storage (**Figure 2**). Treatment and storage time significantly affected total phenolics, anthocyanins, and flavonoids contents of Chinese bayberry (**Table 2**). Treatment with MeJA or EtOH alone remarkably inhibited the decrease in the contents of total phenolics and total anthocyanins and maintained higher levels compared with the controls during 8 days of storage at 1 °C. Similarly, MeJA alone also resulted in significantly higher level of total flavoniods in comparison with the controls during the whole storage. However, EtOH by itself was not as effective in maintaining total flavoniods content as treatment with MeJA alone. The combination-treated fruits showed the highest level of total phenolics, anthocyanins, and flavonoids among all treatments. On the eighth day of storage, the contents of total phenolics, total anthocyanins, and total flavoniods in combination-treated fruit were 64.6, 34.9, and 16.5%, respectively, higher than that in the control.

Effects of MeJA and EtOH on Individual Anthocyanin and Phenolic Compounds in Harvested Chinese Bayberries. Phenolic compounds such as gallic acid, protocatechuic acid, quercetin-3-*O*-rutinoside, myricetin, and cyanidin-3-glucoside, the major

anthocyanin in Chinese bayberries, were detected (**Figure 3**). In general, all four phenolic compounds and cyanidin-3-glucoside increased gradually over the first 4 days and then decreased slightly during the remaining 4 days. Chinese bayberries treated with MeJA alone or in combination with EtOH both demonstrated significantly higher levels of gallic acid, protocatechuic acid, quercetin-3-*O*-rutinoside, myricetin, and cyanidin-3-glucoside during the storage compared with the controls. Significantly higher levels of quercetin-3-*O*-rutinoside, myricetin, and cyanidin-3-glucoside were also observed in EtOH-treated fruit compared with the controls, whereas no significant differences in gallic acid and protocatechuic acid contents were found between EtOH treatment and the control during the storage.

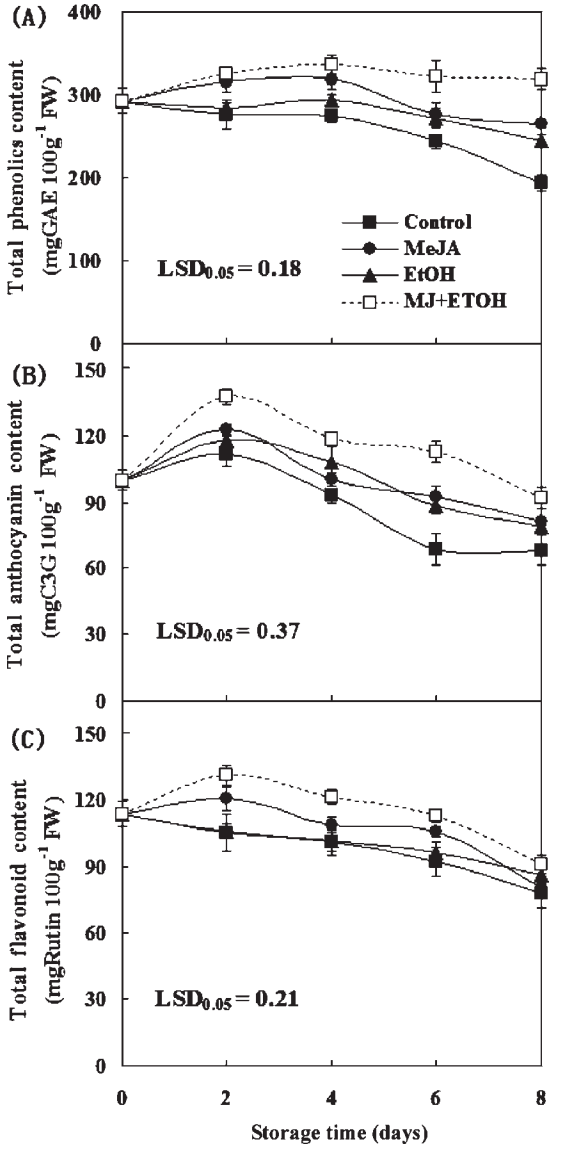

Figure 2. Effects of MeJA at 10 μmol L^{-1} and EtOH at 22.32 μmol L^{-1} on contents of total phenolics (**A**), anthocyanins (**B**), and flavonoids (**C**) in Chinese bayberries during storage at 1 °C for 8 days. Data are expressed as the mean ± SE of triplicate assays. Vertical bars represent the standard errors of the means.

Effects of MeJA and EtOH on Antioxidant Activity in Harvested Chinese Bayberries. We evaluated the antioxidant activity of Chinese bayberries by the scavenging activity against superoxide, hydroxyl, and DPPH radicals and the reducing power test in this study. Superoxide and hydroxyl radical scavenging activities in control fruits decreased rapidly with storage time. Treatment with MeJA or EtOH alone significantly inhibited the decreases of their activities and maintained higher values compared with the controls during the storage. The combined treatment of MeJA with EtOH resulted in higher superoxide and hydroxyl radical scavenging activities in comparison with the treatment of MeJA or EtOH alone (**Figure 4A**,**B**). The DPPH radical scavenging activity and reducing power in control fruit declined with storage time. Treatment with MeJA or EtOH alone maintained significantly higher DPPH radical scavenging activity and reducing power than the controls during the storage. Application of MeJA plus EtOH resulted in enhanced DPPH radical scavenging activity and reducing power compared with the treatment of MeJA or EtOH alone (**Figure 4C**,**D**). The influences of treatments and storage time on antioxidant activity were significant (**Table 2**).

Effects of MeJA and EtOH on Natural Decay and Quality in Harvested Chinese Bayberries. Treatment of Chinese bayberries with MeJA or EtOH alone significantly reduced natural decay after storage at 20 °C for 3 days or at 1 °C for 7 days followed by 20 °C for 1 day. The combined treatment of MeJA with EtOH was most effective in inhibiting fruit decay (**Figure 5**). For example, the decay incidence in the combination treatment was only 13.6% after storage at 1 °C for 7 days followed by 20 °C for 1 day, which was significantly lower than that of MeJA or EtOH alone.

Treatment with MeJA or EtOH alone or in combination had no significant effect on TSS, TA, and pH values compared with the control, regardless of whether the fruits were stored at 20 °C for 3 days or at 1 °C for 7 days plus 1 day at 20 °C. On the other hand, EtOH as stand-alone treatment did not promote the accumulation of acetaldehyde and ethanol in fruit tissue after storage under both conditions. Moreover, acetaldehyde and ethanol concentrations in MeJA- or combination-treated fruit were significantly lower than those in the control fruit (**Table 3**).

Changes in sensory qualities of firmness, color, juiciness, flavor, taste, and visual appearance of the Chinese bayberries after both storages are presented in **Figure 6**. After storage at 20 °C for 3 days or at 1 °C for 7 days followed by 20 °C for 1 day, the control fruit showed a significant decrease in visual appearance scores and had the lowest values (3.7 and 4.8, respectively), to the extent of being unacceptable for consumers, largely due to severe decay and shriveling. MeJA or EtOH alone resulted in higher sensory scores, except for flavor or juiciness, than the control. MeJA in combination with EtOH improved overall

Table 2. Two-Way ANOVA Outcomes for Total Phenolics, Flavonoids, and Anthocyanins Contents and Superoxide, Hydroxyl, and DPPH Radical Scavenging Capacities as well as Reducing Power of Chinese Bayberry with Different Treatments during Storage at 1 °C for 8 Days

		total phenolics content		total flavonoids content		total anthocyanins content		superoxide radical scavenging capacity		hydroxyl radical scavenging capacity		DPPH radical scavenging capacity		reducing power	
source	df	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
treatment (T)	3	79.26	0.01	27.61	0.014	32.16	0.01	79.27	0.01	103.51	0.00	85.19	0.00	226.47	0.01
duration (D)	4	92.15	0.01	19.56	0.032	13.39	0.00	283.15	0.00	365.68	0.012	98.40	0.091	364.30	0.02
T × D	12	35.16	0.00	5.13	0.04	2.29	0.00	12.49	0.00	6.41	0.00	11.08	0.023	21.88	0.01

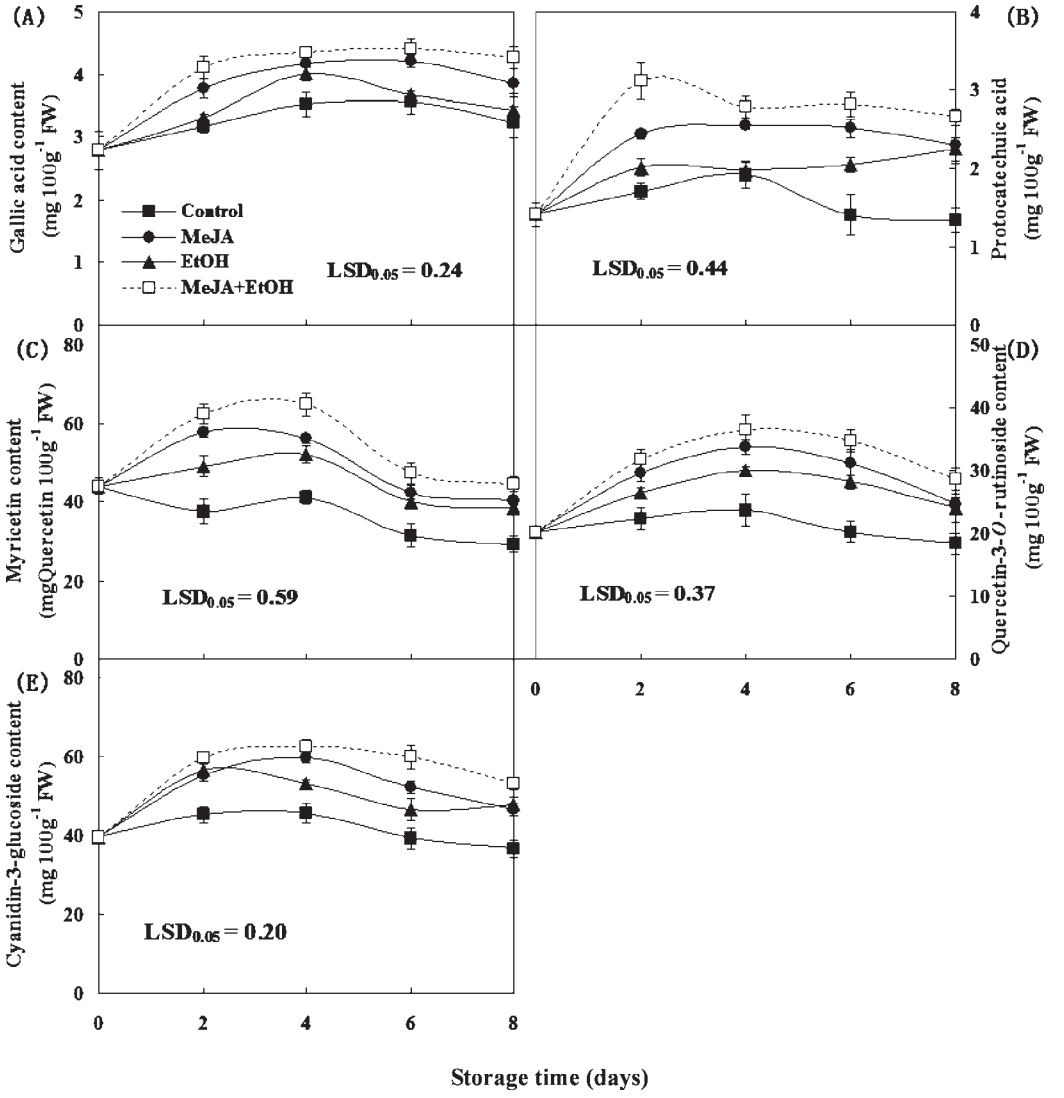

Figure 3. Effects of MeJA at 10 μmol L^{-1} and EtOH at 22.32 μmol L^{-1} on contents of gallic acid (**A**), protocatechuic acid (**B**), myricetin (**C**), quercetin-3-*O*-rutinoside (**D**), and cyanidin-3-glucoside (**E**) in Chinese bayberries during storage at 1 °C for 8 days. Data are expressed as the mean ± SE of triplicate assays. Vertical bars represent the standard errors of the means.

sensory attributes, with firmness, color, juiciness, flavor, taste, and visual appearance scores being higher than either alone.

DISCUSSION

In this study, the combined treatment of MeJA with EtOH significantly reduced green mold rot caused by *P. citrinum* infection and natural decay compared with the treatment of MeJA or EtOH alone (**Figures 1** and **5**). This indicates that the combination of MeJA and EtOH had an additive effect in reducing postharvest decay of Chinese bayberries. This enhanced efficacy of MeJA combined with EtOH in controlling postharvest fungal decay was also observed by Ayala-Zavala et al. in strawberries (*15*). The in vitro tests showed that application of EtOH could significantly inhibit fungal growth compared with the control, whereas treatment with MeJA had no inhibitory effect on the growth of the pathogen (**Table 1**). Therefore, the observed reduction of fruit decay by EtOH or its combination with MeJA was partially due to the direct lethal effect of EtOH on the pathogen. However, EtOH is a volatile compound that quickly evaporates from the berry surface during storage, and so it is ineffective to control secondary infections (*13*). In contrast, MeJA has been shown to induce the synthesis and expression of some pathogenesis-related proteins, which lead to increased resistance to pathogen infection and decreased disease incidence of tomato fruit during storage (*26*). Thus, we postulated that the enhanced persistent effect of the combined treatment in reducing decay of Chinese bayberries during storage was directly because of the inhibitory effect of EtOH on pathogen growth and indirectly because of the induced disease resistance triggered by MeJA.

On the other hand, it has been reported that natural disease resistance of fruits is closely related to the degree of ripeness and senescence. The preformed natural antifungal compounds decreased during ripening with corresponding increase of disease incidence in some subtropical fruits (*27*). Moreover, cell wall disassembly and tissue softening during postharvest ripening render fruit more susceptible to pathogen infection and, hence, higher decay incidence (*28*, *29*). It is possible that MeJA and EtOH might delay fruit ripening and senescence, thereby reducing decay incidence of Chinese bayberry fruit in this study. However, such action mechanisms need to be supported by further investigation.

There is increasing evidence showing that the levels of antioxidants in fruits can be manipulated by pre- or postharvest treatment, such as essential oil, UV irradiation, and high oxygen (*14*, *30*−*32*). It is well-known that MeJA, as a small

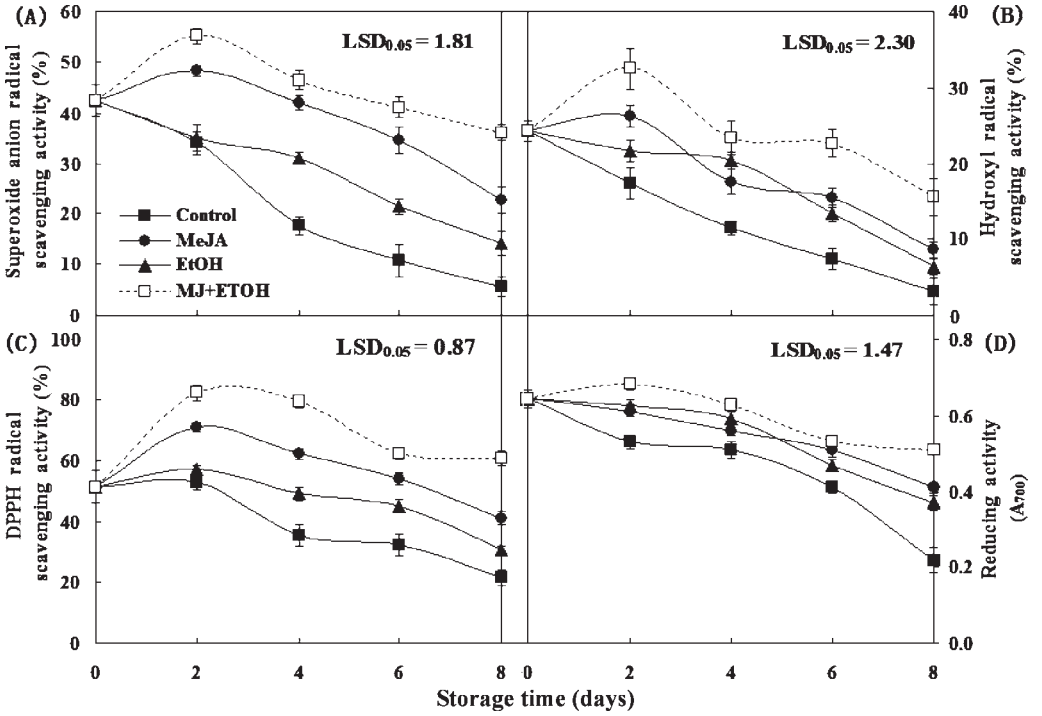

Figure 4. Effects of MeJA at 10 μmol L^{-1} and EtOH at 22.32 μmol L^{-1} on scavenging activities against superoxide anion (**A**), hydroxyl (**B**), and DPPH (**C**) radicals and reducing power (**D**) in Chinese bayberries during storage at 1 °C for 8 days. Data are expressed as the mean ± SE of triplicate assays. Vertical bars represent the standard errors of the means.

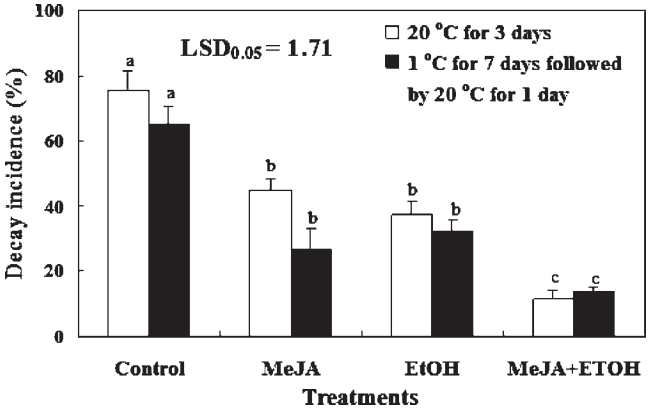

Figure 5. Effects of MeJA at 10 μmol L^{-1} and EtOH at 22.32 μmol L^{-1} on natural decay on Chinese bayberries after storage at 20 °C for 3 days or 1 °C for 7 days plus 1 day at 20 °C. Each column represents the mean of three replicate samples. Vertical bars represent standard error of the mean. Data in columns with different letters for the same storage condition are significantly different according to Duncan's multiple-range test at the $P < 0.05$ level.

signaling molecule in plants, plays key roles in regulating a great diversity of physiological and biochemical processes including stimulating the biosynthesis of secondary metabolites (*4*). Previous studies have demonstrated that MeJA could induce stilbene accumulation in leaves and berries of grapevine plants (*33*), increase the accumulation of anthocyanins and phenolics in raspberry (*14*), strawberry (*15*), blackberry (*16*), and Chinese bayberry (*7*), and promote β-carotene synthesis in apple peel (*34*). Similarly, enhanced anthocyanin biosynthesis by EtOH treatment was also observed in postharvest grape and Chinese bayberry fruit, which was correlated to the increased antioxidant activity (*12*, *17*). In the present study, our data have shown that Chinese bayberries treated with MeJA or EtOH alone both exhibited significantly higher levels of phenolic compounds compared with the controls. Furthermore, the combined treatment of MeJA with EtOH resulted in higher contents of these antioxidant components in fruit than either treatment alone (**Figures 2** and **3**). These results suggest that the combination of MeJA and EtOH also had an additive effect in enhancing antioxidant levels of the fruit by more positively affecting phenolic metabolism.

It has been reported that the harmful action of reactive oxygen species (ROS) can be blocked by antioxidant substances, which scavenge the free radicals and detoxify the organism. Berry fruit with high levels of antioxidants, either constitutive or induced, have been regarded as having greater resistance to oxidative damage of cell lipids, proteins, and nucleic acids; thus, efficient antioxidant activity is essential to maintain the ROS at relatively low levels (*35*). In this study, the combined treatment of MeJA with EtOH showed higher antioxidant activity of the fruit than the treatment of MeJA or EtOH alone (**Figure 4**), indicating that MeJA and EtOH treatments had an additive effect in maintaining high antioxidant activity. This result suggests that a postharvest treatment of MeJA combined with EtOH will improve the health benefit of Chinese bayberry fruit by enhancing the antioxidant activity. Previous studies have shown a significant positive relationship between total phenolic or anthocyanin content and antioxidant activity in some berry fruits (*1*, *31*, *32*, *36*); thus, the higher radical scavenging activity and the reducing power in the combination-treated bayberry fruit in this study could be mainly ascribed to its higher level of total phenolic or anthocyanin compounds.

In conclusion, the data presented in this paper indicate that a postharvest application of 10 μmol L^{-1} MeJA in combination with 22.32 μmol L^{-1} EtOH was most effective in reducing postharvest decay in Chinese bayberries. This combined treatment also maintained highest levels of antioxidant capacity, and total phenolics, anthocyanins, and flavonoids as well as main individual phenolic compounds without impairing fruit quality.

Table 3. Effects of MeJA at 10 μmol L^{-1} and Ethanol at 22.32 μmol L^{-1} on Postharvest Quality in Chinese Bayberries[a]

storage condition	treatment	TSS (°Brix)	TA (mmol 100 g^{-1} of FW)	pH value	acetaldehyde concentration (nmol kg^{-1} of FW)	ethanol concentration (nmol kg^{-1} of FW)
20 °C for 3 days	control	9.63 a	5.08 a	3.37 a	41.07 a	193.30 a
	MeJA	9.67 a	5.27 a	3.38 a	35.27 b	107.59 c
	ethanol	9.71 a	5.16 a	3.35 a	42.41 a	176.78 a
	MeJA + EtOH	9.72 a	5.04 a	3.41 a	30.80 b	137.94 b
1 °C for 7 days followed by 20 °C for 1 day	control	9.21 a	4.14 a	3.20 a	45.09 a	262.05 a
	MeJA	9.25 a	4.41 a	3.26 a	28.57 b	112.51 d
	ethanol	9.17 a	4.28 a	3.21 a	39.73 a	216.96 b
	MeJA + EtOH	9.25 a	4.37 a	3.27 a	25.45 b	143.75 c

[a] Data are expressed as means of triplicate assays. Values in a column followed by different letters are significantly different according to Duncan's multiple-range test at the $P < 0.05$ level.

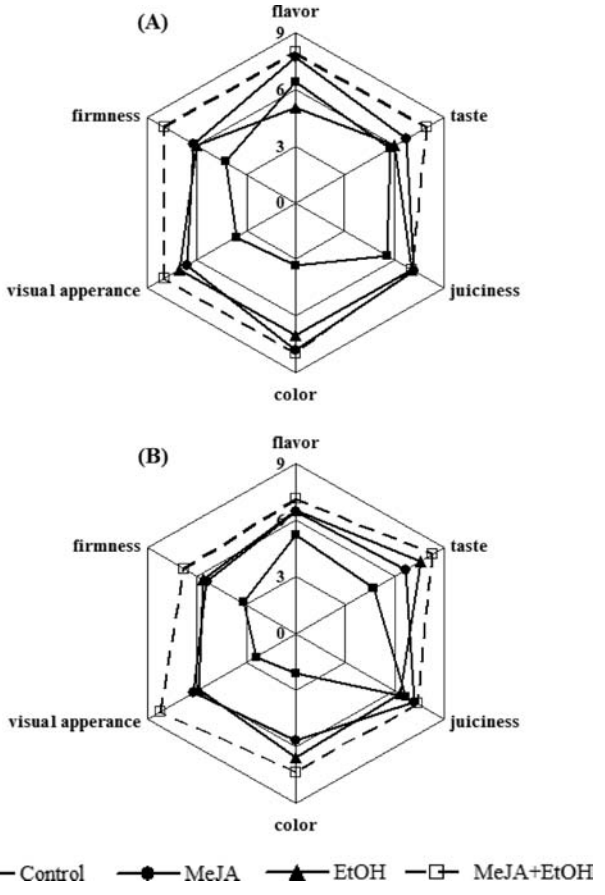

Figure 6. Effects of MeJA at 10 μmol L^{-1} and EtOH at 22.32 μmol L^{-1} on sensory scores of firmness, color, juiciness, flavor, taste, and visual appearance of Chinese bayberries after storage at 20 °C for 3 days (**A**) or at 1 °C for 7 days plus 1 day at 20 °C (**B**).

Thus, MeJA in combination with EtOH has a potential application in postharvest treatment for reducing decay and maintaining a high-quality product in Chinese bayberry during postharvest storage and distribution.

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