

Effects of Exogenous Methyl Jasmonate Treatment with Polyethylene Bag Storage on Decay Reduction and Enhanced Total Ascorbic Acid, Total Phenolic, and Antioxidant Activities in ‘Kamphaeng Saen 42’ Mulberry Fruit

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Abstract

Mulberry fruit has a short shelf life of only 1 - 2 days under room conditions, make it one of the most fragile and extremely perishable fruits. This study investigated the effects of exogenous methyl jasmonate (MeJA) combined with polyethylene bag (PE) on postharvest decay, total ascorbic acid, total phenolic, anthocyanin, and antioxidant activities in 'Kamphaeng Saen 42' mulberry fruits. The study was conducted at Postharvest Quality and Storage System Laboratory, Division of AgriScience and Technology (Postharvest Technology), Faculty of Bioresources and Technology, King Mongkut's University of Technology Thonburi in December 2019. The experiment was laid out in Completely Randomized Design (CRD) with 4 replicates and 2 treatments consisting of 1) untreated with MeJA (control) and 2) fruits treated with 10 $\mu\text{mol L}^{-1}$ methyl jasmonate (MeJA) combination with polyethylene bag (PE) and maintained at 10 ± 2 °C, 90 ± 2 % relative humidity (RH). The results showed that treating the mulberry fruits with 10 $\mu\text{mol L}^{-1}$ MeJA before placing them in the PE bag was the most effective method to reduce fruit decay and also resulted in higher levels of total ascorbic acid, total phenols, and antioxidant activities than in the control at the end of storage (55.14 mg/100 g FW, 43.78 mg GAE /g FW and 28.91 mM TE /100 g FW), respectively. However, the treated fruits showed lower anthocyanin content than the control during the first 8 days of storage. These results suggest that the combination of MeJA with PE bag is beneficial in reducing fruit decay and increasing total ascorbic acid, total phenols, and antioxidant activities in 'Kamphaeng Saen 42' mulberry fruits during storage at 10 ± 2 °C, 90 ± 2 % RH for 10 days.

Keywords: ‘Kamphaeng Saen 42’ mulberry fruit, Methyl jasmonate, Polyethylene bag, Decay, Antioxidant

Introduction

Kamphaeng Saen 42' mulberry is one of the mulberries grown to be eaten as fresh fruit, similar to the mulberry 'Chiang Mai 60'. Nowadays, this fruit is in high demand in the market because of its richness in bioactive components including vitamins, anthocyanins, alkaloids, flavonoids, and polyphenols [1]. The antioxidant compounds present in mulberry fruit, including vitamin C, phenolic compounds, and anthocyanins have potential effects in reducing the risk of cardiovascular disease and cancer [2]. However, mulberry fruit is highly susceptible to physiological deterioration and pathogen attack, which limits its shelf life to 1 - 2 days at room temperature.

Methyl jasmonate (MeJA) is an endogenous regulator that plays a role in plant growth and development and plant defense against biotic and abiotic stresses [3]. Recently, MeJA has been reported to effectively suppress postharvest diseases in various fruits, for example, blueberries [4], strawberries [5], longkong [6], and grapes [7]. Moreover, postharvest MeJA treatment preserved higher bioactive compounds content and improved antioxidant capacity in cherry tomatoes [8], raspberries [9], blueberries [10], jujube [11], and pineapple [12]. In addition, modified atmosphere packaging (MAP) is a practical way to create a suitable gaseous atmosphere around a fresh produce packaged in plastic film to extend shelf life and maintain produce quality. As a result, the atmospheric composition inside the package with a low concentration of O_2 (< 21 %) and a high concentration of CO_2 (> 0.03 %) reduces the respiration

rate of fruits, delays fruit ripening, and suppresses diseases in many fruits [13]. However, the concentration of O₂ and CO₂ in the package depends on the respiration rate of the fruit, storage temperature, and permeability properties of the film material [14]. It has been reported that MAP is one of the most successful techniques for fruit preservation. Moreover, this method is simple and without chemical residues which is safe for human health [15].

Therefore, this research investigated the effect of exogenous methyl jasmonate (MeJA) combination with polyethylene bag (PE) on postharvest decay, total ascorbic acid, total phenol, anthocyanin, and antioxidant activities in 'Kamphaeng Saen 42' mulberry fruits.

Materials and methods

Reagents and chemicals

The reagents and chemicals used in this study were all of analytical grade and were obtained from Sigma-Aldrich, methyl jasmonate (Sigma, 95 %), 2,2-diphenyl-1-picrylhydrazyl (Sigma, 98 %), 2,4-dinitrophenol (Sigma, 98 %), 1-ascorbic acid (Sigma, 98 %), gallic acid (Fluka, 98 %), Folin-Ciocalteu reagent (Sigma, 2N), metaphosphoric acid (Sigma, 36.5 %), thiourea (Sigma, 99 %), sulfuric acid (Sigma, 98 %), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Sigma, 98 %), potassium chloride (Sigma, 98 %), and sodium acetate trihydrate (Sigma, 98 %).

Plant material

Kamphaeng saen 42' mulberry fruit was hand-harvested at the red skin stage (70 % ripe) from Rajamangala Sericulture center, Rajamangala University of Technology Isan, Surin campus, and transported to Postharvest Quality and Storage System Laboratory. The fruits were selected based on uniformity of color and absence of defects or intactness and then randomly divided into 2 lots. All lots were placed in an airtight 45 L plastic container and fumigated with 10 µmol L⁻¹ MeJA and the untreated samples served as a control for 6 h at 20 °C. The MeJA concentration at 10 µmol L⁻¹ was calculated based on the volume of the container. Then all treatments were packed in polyethylene bags (PE)(size 5×8", with a thickness of 0.042 mm, at oxygen transmission rate; OTR of 2,104 cc m⁻² day⁻¹, and water vapor transmission rate (WVTR) of 14.8 g m⁻² day⁻¹) and then kept at 10 ± 2 °C, 90 ± 2 % relative humidity (RH). There were 4 replicates with approximately 500 g of fruit per replicate. Fruit samples were taken randomly from each treatment at 2-day intervals to determine postharvest decay, total ascorbic acid, total phenols, anthocyanins, and antioxidant activities.

Fruit decay

Fruit decay was assessed visually using 100 fruits from each replication. If more than 50 % of visible mold growth occurred on the fruit surface, this was considered decayed. The severity of fruit decay was expressed as the percentage of fruits with fungal symptoms.

Total ascorbic acid content

Total ascorbic acid content was determined according to the method of Roe *et al.* [16]. Five grams of the sample was extracted with 20 mL of 5 % metaphosphoric acid using a homogenizer in an ice bath. The extract was filtered using Whatman # 01 filter paper. Two tenths mL of a 0.02 % indophenol solution was added to 0.4 mL of the sample extract and incubated for 2 - 3 min until a stable reddish-pink color was obtained. Then, 0.4 mL of 2 % thiourea and 0.2 mL of 2 % DNP solution were added and then incubated for 3 h at 37 °C in a hot water bath. Then 1 mL of 85 % sulfuric acid was added and incubated again for 30 min at room temperature. The absorbance was determined at 540 nm using a 1601 UV-visible spectrophotometer (Shimadzu, Tokyo, Japan). A standard curve was prepared using standard ascorbic acid with concentrations of 20, 40, 60, 80 and 100 mgL⁻¹.

Total phenolic content

Total phenol content was determined according to the method of Cheung *et al.* [17] with slight modifications by mixing 1 mL of supernatant with 5 mL of Folin-Ciocalteu's phenol reagent (10 % v/v). After 3 min, 4 mL of 7.5 % Na₂ CO₃ was added to the mixture. The reaction was kept at 30 °C for 1 h, after which the absorbance was measured at 760 nm using a 1601 UV-visible spectrophotometer. The results were expressed as gallic acid equivalents (GAE) in mg GAE /100 g FW. A standard curve was prepared using the above procedure with a range of gallic acid at 20, 40, 60, 80 and 100 mgL⁻¹.

Anthocyanin content

Anthocyanin content was measured according to the method of Horwitz [18]. 500 mg of the samples were homogenized in 20 mL of distilled water. This was followed by sonication (40 °C for 15 - 20 min) and then incubating at room temperature until the temperature of the samples decreased to room temperature. After centrifugation at 8,000 rpm for 5 min, 3 mL of the supernatant was mixed with 7 mL of 0.025 M potassium chloride buffer at pH 1 and 3 mL of the supernatant was mixed with 7 mL of 0.4 M sodium acetate buffer at pH 4. The absorbance was determined at 510 and 700 nm using a 1601 UV-visible spectrophotometer. Anthocyanin content was calculated using the following equations;

$$\Delta \text{ absorbance} = (A_{510 \text{ nm pH1}} - A_{700 \text{ nm pH1}}) - (A_{510 \text{ nm pH4}} - A_{700 \text{ nm pH4}})$$

$$\text{Anthocyanin (\%)} = \frac{\Delta Abs \times MW \times \text{dilution factor} \times V \times 100}{\epsilon \times L \times Wt}$$

ϵ = Absorption coefficient (26,900 Lmol⁻¹cm⁻¹ = pelergo-3-glucoside)

MW = Anthocyanin molecular weight (449.2)

V = Final volume (mL)

L = Width of plant cell (1 cm)

Wt = Sample weight (mg)

Antioxidant activity (DPPH method)

The DPPH assay was performed according to the method of Brand-Williams *et al.* [19] with slight modifications by reacting 150 μ L of fruit extracts with 2,850 μ L of the DPPH solution and then incubated at room temperature for 24 h in the dark. Then the absorbance was measured at 515 nm. The standard curve was linear between 25 and 800 μ M Trolox.

Statistical analysis

The experimental design was a completely randomized design (CRD). All data analyses were performed using SAS statistical software (SAS Institute Inc. Cary, NC). Data were analyzed using a 1-way analysis of variance (ANOVA). Mean separations were performed using Duncan's New Multiple Range Test (DMRT), and differences at $p \leq 0.05$ were considered statistically significant.

Results and discussion

Fruit decay

Mulberry fruit treated with 10 μ mol L⁻¹ MeJA combination with PE bag were the most effective in controlling fruit decay compared to the control lot throughout the storage period. Meanwhile, the control lot showed fruit decay during the first 6 days of storage. At the end of storage, the decay incidence of MeJA combination with PE bag was only 8.60 %, while the control lot showed 14.83 % fruit decay (**Figure 1**). It was reported that MeJA had effects against infection of fruit pathogens. It showed antifungal activity, inhibited spore production, spore germination and germ tube elongation, and induced plant defense against pathogens [20]. In addition, MAP was successful in retarding chilling injury (CI), color development, water loss, and fungal infection in various crops [21,22]. Gonzalez-Aguilar *et al.* [23] reported that papaya treated with 10⁻⁵ M MeJA combination with MAP inhibited fruit decay and reduced CI development during storage for 14 - 32 days at 10 °C and 4 days shelf life at 20 °C. These positive effects can be explained by the fact that MAP enhanced the efficacy of MeJA treatment. Therefore, the combination of MeJA with PE could delay fruit ripening and senescence, thereby reducing the decay of mulberry fruit in this study.

Total ascorbic acid content

Changes in total ascorbic acid content of mulberry fruit treated with 10 μ mol⁻¹ MeJA combination with PE bag and control are shown in **Figure 2A**. After 2 days of storage, there were no significant differences in total ascorbic acid content between treated fruits and the control. However, the content of total ascorbic acid in treated fruits at the end of storage was 55.14 mg/100 g FW higher than that of the control (29.81 mg/100 g FW). In general, total ascorbic acid or vitamin C is most sensitive to destruction when the commodity is exposed to unfavorable handling and storage conditions. Losses are exacerbated by prolonged storage, elevated temperatures, low relative humidity, physical damage, and exposure to cold. It has been reported that treatment with MAP and MeJA reduce CI, fungal attack, and water loss in

various crops and also maintains or increases the production of secondary metabolites [24]. Similarly, the results of Ozturk *et al.* [25] reported that medlar fruits treated with 0.1 mM MeJA combination with MAP reduced the loss of ascorbic acid due to a decrease in O₂ concentration and increase in CO₂ concentration in MAP which slow down the loss of ascorbic acid by inhibiting oxidation. Many studies have confirmed that MeJA also retards water loss, and induces antioxidant capacity of postharvest fruits [26]. In addition, a study on plant cell suspensions found that MeJA can induce the transcription of genes involved in the *de novo* biosynthesis of ascorbic acid [27]. Thus, the total ascorbic acid content of fruits treated with 10 μmol⁻¹ MeJA combination with PE bag was higher than that of the control.

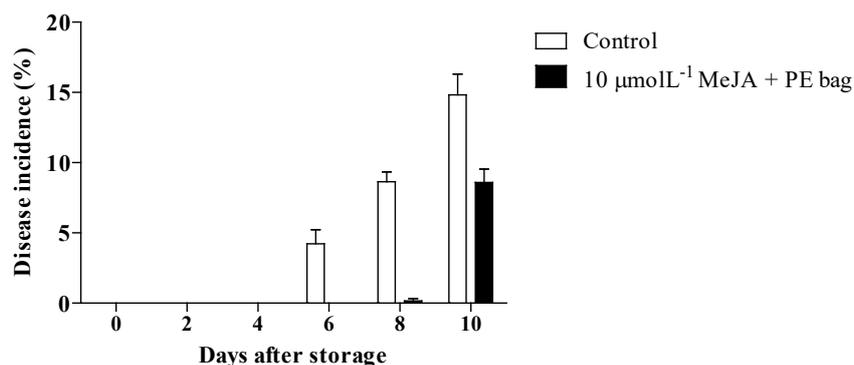


Figure 1 Disease incidence in ‘Kamphaeng Saen 42’ mulberry fruit treated with 10 μmol L⁻¹ MeJA combination with PE bag and control during storage at 10 ± 2 °C, 90 ± 2 % RH for 10 days.

Total phenolic content

Fruits treated with MeJA combination with PE bag had higher total phenolic content than the control throughout the storage period. At the end of storage, the total phenolic content of the treated fruits was 43.73 mg/ GAE g FW, while the total phenolic content in the control was 38.12 mg/ GAE g FW (**Figure 2B**). Wang *et al.* [10,28] found that MeJA treatment increased PAL activity in blueberries and Chinese bayberries, resulting in stimulation of phenol synthesis. It has been reported that MAP with low O₂ and high CO₂ delays the loss of total phenolic content by decreasing polyphenol oxidase activity in sweet cherry fruit [29] and loquat [21], as well as induces their abiotic stress, increasing phenolic compounds in the plant [30]. Thus, when MeJA was applied with MAP, it was the most effective in reducing the total phenolic content in mulberry fruit.

Anthocyanin content

Anthocyanin content was higher in the control than in the treated fruits during the first 8 days of storage, while the fruits treated with 10 μmolL⁻¹ MeJA combination with PE bag had the highest anthocyanin content (34.42 %) at the end of storage (**Figure 2C**). Anthocyanins are colored water-soluble pigments found in mulberry fruit [31]. Many studies have shown that MeJA induces the accumulation of anthocyanins in many fruits [32,33]. However, our results showed that the combination of MeJA with PE bag had lower anthocyanin content than the control. This result suggests that the MAP technique delays fruit ripening and the effect of MeJA treatment on fruit ripening differs depending on the applied concentrations in different plants [32,34]. It was reported that fruits treated with MeJA at 5 μmolL⁻¹ suppressed ethylene biosynthesis and delayed fruit ripening, while the promotion of fruit ripening with MeJA occurred in the range of 100 μmolL⁻¹ to 1 mM [35]. Feng *et al.* [36] reported that apples treated with 1 and 0.1 mM MeJA promoted anthocyanin accumulation by up-regulating the expression of genes encoding enzymes involved in anthocyanin synthesis.

Antioxidant activity

Mulberry fruit treated with 10 μmolL⁻¹ MeJA combination with PE bag exhibited significantly higher levels of antioxidant activity throughout the storage period (**Figure 2D**). At the storage, antioxidant activity of the treated fruits was 28.91 mM TE /100 g FW, while the antioxidant activity in the control was 23.48 mM TE /100 g FW. In various berry fruits, MeJA can increase antioxidant activity through the increase of antioxidant compounds such as ascorbic acid, phenols and flavonoids [28,32,37,38]. Ozturk *et al.* [25] found that medlar treated with MeJA combination with MAP had the

highest content of total phenols, and total flavonoids, which was also reflected in high antioxidant content. Similar results were found in this study.

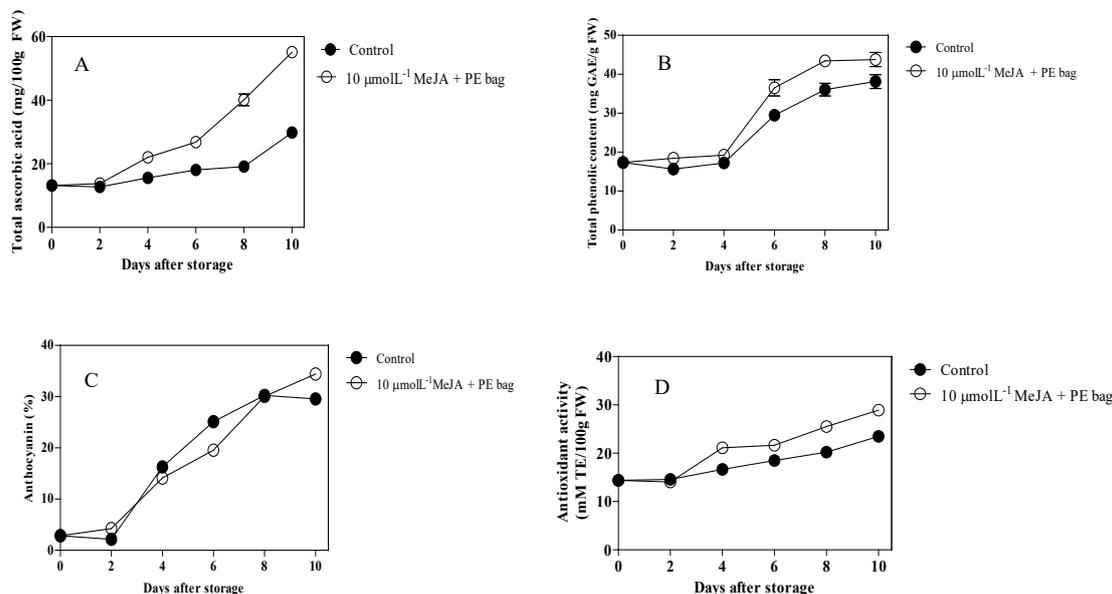


Figure 2 A) Changes in total ascorbic acid content, B) total phenolic content, C) anthocyanin content, and D) antioxidant activity of 'Kamphaeng Saen 42' mulberry fruit treated with 10 µmol L⁻¹ MeJA combined with PE bag and control during storage at 10 ± 2 °C, 90 ± 2 % RH for 10 days.

Conclusions

Mulberry fruits treated with 10 µmolL⁻¹ MeJA combined with PE bag were the most effective in reducing fruit decay and resulted in higher levels of total ascorbic acid, total phenols and antioxidant activities than the control. Thus, the combination of MeJA with PE may improve the postharvest quality of 'Kamphaeng Saen 42' mulberry fruit during storage. Future studies on the activity of PAL and antioxidant enzyme activities, including SOD, CAT, and APX, should be conducted.

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