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# Physiological and biochemical response of harvested plum fruit to oxalic acid during ripening or shelf-life

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ABSTRACT

The effect of oxalic acid application on plum fruit (*Prunus salicina* cv. 'Damili') ripening properties during storage or shelf-life was determined. The fruits were dipped for 3 min in solutions containing 5 mmol/L oxalic acid and then were packed into polyethylene bags and stored at 25 °C for 12 days, or at 2 °C for 20 days and subsequently at 25 °C for 12 days. Ethylene production, fruit firmness, contents of pectin and anthocyanin, specific activities of polygalacturonase (PG), pectin methylesterase (PME) and phenylalanine ammonia lyase (PAL), and chlorophyll fluorescence (Fv/Fm) were measured. The application of oxalic acid reduced ethylene production and delayed softening of plum fruit. The inhibition of softening was associated with decreased PG and PME activities; that is, the retardation of pectin solubilization/degradation. During storage or shelf-life, flesh reddening and anthocyanin synthesis were significantly inhibited in oxalic acid-treated plum fruit, accompanied with decreased PAL activity. Furthermore, it was found that variable:maximalchlorophyll fluorescence (Fv/Fm), an indicator of ripening, senescence or stress injury of fruit and vegetable, decreased much more slowly in oxalic-treated plum fruits than in control fruits. Thus, oxalic acid treatment can be an effective means to extend the shelf life of plum fruit.



## 1. Introduction

The plum fruit is highly perishable. The fruit after harvest undergoes a rapid softening progress, resulting in a short shelf life at ambient temperatures (Menniti, Gregori, & Donati, 2004). Storage at low temperature delayed effectively fruit ripening and extended postharvest life of plums, but the beneficial effects may be limited by the development of chilling injury-associated disorders, including internal browning, flesh translucency, and/or reddening (Crisosto, Garner, Crisosto, & Bowerman, 2004; Manganaris, Vicente, Crisosto, & Labavitch, 2007). Therefore, delaying or reducing flesh softening and low temperature deterioration should be important strategies to extend storage life and maintain quality of plum fruit.

Oxalic acid is an organic acid distributing widely in various organisms, especially in plants. It has been found in potatoes, beans, spinach, beets, tomatoes, cauliflower, onions, mushrooms, and celery root, among the vegetables and in currants, raspberries, grapes, pears and prunes, among the fruits. Oxalic acid has also been reported in meat, liver and kidney and in coffee, cocoa and tea (Franceschi & Nakata, 2005). Recent studies revealed that oxalic acid might play important roles in systemic resistance, stress response, programmed cell death and redox homeostasis in plant (Guo, Tan, Zhu, Lu, & Zhou, 2005; Kim, Min, & Dickman, 2008; Liang, Strelkov, & Kav, 2009; Wang, Lai, Qin, & Tian, 2009). In addition, oxalic acid might have anti-browning and anti-senescence effects. Oxalic acid has been shown to be an anti-browning agent for apple and banana slices (Son & Lee, 2001; Yoruk, Yoruk, Balaban, & Marshall, 2004) and litchi fruit (Zheng & Tian, 2006). Postharvest life extension of mango and peach fruits by oxalic acid also has been reported when 1 or 5 mmol/L of concentrations were applied (Zheng, Tian, Gidley, Yue, & Li, 2007; Zheng, Tian, Meng, & Li, 2007). More recently, Sayyari et al. (2010) reported that application of oxalic acid significantly alleviated chilling injury symptoms of pomegranate after long term storage at 2 °C. The effects of anti-senescence and anti-stress might be associated with antioxidant and ethylene signaling regulated by oxalic acid. However, little information on effect of oxalic acid on ripening properties of harvested plum is available.

The objective of this study was to investigate the effect of postharvest dips of 'Damili' plums with oxalic acid on ripening or senescence during storage or shelf-life.

## 2. Materials and methods

### 2.1. Fruit material and oxalic acid treatment

Plum fruits (*Prunus salicina* Lindley, cv. 'Damili') were harvested at a pre-climacteric stage from a commercial orchard in Guangzhou.

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Fruit were selected for uniformity of shape, color and size, and any blemished or diseased fruits were discarded. Preliminary investigations revealed that, within a concentration range from 0.5–10 mmol/L oxalic acid, treatment with 5 mmol/L oxalic acid was most effective at delaying ripening or extending shelf-life of plum fruit. Fruits were divided into 55 lots of 10 fruits. Twenty-seven lots were immersed for 3 min in 5 mmol/L oxalic solution. Twenty-seven lots of fruit were dipped in water for 3 min as controls. After dipping, the fruit were air-dried for 30 min, packed in 0.03 mm thick polyethylene bags perforated (4×5 mm diameter holes per bag, 10 fruit per bag). The bags were fastened with rubber band. The fruit was divided into two groups. One group was stored at 25 °C for 12 days. The other group was stored at 2 °C for 20 days and subsequently transferred to 25 °C for 12 days. Samples were taken after 0, 3, 6, 9 and 12 days of ripening at 25 °C after (A) harvest, or (B) a 20-day cold storage at 2 °C.

### 2.2. Fruit firmness

Flesh firmness was determined with a penetrometer (Model GY-1, Hangzhou Scientific Instruments, Hangzhou, China) by measuring force required for a 5 mm diameter flat probe to penetrate into the pulp at a depth of 5 mm and the results were expressed as kg $cm^{-2}$. When the firmness is less than 2 kg $cm^{-2}$, the quality of the fruit is unacceptable.

### 2.3. Ethylene production

Nine plum fruit were sealed in a 2.5 L plastic container and then held for 2 h at 25 °C. One mL samples of headspace gas were withdrawn from the container and injected into a gas chromatograph (GC-9A; Shimadzu, Kyoto, Japan) equipped with a 25 m HP-PLOT Q capillary column (Agilent Technologies, USA) and a flame ionisation detector (FID) to measure ethylene concentration. Rates of ethylene production were expressed as microliters per kilogram per hour.

### 2.4. Anthocyanins content

Anthocyanin contents of plum plup tissues were measured using the pH differential method (Zhang, Pang, Yang, Ji, & Jiang, 2004). The results were expressed as milligrams of cyanidin-3-glucoside equivalent per gram of fresh weight.

### 2.5. Pectin content

Water soluble pectin (WSP) and acid soluble pectin (ASP) were extracted according to the method of Cheng et al. (2008). Uronic acid concentrations in WSP and ASP fractions were measured by the m-hydroxydiphenyl method (Blumenkrantz & Asboe-Hansen, 1973) using galacturonic acid (GA) standards.

### 2.6. Assay of polygalacturonase (PG) and pectin methylesterase (PME) activities

Five gram of pulp tissue was homogenized with 20 mL of 50 mmol/L sodium acetate buffer (pH 4.5) containing 7.5% (w/v) NaCl and 0.5 g of polyvinylpyrrolidone (insoluble) at 4 °C. The homogenate was centrifuged at 10,000 ×*g* for 20 min and the supernatant was dialyzed overnight in 50 mmol/L sodium acetate buffer (pH 4.5) for assaying PG activity. The reaction mixture contained 0.4 mL of 200 mmol/L sodium acetate (pH 4.5), 0.3 mL of polygalacturonic acid (PGA, 1% aqueous solution adjusted to pH 4.5), 0.2 mL of distilled water, and 0.1 mL of dialyzed enzyme extract. The reaction was initiated by addition of the PGA substrate. The mixture was incubated at 37 °C for 1 h, followed by addition of 3,5-dinitrosalicylate (DNS) reagent. The reaction was terminated by heating the reaction mixture in a boiling water bath for 5 min. The formed reducing groups were estimated using DNS reagent against galacturonic acid (GalA) standards (Luchsinger & Cornesky, 1962). PG activity was expressed as μg GalA $mg^{-1}$ protein $min^{-1}$.

PME was extracted by homogenizing pulp in 8.8% (w/v) NaCl and 2.5% (w/v) polyvinylpyrrolidone (insoluble) at 4 °C. The homogenate was centrifuged at 10,000 ×*g* for 30 min. The supernatant was collected, adjusted to pH 7.5 and assayed for PME activity. The activity was assayed in a mixture containing 2.0 mL of 0.5% (w/v) pectin, 0.15 mL of 0.01% bromothymol blue, 0.75 mL of water, and 0.1 mL of enzymatic extract. All solutions (pectin, indicator dye, water) were adjusted to pH 7.5 with 2 mol/L NaOH just before each trial was started. After adding the enzyme extract, the decrease in the absorbance at 620 nm was measured spectrophotometrically. Calculation of the activity was carried out against the standard curve as described by Hangermann and Austin (1986). PME activity was expressed as μmol GalA $mg^{-1}$ protein $min^{-1}$.

Protein content was determined according to the method of Bradford (1976) with bovine serum albumin as a standard.

### 2.7. Extraction and assays of PAL activity

Pulp tissue (5 g) was homogenized in 20 mL of 0.1 M Na-borate buffer (pH 8.0) containing 0.5 g of polyvinylpyrrolidone (insoluble), 5 mmol/L β-mercaptoethanol and 2 mmol/L EDTA at 4 °C (Jiang & Joyce, 2003). The homogenate was centrifuged for 20 min at 19,000 ×*g* and 4 °C and then the supernatant was collected for enzyme assay. PAL activity was determined by incubating the mixture of 0.1 mL enzyme extract and 2.9 mL of 0.1 mol/L Na-borate buffer (pH 8.0) containing 3 mmol/L L-phenylanine for 1 h at 37 °C. The increase of absorbance at 290 nm due to the formation of trans-cinnamate was measured spectrophotometrically. One unit of enzyme activity was defined as the amount that caused an increase of 0.01 absorbance per hour.

### 2.8. Chlorophyll fluorescence measurements

Chlorophyll fluorescence was determined using a portable chlorophyll fluorometer (FAM 2100, Walz, Germany). Fruits were dark-adapted for at least 30 min prior to measurements. The chlorophyll fluorescence parameters Fo and Fm were measured. Fo is the minimal or initial fluorescence when all PSII reaction centers are open, while Fm is the maximal fluorescence when all PSII reaction centers are closed and all non-photochemical quenching processes are at a minimum. The maximal variable fluorescence (Fv = Fm − Fo) and PSII quantum yield (Fv/Fm) were calculated from the Fo and Fm values. *Fo* was measured with a measuring beam at a light intensity less than 0.05 μmol $m^{-2}$ $s^{-1}$. Fm was obtained by measuring chlorophyll fluorescence during a 2.5-s pulse of saturating light (18,000 μmol $m^{-2}$ $s^{-1}$).

### 2.9. Statistical analysis

These experiments were arranged in completely randomized design, and each treatment comprised of three replicates. Data were tested by the analysis of variance using SPSS version 7.5. Least significant differences (LSDs) were calculated to compare significant effects at the 5% level.

## 3. Results and discussion

### 3.1. Ethylene synthesis

As shown in Fig. 1, 'Damili' plum fruit stored at 25 °C without refrigeration showed a peak of ethylene production after 9 days (Fig. 1A). When the fruit was stored at 2 °C for 20 days and then transferred to 25 °C, ethylene production peaked earlier than the fruit