



# Limiting the deterioration of mango fruit during storage at room temperature by oxalate treatment

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## ABSTRACT

Effects of oxalate on the incidence of decay and ripening in mango fruit, and its physiological effects on the peel and flesh of mango were investigated after mango fruit (*Mangifera indica* L.) were dipped in different oxalate solutions for 10 min and then stored at 25 °C. Oxalate application decreased the incidence of decay and delayed the ripening process in mango fruit during storage. Potassium oxalate treatment resulted in increased activities of peroxidase (POD) in both the peel and the flesh and polyphenol oxidase (PPO) in the peel, without activation of phenylalanine ammonia-lyase activity, and elevated total phenolic content in the peel. The physiological effects of oxalate in increasing activities of POD and PPO and elevating total phenolic level could be involved in induced resistance of mango fruit against postharvest disease. Oxalate application could be a promising method to suppress postharvest deterioration and extend the useful shelf-life of mangoes.

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## 1. Introduction

Susceptibility of mango fruit (*Mangifera indica* L.) to postharvest diseases increases during storage after harvest, as a result of physiological changes and senescence, favouring pathogen development (Prusky & Keen, 1993). Synthetic fungicides, such as benomyl and prochloraz, alone or in combination with other treatments, have shown efficacy in controlling decay incidence in mango fruit during storage at room and lower temperature (Johnson, Sharp, Milne, & Oosthuysen, 1997; Kobiler et al., 2001). However, increasing public concerns with fungicide toxicity, development of fungicide resistance by pathogens and adverse effects on the environment and human health have lead to intensified worldwide research efforts to develop alternative forms of disease control (Droby, Wisniewski, Macarasin, & Wilson, 2009; Wilson et al., 1994).

Induction of host resistance is one strategy that holds promise for control of postharvest diseases (Adikaram, 1990). In recent years, inducing resistance by chemical, physical or biological elicitors is becoming a great potential approach for the control of postharvest diseases as an alternative to fungicides (Tian, 2006;

Wilson et al., 1994). Previous works have reported that oxalic acid and oxalate induce systemic resistance against diseases caused by fungi, bacteria and viruses in plants (Mucharromah & Kuc, 1991; Toal & Jones, 1999). Oxalic acid treatment inhibits the progress of Alternaria rot in harvested pear fruit associated with an induction of increased defence-related enzyme activities (Tian, Wan, Qin, & Xu, 2006). Wang, Lai, Qin, and Tian (2009) have reported that three proteins related to the defence or stress response are up-regulated by oxalic acid, and contribute to the establishment of systemic resistance induced by oxalic acid in jujube fruits. Moreover, in our previous work, we have found that pre-storage application of oxalic acid can suppress postharvest deterioration and extend the shelf-life of mango fruit, due to a combination of physiological effects associated with delaying the ripening process, and direct effects, including low pH, inhibiting the development of postharvest pathogens such as *Colletotrichum gloeosporioides* (Zheng, Tian, Gidley, Yue, & Li, 2007; Zheng et al., 2007).

In the search for novel treatments to reduce deterioration of mango fruit, as well as to better understand the role of oxalate in improving the limited storage ability of mango fruit, the effects of oxalate on ripening and decay incidence in mango fruit during storage at room temperature, and the effects of potassium oxalate on defence mechanism in association with defence-related enzymes and accumulation of total phenols were further investigated in this study.

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## 2. Materials and methods

### 2.1. Material and treatments

#### 2.1.1. Experiment 1. Postharvest dip treatment with prochloraz, potassium oxalate and ammonium oxalate

Mango (*M. indica* cv. Xiaojinhuang) fruit about 80% matured stage were harvested from a commercial orchard in Hainan island of China. Fruit were selected for uniformity of size and colour, and blemished and diseased fruit were discarded. Transit time by train at a temperature of about 25 °C from harvest to arrival at a Zhangjiang City laboratory (Guangdong Province, China) was approximately 12 h. Upon arrival at the laboratory, 20 selected fruit each were placed in a clean plastic box with fruit touching, and then three plastic boxes each were dipped in water (as control), or 0.1% Prochloraz fungicide (Bayer CropScience, China), or 30 mM potassium oxalate or 30 mM ammonium oxalate solutions for 10 min. After air drying, each box was wrapped in a 0.02 mm polyethylene bag to maintain relative humidity and was held in a room at 25 °C ( $\pm 1$  °C). Fruit disease index and fruit decay of each treatment were evaluated at 2-day intervals. Twenty fruit in the same box was considered as a replicate, and three replicates of each treatment were carried out.

#### 2.1.2. Experiment 2. Postharvest dip treatment with different concentrations of potassium oxalate

Mango (*M. indica* cv. Zill) fruit about 80% matured stage were harvested from a commercial orchard in Panzhihua city, China. Harvested fruit were selected for uniformity of size and appearance. After the selected fruit were cooled for about 2 h in a room at about 25 °C near the orchard, they were dipped in water (as control), 20 or 40 mM potassium oxalate solutions at 25 °C for 10 min. After air drying, each fruit was wrapped with a soft absorbent paper, and then about 15 kg of control and treated fruit were placed in separate cartons. Transit time by plane from harvest to arrival at a Hangzhou laboratory was approximately 24 h. Upon arrival at the laboratory, 20 fruit without injury for control and treatments were placed inside a clean plastic box with fruit touching. Each box was wrapped in a 0.02-mm polyethylene bag and was held in a room at 25 °C ( $\pm 1$  °C). Analysis in triplicate of six fruit each from six plastic boxes was undertaken at 3-day intervals for total soluble solids content (SSC), titratable acidity (TA), enzyme activities of peroxidase (POD), polyphenol oxidase (PPO) and phenylalanine ammonia-lyase (PAL) and total phenols. Another 60 fruit of each treatment (three replicates, each of 20 fruit) were observed to evaluate firmness index, disease index and fruit decay.

### 2.2. Measurement of fruit firmness index

Fruit firmness was assessed by using hand squeeze described by Kobiler et al. (2001) with slight modification, and a firmness index scale from extremely firm to soft ripe (9 = extremely firm, 7 = firm, 5 = sprung, 3 = slight soft and 1 = soft ripe). Firmness index was calculated using the formula:  $\Sigma$  (firmness scale  $\times$  percentage of fruit within each firmness class).

### 2.3. Measurement of disease index

Disease index for fruit was assessed by assessing the extent of total decayed area on each fruit surface using the following scale (Zheng, Tian, Gidley, Yue, & Li, 2007; Zheng et al., 2007): 0 = no visible decay; 1 = <1% decay spots; 2 = 1–20% decayed; 3 = 20–50% decayed; and 4 = >50% decayed. The disease index was calculated using the formula:  $\Sigma$  (disease scale  $\times$  number of fruit in each class)  $\times 100$  / (number of total fruit  $\times$  highest disease scale). The

percentage of fruit including class 2–4 without commercial value was taken as fruit decay.

### 2.4. Measurement of SSC and TA

Juice samples were obtained from 12 discs of flesh (about 5 mm deep under peel, 10 mm thickness  $\times$  13 mm diameter, two discs per fruit on opposite region) from six fruit on the longer transverse axis, and SSC of the fruit juice were determined using a refractometer (Master- $\alpha$ , ATAGO ATC, Japan).

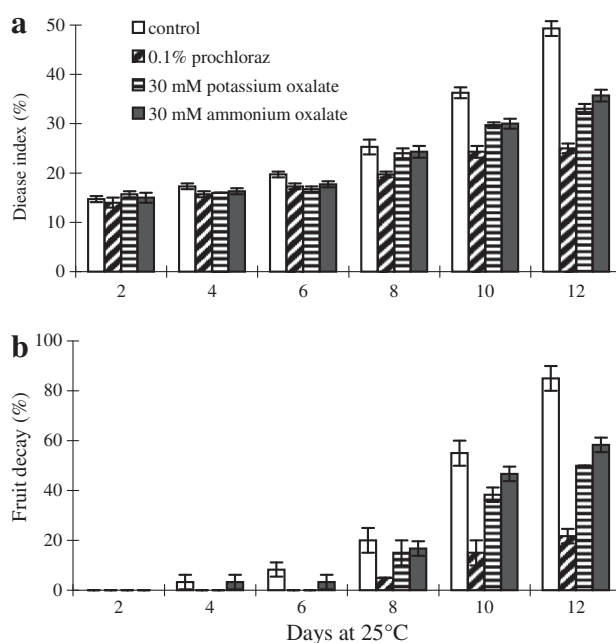
Ten grams of flesh tissue (about 5 mm deep under peel) from six fruit on the longer transverse axis (each fruit on opposite region) were homogenised with 25 ml distilled water and filtered, and then TA of the solution was determined by titration to pH 8.1 with 0.1 M NaOH. TA expressed as the percentage of citric acid per 100 g fresh mass.

### 2.5. Assay for enzymic activities

Five-gram peel and ten-gram flesh samples from six fruit in each treatment were ground separately in 30 ml 100 mM sodium phosphate buffer, pH 7.8, containing 0.3 g PVPP (Sigma, St. Louis, MO) for POD and PPO, and in 25 ml of 50 mM sodium borate buffer, pH 8.8, containing 0.3 g PVPP (Sigma) and 5 mM  $\beta$ -mercaptoethanol for PAL analysis using a Kinematica tissue grinder (Kinematica PT2100, Lucerne, Switzerland) and centrifuged at 20,000g for 45 min (Sigma 3–30 K, Osterode am Harz, Germany). The supernatants were used to assay enzymic activities. All steps in the preparation of extracts were carried out at 4 °C.

POD (EC 1.11.1.7) activity was based on the determination of guaiacol oxidation at 470 nm by  $H_2O_2$ . The change in absorbance at 470 nm was followed every 30 s by a spectrophotometer (Shimadzu UV-2550, Kyoto, Japan) (Lacan & Baccou, 1998). One unit of POD was defined as the amount of enzyme causing a 0.01 absorbance increase per min under the conditions of assay.

PPO (EC 1.10.3.2) activity was measured by incubating 0.5 ml of enzyme extract to 2.5 ml of buffered substrate (100 mM sodium phosphate, pH 6.4 and 50 mM catechol), and then monitoring the



**Fig. 1.** Changes in disease index (a), and fruit decay (b) in untreated (control), prochloraz and oxalate-treated mango cv. Xiaojinhuang fruit during storage. Data are the means of three replicates  $\pm$  SD.

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