Food Chemistry 142 (2014) 72–78

Contents lists available at SciVerse ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Pre-storage application of oxalic acid alleviates chilling injury in mango fruit by modulating proline metabolism and energy status under chilling stress

Peiyan Li [a,b], Xiaolin Zheng [a,*], Yan Liu [a], Yuyan Zhu [a]

[a] College of Food Science and Biotechnology, Zhejiang Gongshang University, Hangzhou 310035, PR China
[b] College of Food Science and Biotechnology, Henan University of Science and Technology, Luoyang 471023, PR China

ARTICLE INFO

Article history:
Received 15 April 2013
Received in revised form 20 June 2013
Accepted 28 June 2013
Available online 6 July 2013

Keywords:
Chilling injury
Energy status
Mango fruit
Oxalic acid
Proline metabolism

ABSTRACT

Effects of oxalic acid on chilling injury, proline metabolism and energy status in mango fruit were investigated after mango fruit (*Mangifera indica* L. cv. Zill) were dipped in 5 mM oxalic acid solution for 10 min at 25 °C and then stored at low temperature (10 ± 0.5 °C) for 49 days thereafter transferred to 25 °C for 4 days. Pre-storage application of oxalic acid apparently inhibited the development of chilling injury, notably elevated proline accumulation actually associated with increase in Δ^1-pyrroline-5-carboxylate synthetase (P5CS) activity and decrease in proline dehydrogenase (PDH) activity in the peel and the flesh, without activation of ornithine-δ-aminotransferase (OAT) activity, and maintained high ATP level and energy charge in the flesh during storage. It was suggested that these effects of oxalic acid might collectively contribute to improving chilling tolerance, thereby alleviating chilling injury and maintaining quality of mango fruit in long term cold storage.

1. Introduction

Mangoes (*Mangifera indica* L.) are economically important tropical fruit with a brief shelf life, and refrigeration has been shown to be an effective method to prolong its storage life (Mitra & Baldwin, 1997). However, harvested mango fruit are susceptible to chilling injury, which limits the advantage for maintaining fruit quality during long term storage at low temperature, especially below 13 °C (Phakawatmongkola, Ketsa, & Doorn, 2004). Typical chilling symptoms in mango fruit include greyish skin discolouration, browning, poor aroma and flavour, surface pitting, uneven ripening, and increased susceptibility to postharvest rot (Nunes, Emond, Brecht, Dea, & Proulx, 2007).

Induction of chilling tolerance by chemical or physical treatments, or by exposure to other stresses such as high and low temperature stress is becoming a great potential approach for protecting harvested fruit from chilling injury, and enhancement of membrance integraty by regulating plasma membrane proteins and lipids (Li et al., 2012; Zhang & Tian, 2010), improvement of antioxidant system and suppression of reactive oxygen species (Chen & Yang, 2012), proline accumulation by modulating its synthesis and degradation (Cao, Cai, Yang, & Zheng, 2012; Shang, Cao, Yang, Cai, & Zheng, 2011), and maintainance of higher ATP content and energy charge (Chen & Yang, 2012; Jin et al., 2012; Zhu, Bai, Liang, & Wang, 2012) are considered as the mechanisms being involved in the acquisition of chilling tolerance.

Oxalic acid is an organic acid ubiquitously occurring in various organisms, especially in plant, and plays several different roles in different living organisms. Previous works have reported that pre-storage application of oxalic acid alleviates chilling injury in mango fruit and in pomegranate fruit associated with an increase in antioxidant capacity (Ding, Tian, Zheng, Zhou, & Xu, 2007; Sayyari et al., 2012). More recently, the physiological effects of oxalic acid in enhancing membrane integrity, inhibiting activity of enzymatic browning, and maintaining higher level of osmotic substances are also considered to be conducive to chilling tolerance in mango fruit during cold storage at 10 °C (Xue, Li, Song, Shen, & Zheng, 2012). In an effort to better understand the mechanism of oxalic acid in improving chilling tolerance in mango fruit during cold storage, the effects of pre-storage application of oxalic acid on proline metabolism and energy status were further investigated in this study.

2. Materials and methods

2.1. Fruit material and treatments

Mango (*M. indica* L. cv. Zill) fruit were harvested at about 80% matured stage from a commercial orchard located in Panzhihua

* Corresponding author. Tel.: +86 571 88071024/7584; fax: +86 571 88053832.
E-mail address: zheng9393@163.com (X. Zheng).

0308-8146/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.foodchem.2013.06.132

city, PR China. The harvest fruit were immediately 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) or 5 mM oxalic acid solution at room temperature for 10 min. After air drying, each fruit was wrapped with a soft absorbent paper, and then about 15 kg fruit of control and treated fruit were placed in separate cartons. The transit time by plane from harvest to arrival at a Hangzhou laboratory was approximately 24 h. Upon arrival at the laboratory, each 30 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 low temperature humidity chambers (Sanyo, MIR-554) at 10 ± 0.5 °C for 49 days and subsequently transferred to 25 °C for 4 days. Analysis in triplicate of ten fruits each was undertaken at 7-day intervals during cold storage.

2.2. Measurement of chilling injury index and internal browning index

The chilling injury index for fruit was assessed by assessing the extent of total chilling symptoms on each fruit surface using the following scale (Xue et al., 2012): 0 = no visible chilling symptoms; 1 = <10% chilling spots; 2 = 10–20% chilling spots; 3 = 20–30% chilling spots; 4 = >30% chilling spots. The chilling injury index was calculated using the formula: $\sum$ (chilling scale × number of fruit in each class)/(number of total fruit × highest chilling scale) × 100.

The internal browning index was evaluated by assessing the extent of total browning symptoms on the mesocarp surface using the following scale (Ding et al., 2007) with slight modifications: 0 = no browning; 1 = browning covering <10% of the surface; 2 = browning covering >10% but <20% of the surface; 3 = browning covering >20% but <30% of the surface; 4 = browning covering >30%. The browning index was calculated using the following formula: Internal browning index (%) = $\sum$ (browning scale × number of mango fruit with that browning level)/(total number of mango fruit × highest browning scale) × 100.

2.3. Measurement of fruit firmness, soluble solid content (SSC) and titratable acid (TA)

Fruit firmness was measured on two pared sides of 10 fruit from each treatment using a durometer (FMH-5, Japan).

The SSC of the fruit juice, which were obtained from 10 fruit on the longer transverse axis (about 5 mm deep under peel, two discs per fruit on opposite region), were determined using a refractometer (Master-a, ATAGO ATC, Japan).

Ten grams of flesh tissue (about 5 mm deep under peel) from 10 fruit on the longer transverse axis (each fruit on opposite region) were homogenised with 25 ml distilled water and filtered. 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.

2.4. Measurement of proline content

The proline content was measured according to the method described by Bates, Waldren, and Teare (1973) with minor modification. The proline content was extracted from mango tissues (peel or flesh) in 30 g/L sulfosalicylic acid at 100 °C for 10 min with shaking. Then, the extract was centrifuged at 10,000×*g* for 15 min, and the supernatant was collected and stored at 4 °C for proline determination. 2 ml supernatant was mixed with 2 ml glacial acetic and 3 ml acid ninhydrin reagent and boiled for 30 min. After cooling, the reaction mix was extracted by 4 ml toluene, and the absorbance of the organic phase was recorded at 520 nm. The proline content was expressed as μg proline g^{-1} fresh weight (FW).

2.5. Measurement of P5CS, PDH and OAT activity

P5CS and PDH activity were assayed as described by López-Carrión, Castellano, Rosales, Ruiz, and Romero (2008) with minor modification. 2 g of peel or flesh tissue was ground with extraction buffer, then the homogenates was centrifuged at 12,000×*g* for 15 min. The supernatant was collected for enzyme activity determination. The extraction buffer was 0.5 M Tris–HCl buffer (pH 7.5) containing 10 mM $MgCl_2$, 2 mM phenylmethanesulfonyl fluoride and 100 mM beta-mercaptoethanol for P5CS extraction, while it was 0.1 M K_2HPO_4–KH_2PO_4 buffer (pH 7.8) with 1 mm EDTA and 10 mM beta-mercaptoethanol for PDH extraction. The P5CS reaction mixture contained 100 mM Tris–HCl (pH 7.2), 25 mM $MgCl_2$, 5 mM ATP and 75 mM sodium glutamate and the enzyme extract. The reaction was initiated by the addition of 0.4 mM NADPH. The activity was measured as the rate of consumption of NADPH monitored by decreased in absorbance at 340 nm. PDH reaction mixture, containing 2.4 ml 0.15 M Na_2CO_3–$NaHCO_3$ buffer (pH 10.3), 0.2 ml 2.67 mM L-proline and 0.2 ml 10 mM NAD^+, was initiated by the addition of 0.2 ml enzyme extraction. PDH activity was assayed by the reduction of NAD^+ at 340 nm. One unit of P5CS or PDH

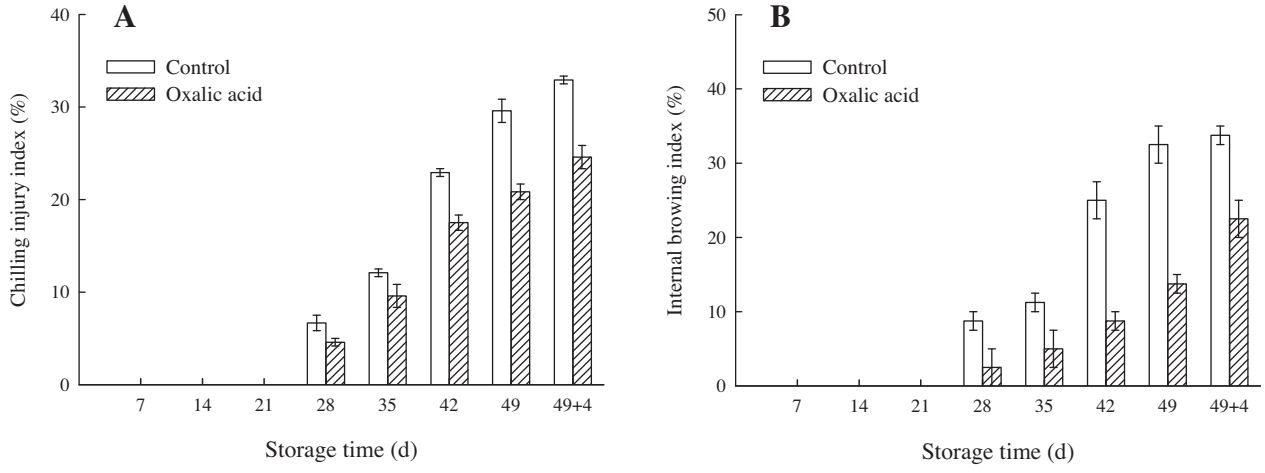

Fig. 1. Effect of oxalic acid on fruit chilling injury index and internal browning index during storage. "49 + 4 d" means fruits had been stored for four days at 25 °C after transferred from at 10 °C for 49 days. Data are the means of three replicates ± SE.