



Changes in sugar metabolism caused by exogenous oxalic acid related to chilling tolerance of apricot fruit



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ABSTRACT

Enhancement of chilling tolerance has been observed in fruits treated with oxalic acid (OA). To learn how OA may play a role in modulating chilling injury in apricot, the fruit were treated with OA (5 mM) and stored at 2 ± 1 °C for five weeks. OA treatment significantly inhibited chilling injury, electrolyte leakage and accumulation of hydrogen peroxide and malondialdehyde in apricot fruit. Sorbitol, sucrose, fructose and glucose were the major soluble sugars in the fruit. Contents of glucose and fructose were enhanced, meanwhile, the levels of sucrose and sorbitole were decreased by OA treatment during storage. OA treatment enhanced activities of the enzymes related to increasing glucose and fructose, and suppressed sucrose synthase synthesis activity which could explain the lower content of sucrose. The activities of sucrose synthase cleavage function, acid invertase, NAD⁺-sorbitol dehydrogenase and sorbitol oxidase were significantly enhanced by OA. Meanwhile, OA-treated apricot fruit showed lower sucrose synthase synthesis activity. The degradation of sorbitol in apricot fruit was accelerated by OA treatment. Our results indicate that higher chilling tolerance of OA treated fruit was associated with higher content of reducing sugars (glucose and fructose).

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1. Introduction

Apricots are popular around the world as a result of their high nutritional value and aroma (Solis-Solis et al., 2007). Various phenolic compounds including caffeic acid, ferulic acid, catechin, epicatechin, p-coumaric acid have been detected in apricot fruit (Sochor et al., 2010). Apricot fruit is usually stored at low temperatures to extend shelf life and maintain quality. During storage at low temperature, apricot fruit show some physiological disorders because apricot fruit is sensitive to low temperatures. Internal browning is one major symptom of chilling injury (CI) in apricot fruit (Saba et al., 2012).

Recently, the metabolism of soluble sugars of fruit during the postharvest storage attracted the interest of numerous scientists (Borsani et al., 2009; Sun et al., 2011). The reason is that the profiles and concentrations of soluble sugars not only contribute to the organoleptic quality of fruit (Borsani et al., 2009), but also affect the chilling tolerance of fruit during cold storage (Agopian et al., 2011; Wang et al., 2013; Cao et al., 2013). In mandarin fruit, the

level of sucrose is considered to enhance chilling tolerance (Holland et al., 2002). However, a higher level of fructose and glucose is believed to enhance the chilling tolerance of loquat fruit (Shao et al., 2013; Cao et al., 2013).

Oxalic acid (OA), a natural organic acid, has been reported to play an important role in systemic resistance and response to environment (Zheng et al., 2012; Jin et al., 2014; Liang et al., 2009). Prestorage application with OA enhanced the antioxidant capacities of pomegranate (Sayyari et al., 2010) and inhibited the decay of mango (Zheng et al., 2012). Moreover, the application of OA enhanced the chilling tolerance of litchi, peach and plum fruit during the postharvest storage under low temperatures (Zheng and Tian, 2006; Jin et al., 2014; Wu et al., 2011). Some work has been reported on the effect of OA on the chilling tolerance of various fruits (Jin et al., 2014; Wu et al., 2011; Zheng and Tian., 2006). The effect of OA on the chilling tolerance of various fruits has been explained by various mechanisms (Jin et al., 2014; Wu et al., 2011; Zheng and Tian., 2006).

To the best of our knowledge, there is no literature focus on the changes of soluble sugar content and metabolism of sugar in fruits caused by OA treatment. Then no work focuses on illuminating the relationship of changes of soluble sugar content caused by OA treatment and higher chilling tolerance of OA-treated fruit during

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the postharvest storage. The objective of this study was to determine changes in sugar content and metabolism caused by exogenous oxalic acid related to chilling tolerance of apricot fruit.

2. Materials and methods

2.1. Materials and treatments

Apricot fruit (*Prunus armeniaca* L. cv. Diaogan) of commercial maturity were obtained from local market in Beijing, PR China. The fruit were packaged in fibreboard cartons, transferred to our laboratory (Beijing, PR China) within 5 h via truck. During the transportation, the fruit was kept at low temperature (4–8 °C). Fruit sorted by hand (uniform size and color, without physical damage) were used in the experiment. Apricots were dipped in 5 mM OA solution for 10 min at room temperature. Control fruit were treated with distilled water. The fruit were then air-dried at room temperature for about 30 min. For each treatment, forty apricot fruit were placed into a plastic boxes (600 × 100 × 70 mm), and then stored at 2 ± 1 °C. The relative humidity (RH) was 85–90%. Each treatment contained 360 apricots. About 60 fruits were collected in each sampling time, and 18 of the collected fruits were used for the biochemical analysis, and 18 of the fruits used for measuring chilling injury (CI) and electrolyte leakage (EL). The measurement of CI was done in three replicates (per replicate consisted of six fruits). For the measurement of EL, flesh tissues from six fruits were used in one measurement. For the biochemical analysis, the flesh from sampling fruits were mixed, immediately frozen in liquid nitrogen and then stored at –80 °C. The flesh from 6 sampling fruits were used as one repeat (3 repeats for each biochemical measurement).

2.2. Determination of internal browning index (IBI), electrolyte leakage (EL), content of hydrogen peroxide (H₂O₂) and malondialdehyde (MDA)

The symptom of chilling injury was expressed as internal browning index (IBI). IBI was determined according to previous method with slight modification (Saba et al., 2012). The severity of browning was visually assessed and classified as 5 grades: 0 = none; 1 = slightly (browning area < 5%); 2 = moderate (browning area 5–25%); 3 = moderate severe (browning area 25–50%); 4 = severe (browning area > 50%). The results were calculated as follows: $IBI = (\sum IBI \text{ level}) \times (\text{the number of fruits at this IBI level}) / (4 \times \text{total number of fruits}) \times 100\%$.

A procedure previously reported was modified to determine the EL (Jin et al., 2014). For the determination of EL, ten flesh disks from fruits (the total weight of flesh was kept about 3 g) were collected with a 3-mm diameter cork borer, rinsed in 25 mL double distilled water for 30 min and was employed to measure the initial conductivity by one DJS-1C conductivity meter (Shanghai Analytical Instrument Co., Shanghai, China). Then the disks were placed at –20 °C for 24 h, incubated in a boiling water for 30 min and then used to determine total electrolyte conductivity. The EL was expressed as relative conductivity: $(\text{the initial conductivity} / \text{the final conductivity}) \times 100\%$.

The content of MDA was determined according to method described by Jin et al. (2014) with some modification. Flesh tissue (2 g) was homogenized on ice with 3 mL trichloroacetic acid (100 g L⁻¹) and then centrifuged at 12,000 × g for 20 min at 4 °C. A 0.5 mL supernatant was mixed with 2.5 mL thiobarbituric acid and then incubated in boiling water for 20 min. The reaction mixture was centrifuged at 12,000 × g for 7 min at 4 °C and absorbance of the supernatant was recorded at 450 nm, 532 nm, 600 nm respectively. MDA content in the supernatant was and

calculated as follow: $c (\mu\text{M}) = 6.45 \times (\text{OD}_{532} - \text{OD}_{600}) - 0.56 \times \text{OD}_{450}$. The MDA content of flesh were expressed as nmol g⁻¹ fresh weight.

The content of H₂O₂ was assayed using the method previously described with slight modification (Patterson et al., 1984). The modification was that we used titanium tetrachloride-hydrochloric acid instead of titanium sulphate. Flesh tissue (2 g) was homogenized with 4 mL acetone (pre-chilled at –20 °C) and then centrifuged at 12,000 × g for 20 min at 4 °C. A 1.0 mL supernatant with 0.1 mL titanium tetrachloride-hydrochloric acid and 0.2 mL ammonia (25%, v/v) was centrifuged at 12,000 × g for 15 min at 4 °C. The precipitation was collected and sufficiently suspended in 3 mL H₂SO₄ (10%, v/v), centrifuged at 12,000 × g for 10 min at 4 °C. Absorbance of the supernatant was recorded at 410 nm and the H₂O₂ content was expressed as nmol g⁻¹ fresh weight.

2.3. Analysis of soluble sugar content

Extraction and assays of soluble sugars were carried out with some modification according to a previous method (Shao et al., 2013). Five grams of frozen fresh from fruit samples were homogenized with 25 mL extraction solution on ice and kept still for 20 min. Special solution contained 3.2 g L⁻¹ acetic acid and 2.4 g L⁻¹ potassium ferrocyanide (dissolved with double distilled water). The mixture was centrifuged at 12,000 × g for 20 min at 4 °C. The supernatant was collected and diluted to 150 mL with double distilled water. Before the HPLC analysis, the resulting extract was filtered through a 0.22 μm membrane filter.

A 10 μL sample was injected into an ultrafast liquid chromatography (UFLC). The UFLC contained HPLC (Agilent 1260, Agilent Corp, America., USA; column: Hadera, NH₂; column temperature: 50 °C) and an evaporative light-scattering detector (Agilent 1260, Agilent Corp, America). The mobile phase composition was acetonitrile and water (80:20). The total flow rate of mobile phase composition was 1 mL min⁻¹. Individual sugars were identified by the retention of time and quantified based on standard curves of individual sugars.

2.4. Extraction and assays of activity of sucrose phosphate synthase (SPS), sucrose synthase synthesis activity (SS-synthesis), sucrose synthase cleavage activity (SS-cleavage), acid invertase (AI) and neutral invertase (NI)

For the assay of relative activity of SPS, SS-synthesis, SS-cleavage, AI and NI, the extraction process was modified from a previous method (Shao et al., 2013). Flesh tissues (3 g) were homogenized on ice with 0.5 g crosslinking polyvinylpyrrolidone (PVPP) and 6 mL buffer containing 50 mM HEPES–NaOH (pH 8.5), 10 mM MgCl₂, 2.5 mM DTT, 0.1% TritonX-100 (v:v) and 10 mM vitamin C, 10 mM β-mercaptoethanol, 20% (v:v) glycerol, 10 μg mL⁻¹ leupeptin, 10 μg mL⁻¹ chymostatin. The homogenate was centrifuged at 12,000 × g for 30 min at 4 °C. A cold pre-equilibrated Sephadex G-25 column was used to desalt the supernatant. These crude extracts were used for the measurement of activity of enzymes.

For the assay of activity of SPS, the reaction system consisted of HEPES–NaOH buffer (50 mM, pH 7.5; containing 15 mM MgCl₂), 25 mM fructose 6-phosphate, 25 mM glucose 6-phosphate, 25 mM UDP-glucose and crude enzyme extract. The reaction system was incubated for 30 min at 37 °C. At last, 0.1 mL 30% (w:v) KOH was added and then the mixture was incubated in boiling water for 5 min to terminate the reaction. The content of sucrose produced by this reaction was determined using the anthrone assay (van Handel, 1963).

SS-synthesis was assayed as SPS but with 60 mM fructose instead of fructose-6-phosphate, and in the absence of glucose-6-phosphate. The content of sucrose was determined using the

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