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Exogenous glycine betaine treatment enhances chilling tolerance of peach fruit during cold storage

Timin Shan[a], Peng Jin[a,*], Yu Zhang[a], Yuping Huang[a], Xiaoli Wang[b], Yonghua Zheng[a]

[a] College of Food Science and Technology, Nanjing Agricultural University, Nanjing 210095, PR China
[b] College of Life Science and Chemical Engineering, Huaiyin Institute of Technology, Huai'an 223003, PR China

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ABSTRACT

The effect of exogenous glycine betaine (GB) treatment on chilling injury (CI), energy status, endogenous GB, γ-aminobutyric acid (GABA) and proline content in cold-stored peach fruit was investigated. The results showed that GB treatment significantly prevented CI and maintained low flesh firmness and high extractable juice in peach fruit during cold storage. The accumulation of endogenous GB, GABA and proline contents in peaches was enhanced by GB treatment resulting from increasing activities of metabolism enzymes, including betaine aldehyde hydrogenase (BADH), glutamate decarboxylase (GAD), Δ^1-pyrroline-5-carboxylate synthetase (P5CS) and ornithine δ-aminotransferase (OAT). Moreover, high level of energy status in GB-treated peach fruit was found. These results indicated that GB treatment enhanced chilling tolerance in peaches due to induction of energy status, endogenous GB, GABA and proline contents, which prevented membrane damage. Thus, exogenous GB treatment could be a useful method to reduce CI in peach fruit during cold storage.

1. Introduction

Chilling injury (CI) is considered to be the main problem in peach fruit refrigeration industry. The main symptoms of CI in peach fruit include flesh browning or mealiness, and even failure to ripen after long time cold storage (Lurie and Crisosto, 2005). The symptoms of CI in peach fruit usually develop during shelf life at ambient temperature, which is not easy to find and control during cold storage period. The development of CI in peach fruit limits refrigeration and cold chain transportation. Thus, it is necessary to develop new technique for controlling or alleviating CI in cold-stored peaches.

Glycine betaine (GB), a kind of quaternary ammonium alkaloid, is an important osmotic adjustment substance that plays an important role in maintaining cell osmotic pressure, protecting protein or enzymes function, and regulating stress response in plant (Mansour, 1998). In plants, GB is synthesized from choline by two steps of dehydrogenation reaction. Choline is oxidized to betaine aldehyde by choline monooxygenase (CMO) first, then betaine aldehyde is oxidized to GB by betaine aldehyde dehydrogenase (BADH) (Burnet et al., 1995). It has been reported that GB accumulation is related to enhancing stress tolerance in some crop plants such as barley, spinach and sorghum under salt and drought stress (Ashraf and Foolad, 2007). Moreover, exogenous GB could be effective in inducing cold tolerance in strawberry plant (Rajashekar et al., 1999) and *Arabidopsis* (Xing and Rajashekar, 2001). Rodríguez-Zapata et al. (2015) also found that preharvest foliar applications of GB had benefit in reducing CI of banana fruit during postharvest cold storage. Recently, exogenous GB has been successful application in increasing cold tolerance in some fruits and vegetables such as loquat (Sun et al., 2014), button mushrooms (Wang et al., 2015) and hot pepper (Ding et al., 2012). However, little information concerning the effect of exogenous GB on reducing CI and inducing cold tolerance in peach fruit has been available.

Accumulation of γ-aminobutyric acid (GABA) and proline in plant tissue is also associated with stress tolerance and is usually considered to be involved in stress resistance mechanism (Ashraf and Foolad, 2007). In cold-stored fruits and vegetables, the increase of GABA and proline content is often regarded as enhancing chilling tolerance. For example, high carbon dioxide treatment reduced CI in tomatoes due to the induction of endogenous GABA accumulation (Deewatthanawong et al., 2010). Salicylic acid improved stress tolerance in mustard related to its enhancement of proline concentration (Nazar et al., 2015). Moreover, exogenous GABA treatment could reduce CI in peach and banana fruits through enhancing endogenous GABA and proline accumulation (Yang et al., 2011; Shang et al., 2011; Wang et al., 2014). In contrast, a reduction of endogenous GABA was found in

* Corresponding author. Fax: +86 25 8439 5618.
E-mail address: pjin@njau.edu.cn (P. Jin).

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zucchini fruit, which had high tolerance to cold stress (Palma et al., 2014). However, the accumulation of GABA and proline content induced by GB is not clear. Studying the effect of exogenous GB treatment on regulation of proline and related enzymes activities could provide new evidence on its physiological function under cold stress.

The object of this study was undertaken to evaluate the effects of exogenous GB treatment on CI, firmness, extractable juice in peach fruit during cold storage. Moreover, the effects of exogenous GB treatment on endogenous GB, GABA, proline and energy status were also investigated to elucidate the possible mechanism in cold-stored peaches.

2. Materials and methods

2.1. Fruit material and treatment

Peach fruit (*Prunus persica* Batsch cv. 'Yuhua No. 2') were harvested at commercial maturity (firmness about 8.5 N, total soluble solids about 10.5%) from a local orchard in Nanjing, China. The peaches were selected for uniformity without any damage and randomly divided into two groups with 360 fruit for each. Based on our previous study (Shan et al., 2015), 10 mM GB was chosen as the option concentration for treatment. The GB treatment group was immersed in 10 mM GB solution for 10 min, and the control group was soaked in sterile deionized water for 10 min. All fruit were then air dried for approximately 30 min. After treatment, all peaches were air dried for approximately 30 min and transferred to 0 °C for 5 weeks. Mesocarp samples were taken from 10 fruit each week and frozen in liquid nitrogen, then stored at −80 °C until biochemical analysis. Another 10 peaches were removed from 0 °C to 20 °C for 3 d to simulate shelf condition after each week, and evaluated CI index, firmness and extractable juice.

2.2. CI index, firmness and extractable juice measurement

CI index was evaluated by rating the browning severity of 10 peaches flesh according to the browning area of peach flesh. The severity of CI was evaluated by rating a five-grade scale with 0 = none; 1 = slight; 2 = moderate; 3 = moderately severe; and 4 = severe. Results were expressed as CI calculated using the following formula: CI = $\sum$ [(CI scale) × (number of fruit at that CI)]/(4 × total number of fruit in each treatment) × 100%.

Fruit firmness was measured on two paired sides of 10 fruit with a TA-XT2i texture analyser (Stable Micro System Ltd., UK) with a 5 mm diameter probe at a speed of 1 mm s^{-1}.

Extractable juice was evaluated by measuring the weight loss from mesocarp disks (10 mm in diameter and 10 mm in thickness) after centrifuging for 10 min at 1500 × *g*. Mesocarp disks were supported over a wad of absorbent cotton in centrifugal tubes. The result was expressed as percent fresh weight loss of the tissue plugs after centrifugation.

2.3. Ion leakage and MDA content measurement

Ion leakage was measured using 20 flesh disks (5 mm thickness × 10 mm diameter) obtained with a puncher (diameter 1 cm). Then the disks were placed in 25 mL of distilled water and immersed for 30 min. The initial conductivity of solution (L_0) was determined using a conductivity meter (DDS-11A, Shanghai, China). Then the solution was boiled for 5 min and re-adjusted to a volume of 25 mL before the final conductivity of the solution (L_1) was measured. The Ion leakage were calculated as the following formula: Ion leakage (%) = (L_0/L_1) × 100%.

Mesocarp (2 g) was taken for malondialdehyde (MDA) content assay, and homogenized with 5 mL of 0.5% (w/v) trichloroacetic acid (TCA) and then centrifuged at 10,000 × *g* for 10 min at 4 °C. MDA content were determined following the method of Hodges et al. (1999). MDA content was expressed as nanomole per gram.

2.4. GB content and BADH activity measurement

Mesocarp (5 g) was homogenized with 20 mL of methanol–chloroform extraction buffer (60% methanol, 25% chloroform) and then centrifuged at 10,000 × *g* at 4 °C for 20 min. The entire aqueous phase (top) was purified by a sole cationic ion-exchange resin (Dowex AG1 OH^-, 200–400 mesh, Dow Chemical Co., Midland, MI, USA). The GB fraction was eluted with 20 mL of 6 mmol L^{-1} NH_4OH and then dried under nitrogen at 40 °C. The residues were then dissolved with 3 mL of methanol. The GB content was assayed using high-performance liquid chromatography (Agilent 1100, Agilent Corp., Santa Clara, CA) according to the method of Bessieres et al. (1999). The GB content was expressed as microgram per gram FW.

For betaine aldehyde hydrogenase (BADH) activity assay, mesocarp tissue (5 g) was homogenized with 20 mL of pH 7.4 potassium phosphate buffer and centrifuged at 13,000 × *g* at 4 °C for 20 min. The supernatant was used for BADH activity assay. The reaction mixture contained 0.5 mL of crude enzyme extract, 0.2 mL of 30 mmol L^{-1} NAD^+ and 2.9 mL of 100 mmol L^{-1} Tris–HCl buffer (pH 8.0) containing 5 mmol L^{-1} dithiothreitol (DTT). 0.5 mL of 10 mmol L^{-1} betaine aldehyde was added to initiate the reaction (Arakawa et al., 1990). BADH activity was calculated as NADH production and one unit of enzyme activity was expressed as the release of 1 μmol of NADH per second.

2.5. GABA content and GAD activity measurement

GABA content and glutamate decarboxylase (GAD) activity were measured according to the method of Deewatthanawong et al. (2010). Mesocarp tissue (5 g) was extracted with 0.5 mL of methanol for 10 min, and then centrifugation at 12,000 × *g* for 10 min. The supernatant was transferred to a new tube and mixed with 0.5 mL of 1.5 M potassium hydroxide. The mixture was shaken for 10 min, and centrifuged at 12,000 × *g* for 10 min. The supernatant was used for GABA measurement. The reaction mixture contained 550 μL of supernatant, 100 μL of 0.6 mM of $NADP^+$, 0.1 unit of GABase (Sigma, St. Louis, MO), 2 mL of 0.1 M potassium pyrophosphate buffer (pH 8.6) and 250 μL of 1 mM α-ketoglutarate. The result was calculated using GABA standard curve and expressed as microgram per gram FW.

For GAD activity assay, mesocarp tissue (2 g) was grinded with 10 mL of Tris–HCl buffer (pH 9.0) containing 10% (v/v) glycerol, 1 mM DTT, 5 mM EDTA, 0.5 mM pyridoxal phosphate (PLP) and 1 mM phenylmethylsulfonyl fluoride. The grinded mixture was centrifuged at 12,000 × *g* for 30 min at 4 °C. The supernatant was used for GAD activity assay. GAD activity reaction mixture contained 0.5 mL of supernatant, 2 mL of 0.1 M potassium phosphate buffer (pH 6.0), 100 μL of 0.04 mM PLP and 0.4 mL of 3 mM glutamate. The reaction was incubated at 30 °C for 40 min, and stopped by adding 0.2 mL 1 M HCl. GAD activity was calculated as GABA production and one unit enzyme activity was defined as the amount of 1 μmol GABA formation per second.

2.6. Proline content and P5CS, PDH and OAT activities measurement

The proline content, Δ^1-pyrroline-5-carboxylate synthetase (P5CS), ornithine δ-aminotransferase (OAT) and proline dehydrogenase (PDH) activities were measured according to the method of Sánchez et al. (2001). Mesocarp tissue (2 g) was grinded and then was shaking extracted with hot sulfosalicylic acid solution for 10 min. The homogenate was centrifuged at 12,000 × *g* for 20 min