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Glycine betaine treatment attenuates chilling injury and maintains nutritional quality of hawthorn fruit during storage at low temperature

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ABSTRACT

Low-temperature storage delays senescence and helps to maintains nutritional quality, which recommended for extending the postharvest life of fruit and vegetable. But, under low temperature storage, hawthorn fruit pitting as a physiological manifestation of chilling injury (CI) symptom can develop visually. In this study, the effects of glycine betaine (GB) treatment applied by immersion (0, 2.5, 5 and 10 mM, for 15 min at 20 °C) on chilling injury and nutritional quality of hawthorn fruit during storage at 1 °C for 20 days was investigated. The results showed that GB treatment, especially at 10 mM, significantly delayed fruit pitting development. Also, GB treated hawthorn fruit exhibited significantly higher endogenous GB and proline accumulation, which was concurrent with higher antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX) activity leading to lower H_2O_2 accumulation. Also, hawthorn fruit treated with GB exhibited significantly higher phenols, flavonoids and anthocyanins accumulation resulting from higher phenylalanine ammonia lyase (PAL) enzyme activity, which concomitant with higher ascorbic acid accumulation leading to higher DPPH scavenging capacity during storage at 1 °C for 20 days. These results suggested that GB treatment not only can be used as a useful strategy for attenuating chilling injury of hawthorn fruit by enhancing antioxidant enzymes activity leading to lower reactive oxygen species (ROS) accumulation, but also is useful for maintaining nutritional quality of hawthorn fruit by triggering antioxidant molecules accumulation which is beneficial for human health.

1. Introduction

Owing to rich phytonutrient status such as phenols, flavonoids, anthocyanins and ascorbic acid with ROS scavenging capacity in hawthorn fruit, its consumption is beneficial for human health (Özcan et al., 2005; Yang and Liu, 2012). However, the postharvest life of hawthorn fruit is very short (9 days) at room temperature accompanying with phenols, flavonoids, anthocyanins loss leading to falling 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) and ferric reducing antioxidant power (FRAP) scavenging capacity (Samec and Piljac-Zegarac, 2011). Low-temperature storage (1 °C) delays senescence and helps to maintain nutritional quality by enhancing phenols, flavonoids, anthocyanins accumulation leading to higher DPPH, ABTS and FRAP scavenging capacity, which recommended for extending the postharvest life of hawthorn fruit (Samec and Piljac-Zegarac, 2011). However, under low temperature storage for 5 days, hawthorn fruit pitting as a physiological manifestations of chilling injury (CI) symptom can be observed visually.

Chilling temperature directly promotes membrane phase transition from a fluid liquid-crystalline to rigid solid-gel leading to declining membrane selective permeability, which can be assayed by electrolyte leakage. Also, chilling temperature as oxidative stress indirectly promotes reactive oxygen species (ROS) accumulation leading to membranes unsaturated fatty acids (unSFA) peroxidation, which can be assayed by malondialdehyde (MDA) accumulation (Aghdam and Bodbodak, 2013; Sevillano et al., 2009). Zhou et al. (2014) reported that the chilling injury manifested by external pitting in blueberry fruits during cold storage (0 °C for 60 days) was accompanied with higher O_2^- and H_2O_2 accumulation, which results from lower superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) enzymes activity along with higher LOX enzyme activity. Lower membrane integrity revealed by higher electrolyte leakage and MDA accumulation in blueberry fruits during cold storage results from lower antioxidant system activity Zhou et al. (2014). According to Zhou et al.

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(2014), higher antioxidant system activity leading to lower ROS accumulation is crucial for attenuating chilling injury in blueberry fruit manifesting with fruit pitting.

In fruit during postharvest life, endogenous glycine betaine (GB) accumulation occurs in response to chilling stress (Shan et al., 2016). Glycine betaine is synthesized from choline catalyzed by choline monooxygenase (CMO) and then catalyzed by betaine aldehyde hydrogenase (BADH) that is the key enzyme during the GB biosynthesis (Kumar et al., 2017; Kurepin et al., 2015). But, endogenous GB accumulation is not usually enough for attenuating postharvest senescence and chilling stress with oxidative facet (Shan et al., 2016). Therefore, GB treatment would be a promising strategy for attenuating postharvest senescence and chilling stress in fruit and vegetable, which results from higher endogenous proline and GB accumulation. Higher endogenous proline and GB accumulation are crucial for triggering ROS avoiding and scavenging system activity which maintains membrane fluidity and integrity by attenuating unSFA peroxidation (Kumar et al., 2017; Shan et al., 2016; Wang et al., 2016; Wang et al., 2015; Zhang et al., 2016).

In this study, the impact of postharvest GB treatment on the fruit pitting as chilling injury symptom, endogenous GB and proline accumulation, antioxidant enzymes CAT, APX, and SOD activity along with ascorbic acid and H_2O_2 accumulation, PAL enzyme activity associated with phenols, flavonoids and anthocyanins accumulation associated with DPPH· scavenging capacity of hawthorn fruit were evaluated.

2. Materials and methods

2.1. Fruits and treatments

Hawthorn fruit (*Crataegus monogyna*) were picked at commercial maturity (TSS = 5) in Zanjan Province, Iran, and transported to the fruit analysis laboratory at Zanjan University. In the laboratory, the fruit was screened for uniform size, maturity, and absence of mechanical damage. Then, health fruit randomly divided into four groups of 800 fruit each for one replication and subjected to GB treatment by fruit immersing in 0, 2.5, 5 and 10 mM GB solution at 20 °C for 10 min. After treatment, all hawthorn fruit were taken out and air dried for 30 min at 25 °C, and then transferred to 1 °C and about 95% relative humidity for 20 d. Each treatment was replicated three times, and the experiment was conducted twice. A 100 hawthorn fruit, from each treatment from each replicate, was taken every 5 days during storage to frozen in liquid nitrogen and stored at − 80 °C until biochemical analysis. Another 100 hawthorn fruit were removed from 1 °C and placed into 20 °C for 3 days to simulate shelf conditions every 5 days during storage and fruit were evaluated for pitting incidence and nutritional quality attributes.

2.2. Fruits pitting incidence

Pitting incidence as chilling injury symptom was assessed after 0, 5, 10, 15, and 20 days' storage at 1 °C followed by 3 days of shelf life at 20 °C. Three independent replicates (n = 100 fruit each) for each treatment were conducted. Pitting incidence was calculated as follows: pitting incidence = An/Am × 100%, where An is the number of hawthorn fruit with pitting and Am is the total number of hawthorn fruit.

2.3. GB and proline content

The GB content was measured according to the method of Grieve and Grattan, (1983). The fruit tissue (2 g) was finely ground, mechanically shaken with 20 mL deionized water for 24 h at 25 °C. The samples were then filtered, and filtrates were diluted to 1:1 with 2 N H_2SO_4. Aliquots were kept in centrifuge tubes and cooled in ice water for 1 h. Cold KI-I_2 reagent was added, and the reactants were gently stirred with a vortex mixture. The tubes were stored at 4 °C for 16 h and then centrifuged at 10,000 × *g* for 15 min at 4 °C. The supernatant was carefully aspirated with a fine glass tube. The periodide crystals were dissolved in 9 mL of 1,2-dichloroethane. After 2 h, the absorbance was measured at 365 nm using GB as standard and GB content was expressed as mg g^{-1} dry weight (DW). The proline content was measured according to the method of Sanchez et al. (2001). Proline in fruit tissues (2 g) was extracted with 30 mL L^{-1} sulfosalicylic acid at 100 °C for 10 min with shaking. The extract was mixed with an equal volume of glacial acetic acid and acid ninhydrin and boiled for 30 min. After cooling, the reaction mix was toluene, and the absorbance of the organic phase was recorded at 520 nm. The resulting values were compared with a standard curve constructed using proline and expressed as μg $^{-1}$ fresh weight (FW).

2.4. Antioxidant system activity

To analyze antioxidant enzymes activity, fruit tissue (5 g) was homogenized in 50 mM phosphate buffer (pH 7.8) containing 0.2 mM EDTA and 2% polyvinyl polypyrrolidone (PVPP). The homogenate was centrifuged at 12,000*g* for 20 min at 4 °C, and the supernatant was used for SOD, CAT, and APX, enzymes activity measurements. SOD, CAT, and APX activity was measured according to Zhang et al. (2013). One unit of CAT activity was defined as a decrease in absorbance at 240 nm of 0.01 per min. One unit of APX activity was defined as the amount of enzyme that oxidizes 1 μmol of ascorbate per minute. One unit of SOD activity was defined as the amount of enzyme that causes a 50% inhibition of nitro blue tetrazolium (NBT) reduction under assay conditions. SOD, CAT, and APX activity was expressed as U mg^{-1} protein. Protein content was estimated according to Bradford (1976) using bovine serum albumin (BSA) as a standard. The content of H_2O_2 was measured according to Patterson et al. (1984). Fruits tissue (2 g) was ground in liquid nitrogen and extracted with 5 mL of cold acetone. After centrifugation at 6000 × *g* for 15 min at 4 °C, the supernatant was collected. One mL of supernatant was added to 0.1 mL of 5% titanium sulphate and 0.2 mL ammonia, and then centrifuged for 10 min at 6000 × *g* and 4 °C. The pellets were dissolved in 3 mL of 10% (v/v) H_2SO_4. The mixture was centrifuged for a further 10 min at 6000 × *g*. After that, the absorbance of the solution was recorded at 410 nm. The H_2O_2 content was expressed as μmol g^{-1} FW. The total ascorbic acid content was determined using the dinitrophenyl hydrazine (DNPH) method (Terada et al., 1978). The total ascorbic acid content was expressed as mg 100 g^{-1} FW.

2.5. Phenols metabolism

Total phenol content was assayed according to the Folin–Ciocalteu procedure (Singleton and Rossi, 1965), in which methanol/water (70:30, v/v) was used as the extraction solvent. 0.1 mL aliquots of extract were mixed with 0.1 mL of 50% (v/v) Folin–Ciocalteu reagent and allowed to react for 2 min at room temperature. Then, 2 mL of 2% Na_2CO_3 solution was added and left to stand for 30 min. After that, the absorbance of the mixture was recorded at 720 nm. Total phenol content was expressed as mg gallic acid equivalent (GAE) 100 g^{-1} FW. The pH-differential method reported by Giusti and Wrolstad (2001) was used for assaying total anthocyanin content. Total anthocyanin content was expressed as mg cyanidin-3-glucoside per 100 g^{-1} FW. Total flavonoids content was determined spectrophotometrically, using the method of Zhishen et al. (1999). Briefly, 0.25 mL of the sample was mixed with 75 μL of aqueous $NaNO_2$ (5%) and incubated at room temperature for 5 min. Then 0.15 mL of 10% $AlCl_3$ was added and was vortexed. After 6 min, 0.5 mL of 1 mol L^{-1} NaOH was added to this mixture. The final volume was set to 2.5 mL with distilled water, and the absorbance was measured against a blank at 510 nm. Total flavonoids content was expressed as mg quercetin equivalents per 100 g^{-1} FW.

PAL was extracted and assayed according to Nguyen et al. (2003). For PAL activity a sample of one gram of frozen tissue was homogenized in 20 mL of 50 mM borate buffer (pH 8.5) containing 5 mM 2-