Postharvest Biology and Technology 137 (2018) 31–37

Contents lists available at ScienceDirect

Postharvest Biology and Technology

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

Arginine treatment attenuates chilling injury of pomegranate fruit during cold storage by enhancing antioxidant system activity

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ARTICLE INFO

Keywords:
Antioxidant enzymes
DPPH scavenging capacity
Husk browning
Nutraceutical properties
Postharvest

ABSTRACT

In this study, the impacts of arginine treatment at 0, 0.5, 1 and 2 mM applied by preharvest spray in combination with postharvest immersion, on postharvest chilling injury and nutraceutical properties of pomegranate fruit during storage at 4 ± 0.5 °C for 120 d was investigated. Pomegranate fruit treated with arginine at 1 mM, displayed significantly lower chilling injury symptom manifested by external husk browning. Lower husk browning in pomegranate fruit treated with arginine, caused by lower husk H_2O_2 accumulation, was partially due to higher antioxidant enzymes, superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) activities, leading to higher membrane integrity revealed by lower electrolyte leakage and malondialdehyde (MDA) accumulation. Also, lower husk browning in pomegranate fruit treated with arginine resulted from higher husk phenylalanine ammonia-lyase (PAL)/polyphenol oxidase (PPO) enzymes activity ratio. Moreover, pomegranate fruit treated with arginine displayed higher arils DPPH scavenging capacity, due to higher arils total phenols and anthocyanins accumulation. Also, higher arils PAL/PPO enzymes activity ratio together with lower arils H_2O_2 accumulation was partially due to higher antioxidant enzymes, SOD, CAT and APX activities which are crucial for higher arils DPPH scavenging capacity. In addition, higher arils ascorbic acid content in pomegranate fruit treated with arginine may be attributed to higher glutathione reductase (GR)/APX system activity or higher anthocyanin accumulation. The results of this study suggest that arginine treatment can be used as promising technology not only for attenuating chilling injury but also for maintaining nutraceutical properties of pomegranate fruit partially by promoting antioxidant system activity.

1. Introduction

Low temperature storage is widely employed to prolong postharvest life of horticultural commodities together with maintaining nutraceutical properties (Aghdam and Bodbodak, 2013). Pomegranate fruit with high economic value, endemic to subtropical climates, is highly vulnerable to chilling injury (CI), which limits the usage of low temperature storage (Sayyari et al., 2011; Pareek et al., 2015). Owing to the economic value of pomegranate fruit and also its health benefits, great efforts have been made by researchers to attenuate its chilling injury during low temperature storage by exerting postharvest strategy such as polyamines (Mirdehghan et al., 2007a) and salicyloyl chitosan treatments (Sayyari et al., 2016).

Arginine as a metabolically versatile amino acid with high N/C ratio, in addition to being a structural unit of proteins, is crucial to enhance tolerance of horticultural commodities to chilling stress by biosynthesizing signaling molecules such as polyamines (PAs), nitric oxide (NO), γ-aminobutyric acid (GABA) and proline achieved by arginase, arginine decarboxylase (ADC) and nitric oxide synthase (NOS) enzymes (Aghdam and Bodbodak, 2013). Enhancing arginine pathways by pre and postharvest treatments such as arginine, methyl salicylate, methyl jasmonate and heat treatments, attenuates chilling injury caused by higher signaling molecules accumulation, which is crucial for chilling tolerance (Zhang et al., 2010, 2011, 2012, 2013a,b,c). Zhang et al. (2010) reported that the higher arginase genes expression and enzyme activity in cherry tomato fruit in response to chilling stress might act as a powerful chilling tolerance strategy functioning by promoting polyamines (PAs), proline or GABA accumulation. Zhang et al. (2010) reported that tomato fruit displayed higher chilling tolerance together with lower electrolyte leakage and malondialdehyde (MDA) accumulation, in response to arginine treatment. Additionally, higher membrane integrity in tomato fruit, treated with arginine, revealed by lower electrolyte leakage and MDA accumulation, may be caused by higher antioxidant enzymes, superoxide dismutase (SOD),

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https://doi.org/10.1016/j.postharvbio.2017.11.012
Received 16 April 2017; Received in revised form 11 November 2017; Accepted 12 November 2017

catalase (CAT) and ascorbate peroxidase (APX) activities, leading to lower reactive oxygen species (ROS) accumulation. Zhang et al. (2013a) reported that attenuating postharvest chilling injury of tomato fruit by arginine treatment was caused by higher polyamines accumulation via higher ADC and ornithine decarboxylase (ODC) enzymes activity, and higher proline accumulation via higher ornithine aminotransferase (OAT) enzyme activity concurrent with higher NO accumulation via higher NOS enzyme activity. Wills and Li (2016) reported that the fresh cut granny smith apple and iceberg lettuce, displayed delayed browning development and extended postharvest life in response to arginine treatment. Owing to nutritional and therapeutic roles of arginine as a promising, safe and cost-effective amino acid in human health (McKnight et al., 2010), the usage of its treatment may provide a commercially safe strategy with GRAS status to attenuate chilling injury and enhance nutraceutical properties of horticultural commodities, leading to extension of commodities postharvest life.

For the first time, in this study, the impact of arginine treatment applied by preharvest spray in combination with postharvest immersion, on chilling injury and its relation with husk electrolyte leakage and MDA accumulation as indirect membrane integrity indicators, husk and aril phenylalanine ammonia-lyase (PAL)/polyphenol oxidase (PPO) enzymes activity and antioxidant system activity, together with nutraceutical properties of aril during storage at 4 ± 0.5 °C for 120 d were evaluated. It was proposed that attenuation of chilling injury in pomegranate fruit in response to arginine treatment which is partially caused by higher antioxidant system activity, resulted in lower electrolyte leakage and MDA accumulation which indicates maintenance of membrane integrity. Also, higher PAL/PPO enzymes activity, which is concurrent with higher total phenols, anthocyanins and ascorbic acid accumulation, caused higher DPPH scavenging capacity.

2. Materials and methods

2.1. Fruit and treatments

In the current experiment, arginine treatment was applied by preharvest spray in combination with postharvest immersion. Six trees were selected for each arginine concentration and 25 fruit were labeled from each tree. For treatment, 0, 0.5, 1 and 2 mM arginine was sprayed on pomegranate fruit cv. Malase Saveh, from the Saveh Pomegranate Research Center by using a hand-sprayer until fruits were wet to runoff. The sprays were applied three times at 20 d interval before commercial harvest. Preharvest treatment contained 600 fruits which were divided into 4 lots of 150 fruits, treated in triplicate (50 fruits per replicate): control (0) and arginine at 0.5, 1 and 2 mM. At commercial harvest, fruits were harvested and transported to the laboratory. Postharvest treatment was applied by dipping fruits in 0 (control), 0.5, 1 and 2 mM arginine solution for 15 min at 20° C and then fruits were removed from the arginine solution and allowed to air-dry at room temperature, and stored at 4 ± 0.5 °C (85–90% RH) for 120 d. After evaluation of chilling injury every 30 d during storage at 4 ± 0.5 °C by determining 10 fruits husk browning, husk electrolyte leakage and MDA content as membrane integrity indicators, husk and aril antioxidant enzymes, SOD, CAT and APX activities and H_2O_2 content, husk and aril PAL and PPO enzymes activity associated with aril total phenols and anthocyanins content and DPPH· scavenging capacity, were evaluated.

2.2. Husk browning

Fruit chilling injury was assessed by visualizing 10 fruits husk browning using a scale of 1 to 3 according to Sayyari et al. (2009): 0 (no symptom), 1 (1–25%), 2 (26–50%) and 3 (> 51%). Chilling injury was calculated using the following formula:

CI = Σ (value of hedonic scale) × (number of fruits with the corresponding scale number)/(total number of fruits in the sample × 4).

2.3. Electrolyte leakage and MDA content

Husk electrolyte leakage was measured according to Mirdehghan et al. (2007b). Electrolyte leakage was calculated using the following formula:

Electrolyte leakage (%) = (Initial electrolyte leakage/Final electrolyte leakage) × 100

MDA content was measured by TBA method described by Hodges et al. (2004). MDA content was expressed on a fresh weight basis in μmol kg^{-1}.

2.4. Antioxidant enzymes activity

For analysis of antioxidant enzyme activities, 5 g of husk or aril tissue was homogenized in 50 mM phosphate buffer (pH 7.8) containing 0.2 mM EDTA and 2% PVP. The homogenate was centrifuged at 12,000 × *g* for 20 min at 4 °C, and the supernatant was used for CAT, APX and SOD activity measurements. CAT, APX and SOD activities were measured according to Zhang et al. (2013b). 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 enzyme that oxidizes 1 μmol of ascorbate per minute. One unit of SOD activity was defined as enzyme that causes a 50% inhibition of nitro blue tetrazolium (NBT) reduction under assay conditions. CAT, APX and SOD activities were expressed as katals produced per mass of protein, kat kg^{-1}. Protein content was estimated according to Bradford (1976) using bovine serum albumin (BSA) as a standard. H_2O_2 content was measured according to Patterson et al. (1984). H_2O_2 content was expressed on a fresh weight basis in mmol kg^{-1}, according to a standard curve. Free radical DPPH· scavenging activity was measured according to Nakajima et al. (2004). The reduction of DPPH· was calculated according to the following formula in percent:

% inhibition of DPPH· = (Abs control − Abs sample)/Abs control × 100

2.5. PAL/PPO enzymes activity and arils nutraceutical properties

PAL and PPO were extracted and assayed according to Nguyen et al. (2003). PAL and PPO activities were expressed as katals produced per mass of protein, kat kg^{-1}. Total phenol content was assayed according to the Folin–Ciocalteu procedure (Chen et al., 2008). The total phenols content was expressed as gallic acid equivalents (GAE) on a fresh weight basis, g kg^{-1}. Total anthocyanins content was assayed according to the pH differential method, as described by Lako et al. (2007). Total anthocyanin content was expressed as cyanidin-3-glucoside (C3G) on a fresh weight basis, g kg^{-1}. Total ascorbic acid content was determined using the 2,4-dinitrophenylhydrazine (DNPH) method (Terada et al., 1978). Total ascorbic acid content was expressed on a fresh weight basis, g kg^{-1}.

2.6. Statistical analysis

The experiment was arranged as split plots for time on the basis of completely randomized design with three replications. Analysis of variance (ANOVA) was carried out with SPSS software. Differences between means were assessed by Tukey-Kramer's multiple range test with differences considered significant at $P < 0.05$.