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Salicyloyl chitosan alleviates chilling injury and maintains antioxidant capacity of pomegranate fruits during cold storage

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ABSTRACT

In this study, the effects of postharvest salicylic acid, chitosan and salicyloyl chitosan treatments on chilling injury and nutritional quality of pomegranate fruits during storage at 2 °C for 5 months was investigated. Chilling injury symptoms in pomegranate fruits were manifested by external husk browning and internal segment browning. Alleviation impacts of salicyloyl chitosan treatment on pomegranate fruits chilling injury was higher than salicylic acid and chitosan treatments along, which results to delay external and internal browning and increases in electrolyte leakage. Also, pomegranate fruits treated with salicyloyl chitosan exhibited higher membrane unsaturated/saturated fatty acids (unSFA/SFA) ratio. Higher hydrophilic (H-TAA) and lipophilic (L-TAA) antioxidant capacity in pomegranate fruits treated with salicyloyl chitosan results from higher total phenols, anthocyanins and ascorbic acid accumulation. In addition to nutritional quality, pomegranate fruits treated with salicyloyl chitosan exhibited lower weight loss, respiration rate and ethylene production associated with higher firmness, total soluble solids, and titrable acidity as sensory quality. These results suggest that salicyloyl chitosan treatment can be used as promising strategy not only for alleviating chilling injury by maintaining membrane integrity results from higher unSFA/SFA ratio but also for enhancing antioxidant capacity by total phenols, anthocyanins and ascorbic acid accumulation.

1. Introduction

Cold storage is employed widely to extending postharvest life of fruits and vegetables, associated with maintaining their sensory and nutritional quality (Aghdam and Bodbodak, 2013). Pomegranate fruits, endemic to subtropical climates, cannot simply be stored at low temperatures, due to the risk of chilling injury (Pareek et al., 2015; Sayyari et al., 2011b). Due to its economic impact and also human health, great efforts have been done by researchers for alleviating postharvest chilling injury of pomegranate fruits during cold storage by applying postharvest treatment such as polyamines (Mirdehghan et al., 2007), heat (Mirdehghan et al., 2006), methyl salicylate or methyl jasmonate (Sayyari et al., 2011a), salicylic acid and acetyl salicylic acid (Sayyari et al., 2009; Sayyari et al., 2011b), oxalic acid (Sayyari et al., 2010), putrescine and carnauba wax (Barman et al., 2011), salicylic acid, jasmonic acid, and calcium chloride (Mirdehghan and Ghotbi, 2014).

By function as a barrier to O_2, CO_2, and ethylene gasses exchange via building modified atmosphere surrounding the fruits, postharvest fruits coating can be used for delaying fruits ripening, maintaining fruits sensory and nutritional quality, and extending fruits postharvest life (Jongsri et al., 2016). Due to non-toxicity, biodegradability, biocompatibility, antioxidant and antimicrobial activity, chitosan can be used as promising edible and biologically safe preservative coating for maintaining sensory and nutritional quality of fruits (Hu et al., 2016; Jongsri et al., 2016; Kerch, 2015; Petriccione et al., 2015). Insolubility of chitosan in water in neutral pH restricted its using as an edible and biologically safe fruits coating. In order to the improvement of chitosan water solubility, gallic acid, caffeic acid, ferulic acid and salicylic acid polyphenols grafted chitosan have been synthesized in recent years (Hu et al., 2016; Yang et al., 2016).

Salicylic acid (SA) as a natural and safe signaling molecule was applied for boosting tolerance of fruits and vegetables to postharvest chilling injury and also for maintaining their sen-

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sory and nutritional quality, leading to extending postharvest life (Asghari and Aghdam, 2010). Alleviation of postharvest chilling injury in fruits and vegetables treated with salicylic acid has been results from (1) higher membrane integrity due to higher unsaturated/saturated fatty acids (unSFA/SFA) ratio caused by lower membrane deterioration enzymes phospholipase D (PLD) and lipoxygenase (LOX)activities, higher fatty acid desaturase (FAD) gene expression and higher endogenous energy status, (2) higher HSPs gene expression and accumulation (3) higher antioxidant system activity (4) higher arginine pathway activity leading to higher polyamines, NO, and proline accumulation (5) higher phenylalanine ammonia lyase (PAL)/polyphenol oxidase (PPO) enzymatic activity ratio along with higher DPPH scavenging capacity leading to lower browning and (6) higher γ-aminobutyric acid (GABA) shunt pathway activity (Aghdam and Bodbodak, 2013; Aghdam et al., 2016b). For proffering promising biological function of salicylic acid to chitosan, efforts have been done to synthesize salicylic acid grafted chitosan so called salicyloyl chitosan and utilize its potential superiority such as water solubility (Hu et al., 2016). Due to lack attaching ability of salicylic acid to fruits surface, its protective effects is not sustained. But chitosan exhibit superior adhesive attribute, so salicyloyl chitosan coating exhibits synergistimpacts (Hu et al., 2016). Zhang et al. (2015) reported that the salicyloyl chitosan coating alleviated chilling injury in cucumber fruits, which was associated with lower electrolyte leakage and malondialdehyde (MDA) accumulation. Cucumber fruits treated with salicyloyl chitosan exhibited higher total soluble solids, chlorophyll and ascorbic acid contents and lower weight loss and respiration rate. Also, salicyloyl chitosan treated cucumber fruits exhibited higher endogenous salicylic acid accumulation which may result from releasing salicylic acid from salicyloyl chitosan via amidase enzyme action. Higher endogenous salicylic acid accumulation in cucumber fruit treated with salicyloyl chitosan was associated with higher antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR) activities. Zhang et al. (2015) suggested that the lower chilling injury in cucumber fruit treated by salicyloyl chitosan results from synergistic action of chitosan with salicylic acid and enhanced endogenous salicylic acid accumulation.

In this study, the impact of postharvest salicylic acid, chitosan and salicyloyl chitosan treatments on the external husk browning and internal segment browning as chilling symptoms and its relation with membrane unSFA/SFA ratio, along with nutritional quality of pomegranate fruits during storage at 2 °C for 5 months were evaluated. We propose that alleviation of chilling injury in pomegranate fruits in response to salicyloyl chitosan treatment results from higher unSFA/SFA ratio, concurrent with higher total phenols, anthocyanins and ascorbic acid accumulation leading to higher hydrophilic and lipophilic antioxidant capacity.

2. Materials and methods

2.1. Pomegranate fruits and treatments

Pomegranates (Mallas Saveh cultivar, a late ripening with delicious, sour and semi-soft arils and commercial production in central part of Iran) were harvested at fully mature stage according to commercial practice in a private orchard in Saveh (Markazi province, Iran). Fruits immediately transported to the laboratory and those with defects (sunburn, crack, bruise, and cut in the husk) were discarded. The remaining fruits were randomized and divided into five lots of 96 fruits for the following treatments in triplicate (each treatment contained 24 individual fruits for any sampling date): dipped in distilled water (control), treated with 2 mM salicylic acid, 0.5% chitosan and 0.57% salicyloyl chitosan. Following treatments, fruits were allowed to completely dry at room temperature for 2 h before transfer to cold storage.

After 1–5 months, 24 fruits for each treatment (8 from each replicate) were sampled and further stored at 20 °C for 3 days as shelf life. Chilling injury, ethylene production, respiration rate and electrolyte leakage were assessed immediately and then each husk was carefully cut at the equatorial zone and arils of central part were manually separated. The arils of each replicate were combined, frozen in liquid N_2, milled and stored at −20 °C until analytical determinations.

2.2. Chilling injury and electrolyte leakage

The degree of CI was visually assessed on the husk surface pitting and browning for external CI and following cut the fruits and assessed internal CI based on segment separating thin layers and arils discoloration. The extent of internal and external CI was divided into five classes: 0, no browning; 1, extensive browning covering <25% of the cut surface; 2, extensive browning covering = 25% but <50% of cut surface; 3, extensive browning covering = 50% but 75% of cut surface; 4, extensive browning covering = 75% of cut surface. From this, a CI index was expressed as: CI index = [(CI level) × (number of fruit at the CI level)]/(4 × total number of fruit in the treatment) (Sayyari et al., 2009).

The rate of electrolyte leakage was determined as described by Mirdehghan et al. (2007) in duplicate for each replicate, using 6 discs (10 mm) of peel tissue (1.50 ± 0.02 g) cut with a cork borer. Electrical conductivity was measured after 4 h incubation in 25 mL of 0.4 M mannitol under constant shaking, using a Crison conductivity meter (Met Rohm, 664). After readings were taken, the vials were autoclaved at 121 °C for 20 min, held for 24 h at room temperature and conductivity was measured again for total electrolytes. The rate of electrolyte leakage was expressed as a percentage of the total and results were the mean ± SE (n = 6).

2.3. Respiration rate and ethylene production

Respiration rate was measured during storage at 25 °C. Three fruit from each replication were randomly selected to measure respiration rate and ethylene production. Fruits were kept in an airtight glass jars (1000 mL) fitted with a rubber septum for collecting the gasses. After 1 h incubation of the fruits, one mL of the head-space atmosphere was withdrawn with a gas syringe and CO_2 quantified using a gas chromatograph (GC; Shimadzu, model 2014, Kyoto, Japan). Results were expressed as nmol CO_2 kg^{-1} h^{-1}. Again, one mL of the headspace atmosphere of jars was used to measure ethylene concentration using GC. Results were expressed as nL g^{-1} h^{-1}(Zapata et al., 2014).

2.4. Total phenols, anthocyanins and total antioxidant activity

Total phenols and anthocyanins were determined according to Tomás-Barberán et al. (2001) and Serrano et al. (2005) methods, respectively. Extraction of total phenolic compounds was performed using water:methanol (2:8) containing 2 mM sodium fluoride (to prevent phenolic degradation and polyphenol oxidase activity) and quantified using the Folin–Ciocalteu reagent. Results were expressed as mg pyrogallol equivalent 100 g^{-1} FW.

For determination of anthocyanins three grams of arils was homogenized in 5 mL of methanol and left 1 h at −18 °C. Extracts were centrifuged at 15,000 rpm for 15 min at 4 °C. The supernatant was loaded onto a C18 Sep-Pak cartridge that conditioned with 5 mL of methanol, 5 mL of pure water and 5 mL of 0.01 N HCl. Cartridge was then washed with 5 mL pure water and eluted with acidified methanol (with 0.01% HCl). Absorbance of the collected fraction was measured at 530 nm with spectrophotometer (Varian model