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Pre-storage salicylic acid treatment affects functional properties, unsaturated/saturated fatty acids ratio and chilling resistance of pomegranate during cold storage

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Abstract

This research was conducted for assessment of salicylic acid (SA) effects on functional properties of pomegranate during cold storage. Pomegranate fruits were dipped in 2 mM solution of SA and stored at 2°C for 90 days. Every month, samples were taken and further stored 3 days at 20°C for shelf life study. SA-treated fruits were compared with chilled and non-chilled fruits (stored at 2 and 10°C, respectively). Arils of treated fruits exhibited higher antioxidant activity (both in hydrophilic and lipophilic fractions) than chilled and non-chilled fruits. The chilling index, ion leakage and respiration rate of treated fruits were lower than chilled fruits but higher than non-chilled fruits. Severity of damage in control fruit was related to softening and loss of fatty acids with a concomitant reduction in the ratio of unsaturated/saturated fatty acids during storage. These chilling injury symptoms reduced in SA-treated pomegranates.

Introduction

Pomegranate (*Punica granatum* L.) is considered one of the oldest known edible fruit that associated with ancient civilizations of the Middle East and originated in the area now occupied by Iran and Afghanistan (Heber *et al.*, 2006). During postharvest, pomegranate exhibits important quality loss due to several physiological and enzymatic disorders, the major storage problem being water loss leading to browning symptoms in both peel and arils. These symptoms increase with storage of fruits below than 5°C (Elyatem and Kader, 1984; Mirdehghan *et al*., 2006).

To avoid excessive desiccation and decay occurrence, storage at low temperatures is therefore necessary. Moreover, loss of firmness, aril color, vitamin C, and acidity was reported, which were accompanied by reduction of acceptability in terms of freshness, juiciness, and taste (Defilippi *et al*., 2006). To extend storability and marketing of pomegranate fruits, good results were obtained with pre-storage application of salicylic acid (Wang *et al*., 2006; Sayyari *et al*., 2009; Siboza *et al*., 2014). This treatment was highly effective in reducing chilling injury and electrolyte leakage in the husk of pomegranate, as well as the ascorbic acid loss compared with that observed in control fruit.

However, no information is available about SA treatment effects in pomegranate on the functional properties of the arils. In this sense, the aim of this work was to study the effect of pre-storage SA treatment on functional properties (total phenolic compounds, total anthocyanins, and total antioxidant activity), saturated and unsaturated fatty acids composition and chilling injury symptoms in comparison of chilled and non-chilled fruits during postharvest storage of pomegranate.

Materials and Methods

Plant material and experimental design

Mollar de Elche cultivar (a late ripening with delicious, sweet and soft arils) of pomegranate were harvested when fully mature according to commercial practice in a private orchard in Elche (Alicante). 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 three lots of 45 fruits for the following treatments in triplicate (each replicate contained 35 individual fruits): non-chilled control

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(stored at 10°C), chilled control (stored at 2°C) and SA-treated chilled fruits (dipping for 10 min in 2mM SA solution and stored at 2°C). Relative humidity (RH) was 90±3% at all storage conditions and the SA concentration was based on previous experiments (Sayyari *et al*., 2009). Following treatments, fruit were allowed to completely dry at room temperature for 2 hours before transfer to cold storage.

After 1, 2 and 3 months 15 fruits for each treatment (5 from each replicate) were sampled and further stored at 20°C for 3 days (as shelf life, SL). After assessment of chilling injury, respiration rate and electrolyte leakage, each husk was carefully cut at the equatorial zone and then arils of central part were manually extracted. The arils of each replicate were combined, frozen in liquid N2, milled and stored at -20°C until analytical determinations.

Respiration rate

Respiration rate was measured during storage at 25°C. Three fruit from each replication were randomly selected to measure respiration rate. Three fruits from each replication were kept in airtight glass jars (1000 mL) fitted with a rubber septum for collecting the gases. After 1 hr incubation of the fruits, one mL of the head-space atmosphere was withdrawn with a gas syringe, and CO_2 quantified using a Shimadzu TM 14A gas chromatograph (Kyoto, Japan), with a thermal conductivity detector and a molecular sieve 5A column, 80–100 mesh (Carbosieve SII. Supelco Inc., Bellefonte, USA), of 2 m length and 3mm i.d. Oven and injector temperature were 50 and 110°C, respectively. Helium was used as carrier gas at a flow rate of 50 $mLmin^{-1}$ (Sayyari *et al*., 2011). Results are the means±S.E. of two determinations for each fruit and expressed as nmol CO_2 kg^{-1} h^{-1}.

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 on segment separating thin layers and discoloration of arils. 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 (EL) was determined as described by Mirdehghan *et al.* (2007) in duplicate for each replicate, using 6 discs (10mm) of peel tissue (1.50±0.02 g) cut with a cork borer. Conductivity was measured after 4 h of incubation in 25mLof 0.4M manitol 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).

Total antioxidant activity, total phenolic and total anthocyanin determination

Total antioxidant activity (TAA) was quantified according to Arnao *et al.* (2001) which enables to determine TAA due to both hydrophilic and lipophilic compounds in the same extraction. Briefly, for each sub-sample, five grams of tissue were homogenized in 5 mL of 50 mM phosphate buffer pH=7.8 and 3 mL of ethyl acetate, then centrifuged at 10,000g for 15 min at 4°C. The upper fraction was used for total antioxidant activity due to lipophilic compounds (L-TAA) and the lower for total antioxidant activity due to hydrophilic compounds (H-TAA). In both cases, TAA was determined using the enzymatic system composed of the chromophore 2,2´-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), the horse radish peroxidase enzyme (HRP) and its oxidant substrate (hydrogen peroxide), in which ABTS radicals are generated and monitored at 730 nm. The decrease in absorbance after adding the extract was proportional to TAA of the sample. A calibration curve was performed with Trolox ((R)-(+)-6-hydroxy-2, 5, 7, 8-tetramethyl-croman-2-carboxylic acid) (0-20 nmol) from Sigma (Madrid, Spain), and results are expressed as mg of Trolox equivalent 100 g^{-1}.

Total phenolics were extracted according to Tomás-Barberán *et al.* (2001) using water: methanol (2:8) containing 2mM sodium fluoride and quantified using the Folin-Ciocalteu reagent and results (mean ± SE) were expressed as mg pyrogallol equivalent 100 g^{-1} fresh weight.

Total anthocyanins were determined according to the method described by Serrano *et al.* (2005). Five grams of arils was homogenized in 4 mL of methanol and left 1 h at -18°C. Extracts were centrifuged at 15000 rpm for 15 min at 4°C. The supernatant was loaded onto a C18 Sep-Pak cartridge, previously conditioned with 5 mL of methanol, 5 mL of pure water, and then 5 mL of 0.01 N HCl. Cartridge was washed with 5 mL of pure water and then eluted with acidified MeOH (0.01% HCl). Absorbance

of the collected fraction was measured at 530 nm. Total anthocyanin was calculated using cyanidin-3-glucoside, and results were expressed as mg100g-1 FW and were the mean of determinations made in duplicate in each one of the five samples.

Fatty acid determination

Total lipids were extracted according to method described by Mirdehghan *et al.* (2006). 2 g arils were homogenized in 10 ml chloroform:methanol:0.1N HCl (200:100:1) and then 10 ml of 0.1N HCl were added before centrifugation at 4000×g for 10 min. The organic phase was collected and taken to dryness. Methylation of fatty acids was carried out by adding 1ml boron trifluoride/methanol at boiling temperature for 10 min. Methylated fatty acids were extracted with hexane, taken to dryness and redissolved in 200 µL chloroform before injection. Fatty acids were separated and quantified by gas chromatography (GC, Shimadzu model 14B) equipped with flame ionization detector (FID). Five micro liters in split mode was injected into a capillary column (HP-Innowax Polyethylene glycol, 30m×250_m×25_m). A gradient of temperature was used for fatty acid separation: initial temperature 120°C for 2 min and then a rate at 4°C /min to 190°C, which was held 5 min, and final rate at 4°C /min to 242°C. Identification of fatty acids was performed by comparing retention times with authentic standards (purchase from Sigma, Sigma–Aldrich, Madrid, Spain) and quantification was carried out using C11 as internal standard. Results were expressed as mg 100 g^{-1}.

Statistical analysis

Data from the analytical determinations were subjected to analysis of variance (ANOVA). Sources of variation were storage time and treatments. Mean comparisons were performed using Duncan's test ($P<0.05$). All analyses were performed with SAS software package.

Results and Discussion

Chilling index (CI), electrolyte leakage (EL) and respiration Rate (RR)

Chilled control fruits of pomegranates exhibited CI symptoms manifested as browning, pitting, and dehydration of the husk surface, reaching a CI index value of 0.36, that is, CI symptoms affected from 25 to 50% of the fruit surface (Figure 1). These CI symptoms were similar to those observed in other pomegranate cultivars, such as 'Wonderful' (Defilippi *et al*., 2006) or 'Malas Saveh' (Sayyari *et al*., 2009),

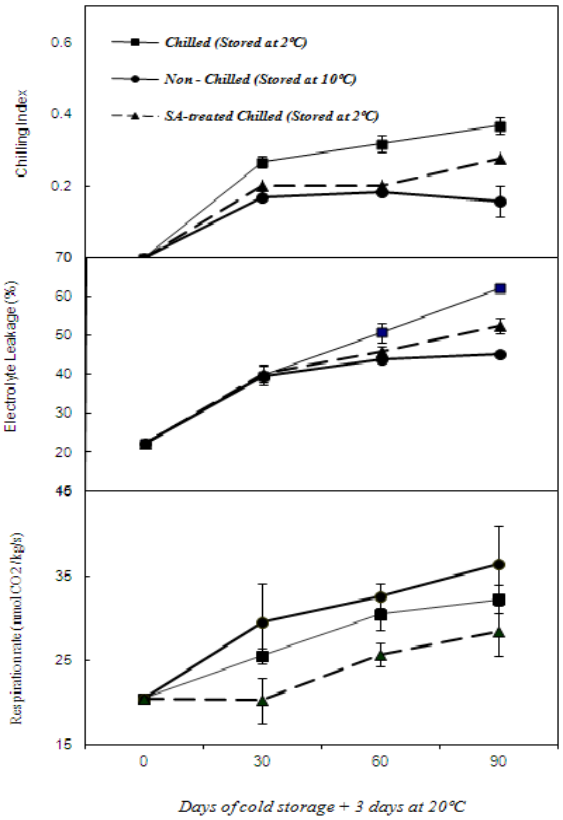

Figure 1. Chilling index, electrolyte leakage and Respiration rate of fruits during cold storage + 3 days at 20°C (SL) of chilled, Non-chilled and salicylic acid-treated pomegranates.

although significant differences were found between cultivars. Thus, 'Wonderful' held at 5°C for 8 weeks showed only slight CI symptoms manifested as brown discoloration of the locular septa (Elyatem and Kader, 1984). Moreover, differences exist also between harvest dates, with late-season fruit being more sensitive to CI than mid-season, indicating that this disorder may be associated with senescence (Defilippi *et al*., 2006). In addition, EL in chilled controls fruits was significantly high after 2 and 3 month of cold storage (Figure1). Respiration rate of non-chilled control fruits were higher than SA-treated and chilled control fruits, but SA decreased the respiration rate of fruits in comparison of chilled controls (Figure1). Similar results have been reported in peaches, in which the alleviation of CI was achieved at 1mM but failed at 0.7 mM or lower SA concentrations (Wang *et al*., 2006). It is known that CI is characterized by membrane disruption resulting in loss of tissue integrity accompanied by skin browning, which was reduced by the SA treatments. This effect of SA could be attributed to the inhibition of lipid peroxidation, as has been reported in watermelon seedling (Jing-Hua *et al*., 2008).

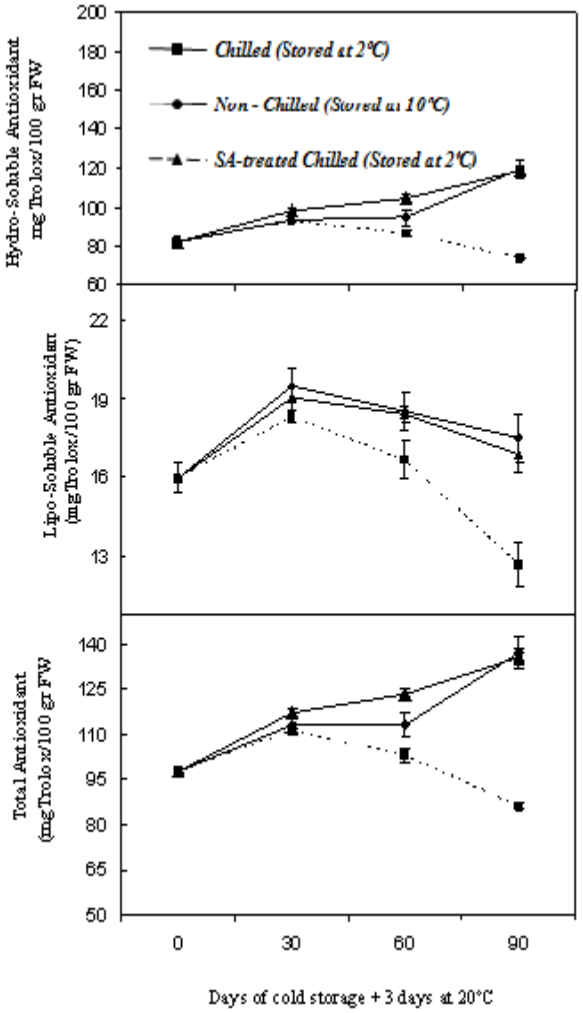

Figure 2. Hydro and lipo fraction antioxidant and Total antioxidant content in arils during cold storage+3 days at 20°C (SL) of chilled, Non-chilled and salicylic acid-treated pomegranates.

Total phenolic and antocyanine compounds and antioxidant activity

During storage, chilled control pomegranates showed significant reduction in the content of total phenolics and antocyanine content, as well as in TAA, in both hydrophilic (H-AA) and lipophilic (L-AA) fractions (Figures 2 and 3). The application of SA and keeping of fruits at 10°C led to lower losses of total phenolics and to a significant increase in antocyanine, phenolics and TAA after 3 month of cold storage. SA-treated and con-chilled control fruits showed higher total anthocyanins and phenolic compounds at the end of the experiment (Figure 3). The increase in anthocyanin concentration in non-chilled fruits is in agreement with previous result (Sayyari *et al*., 2010) and was associated with the advancement of the ripening process during postharvest storage. and increase toxic species scavenging capacity of fruits tissues (Rice-Evans *et al*., 1991; Bowler *et al.*, 1992; Kong *et al*., 2003; Narayan *et al*., 1999). Compared with chilled controls, SA pretreatment significantly

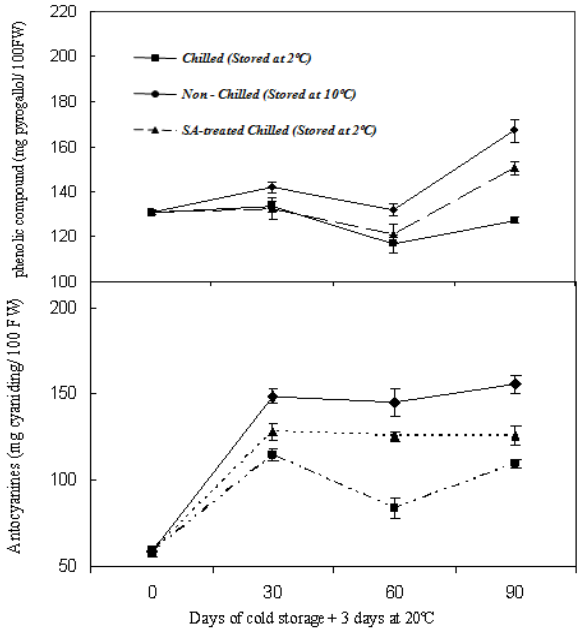

Figure 3. Phenolic compound and antocyanines content in arils during cold storage+3 days at 20°C (SL) of chilled, Non-chilled and salicylic acid-treated pomegranates

increased antioxidant activities, both in hydrophilic and lipophilic fractions, (Figure 2). Antioxidants scavenged reactive oxygen species (ROS) and maintained cell membrane health (Figure 1).

These results suggest that SA pretreatment could directly or indirectly activate antioxidant system during chilling stress, which had a higher ability to withstand chilling-induced injuries. ROS react with cell membrane un-saturated fatty acid and peroxide them followed losses of cell membrane integrity and increasing of EL. To alleviate or prevent low temperature-induced oxidative injury, plants have evolved mechanisms to scavenge these toxic and reactive species by antioxidant compounds and by enzymatic antioxidant systems (Wise, 1995). As ROS concentration during low temperature exposure depends on the balance between their production and scavenging, the correct functioning and cooperation of antioxidant systems, and their low temperature stability were supposed to be important requirements for plants to survive (Gill and Tuteja, 2010; Scebba *et al*., 1999). An efficient antioxidant activity is essential in order to maintain the concentration of ROS at relatively low levels.

Fatty acid composition

In pomegranate arils, palmitic acid (C16) as dominant saturated fatty acid, oleic acid (C18:1), erucic acid (C20:1) and gadoleic acid (C22:1) as mono-unsaturated fatty acids and linoleic acid (C18:2) and linolenic acid (C18:3) as poly-unsaturated fatty acids were identified. During storage, fatty acids

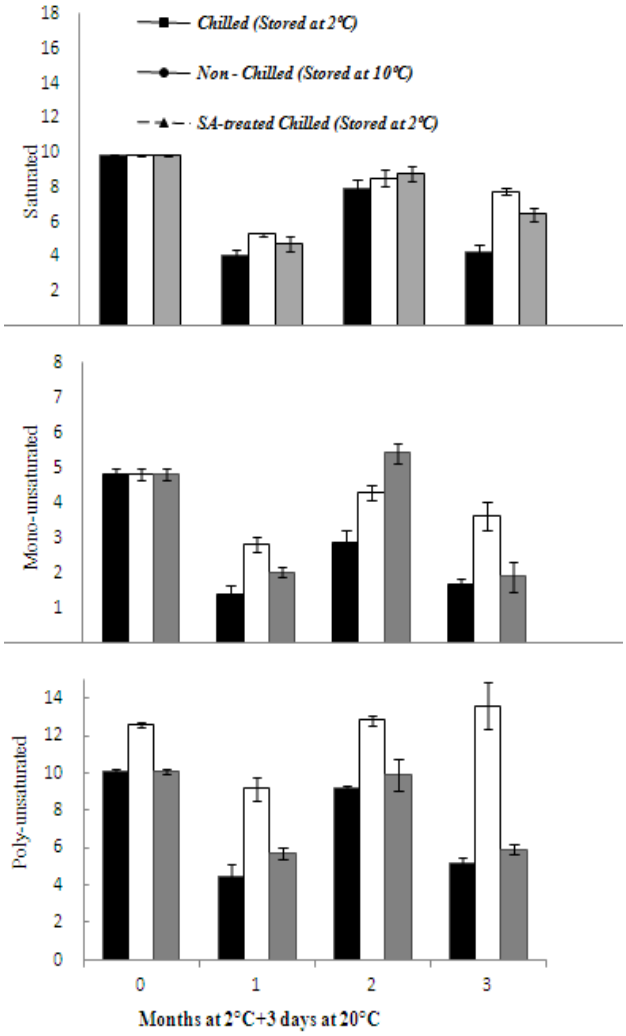

Figure 4. Saturated, mono-unsaturated and poly-unsaturated fatty acids (mg 100^{-1}g FW) in arils during cold storage + 3 days at 20°C (SL) of chilled, Non-chilled and salicylic acid-treated pomegranates.

decreasing rate of chilled control fruits was greater than SA-treated and non-chilled control fruits and all fatty acids (saturated, mono-unsaturated and poly-unsaturated) of SA-treated and none-chilled fruits were in higher ratio in comparison of chilled control fruits (Figure 4). The ratio of unsaturated/saturated fatty acids decreased in chilled control fruits while higher ratios were found in SA-treated and non-chilled fruits (Figure 5).

It has been reported that low temperatures induce changes in cell membrane lipids from a liquid-crystalline to a solid-gel state, which lead to an increase in membrane permeability and leakage of ions (Galindo *et al.*, 2004). In fact, we have found that membrane lipid composition changed during storage, with significant losses in both saturated and unsaturated fatty acids in control fruit, while in SA-treated fruit maintenance or slight increases for all fatty acids occurred. Thus, Arils from SA-treated pomegranates exhibited a significantly higher

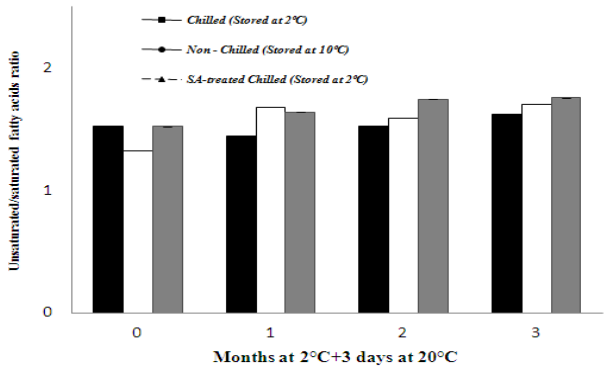

Figure 5. Unsaturated/saturated fatty acids ratio in arils during cold storage + 3 days at 20°C (SL) of chilled, None-chilled and salicylic acid-treated pomegranates.

ratio of unsaturated/saturated than control fruits over all storage. This increase in the degree of unsaturation of membrane lipids has been described as a mechanism of acclimation to low temperatures (Campos *et al.*, 2003; Lurie *et al.*, 1987; Stanley and Parkin, 1991), which would lead to maintenance of membrane fluidity at low temperature of storage and could be responsible of the lower electrolyte leakage and skin browning. Thus, our results show that control pomegranates were not able to develop this adaptation mechanism and thus chilling injury occurred in greater extent, corroborated by the high relationship found between the decrease of unsaturated/saturated fatty acids and the increase in electrolyte leakage during cold storage.

Conclusion

SA pre-treatment maintained greater antocyanines, polyphenols, antioxidant activity, reduced chilling injury indices, respiration rate and delayed membrane lipid peroxidation in pomegranate fruits during cold storage. The effect of SA on alleviating chilling injury of pomegranates during cold storage may be attributed to its ability to induce antioxidant systems and maintenance of unsaturated fatty acids during cold storage. Pre-storage application of SA may provide a useful means of extending pomegranates postharvest life during cold storage.

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