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Acetyl salicylic acid alleviates chilling injury and maintains nutritive and bioactive compounds during cold storage

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Abstract. Pomegranates are highly perishable and to extend storability the refrigeration is therefore necessary, but this fruit is susceptible to chilling injury (CI) if stored longer at temperatures lower than 5ºC. Acetyl salicylic acid (ASA) is present in vegetable tissues, its concentration being below 0.2 mg kg^{-1} in some fruits. In this experiment the effect of ASA treatments at three concentrations (0.1, 0.5 and 1.0 mM) on pomegranate quality and nutritive and bioactive compounds after storage for 14-84 days at 2ºC plus 4 days at 20ºC was assayed. Control fruit exhibited more CI symptoms (manifested by pitting and browning) than treated fruit during storage, which were accompanied by increased softening, ion leakage and respiration rate. The ASA treatments were also effective in maintaining higher contents of nutritive (sugars and organic acids) and bioactive compounds (acid, total phenolics and anthocyanins), ascorbic acid and total antioxidant activity. These results suggest that ASA could have potential postharvest application to reduce CI, maintain quality and improve the health benefits of pomegranate fruit consumption by increasing their antioxidant capacity.

Keywords. Pomegranate – Chilling injury – Acetyl salicylic acid – Quality – Bioactive compounds.

I – Introduction

The juice of pomegranate arils contains high concentration of sugars, organic acids, vitamins, polysaccharides, and essential minerals, as well as bioactive compounds with antioxidant activity and health beneficial effects (Mertens-Talcott *et al.*, 2006). Pomegranate is a highly perishable fruit and then to extend shelf life refrigeration is therefore necessary, but the fruits are susceptible to chilling injury (CI) with symptoms such as browning of the husk, pitting, husk-scald, loss of firmness, and higher sensitivity to decay (Mirdehghan, *et al.*, 2007a; 2007b). Acetyl salicylic acid (ASA) is a closed analogue of salicylic acid (SA) and has been shown to be effective on reducing CI in loquat (Cai *et al.*, 2006). Thus, the main objective of this paper was to study the effect of ASA on pomegranate fruit quality attributes during storage under chilling conditions, as well as their role on the content of nutritive (sugars and organic acids) and bioactive compounds (polyphenol and anthocyanins) and the antioxidant capacity determined in both hydrophilic (H-TAA) and lipophilic (L-TAA) fractions, separately.

II – Materials and methods

Pomegranates (*Punica granatum* L. cv. Mollar de Elche) were harvested when fully mature from a commercial plot in Elche (Alicante, Spain) and 195 homogeneous fruits were selected, from which 15 were used to determine the characteristics at harvest, and the remained 180 fruits were randomized and divided into 4 lots for the following treatments: control (no treatment) and acetyl salicylic acid (ASA) at 0.1, 0.5 and 1.0 mM concentration. Treatments were performed by dipping the pomegranates in 20-L solution for 10 min before storage at 2°C, in permanent

darkness and with relative humidity of 90%. Every 2 weeks 1 lot from each replicate and treatment was transferred to a chamber at 20°C for 4 days for analytical determinations. Chilling injury (CI), fruit firmness and skin colour were assayed in the whole fruit. Then, each husk was carefully cut at the equatorial zone with sharpened knives, the skin was used to determine ion leakage according to Mirdehghan *et al.*. (2007b), and arils were manually extracted. The arils of each replicate, obtained from equatorial fruit zones, were combined and frozen in liquid N_2, milled and stored at -20°C, in which total soluble solids (TSS) and total acidity (TA) were determined as reported by Mirdehghan *et al.* (2007b), and total phenolics, total anthocyanisns and total antioxidant activity according to Mirdehghan *et al.* (2007c).

III – Results and discussion

CI increased during storage although scores were always significantly higher in control than in treated pomegranates the 1 mM ASA doses being the most effective in reducing CI symptoms, which included husk pitting, browning and desiccation being responsible for extensive postharvest losses and limiting the fruit storability. Generally, CI leads to damage of the cell membranes that can be measured by the ion leakage, which was significantly higher in the skin of control than in treated fruit, ≈65 and ≈55%, respectively at the last sampling date (data not shown). These results show a role of ASA on maintaining membrane integrity, as has been reported for loquat fruit (Cai *et al*., 2006). In addition, the application of ASA was able to reduce softening and acidity losses (data not shown) that occurred in control fruit during postharvest storage as consequence of the advance of the ripening process (Mirdehghan *et al.*, 2007a; Sayyari *et al.*, 2011), showing an effect on delaying the ripening process, according to previous reports in climacteric fruit such as kiwifruit (Zhang *et al*., 2003), banana (Srivastava and Dwivedi, 2000), and sugar apple (Mo *et al.*, 2008), SA reduced the ripening process during storage by suppressing and/or delaying ethylene production and respiration rate, and the related parameters such as firmness, TA and TSS.

The content of total phenolic compounds at harvest was 261.19±6.97 mg 100 g^{-1} and diminished throughout storage in control fruit with final concentration of 234.10±2.59 mg 100 g^{-1} (Fig. 1), while no significant changes were observed in ASA-treated pomegranates for any of the applied dose with values ≈270 mg 100 g^{-1} at the end of the experiment. With respect to total anthocyanins, the levels at harvest (59.03±9.52 mg 100 g^{-1}) significantly increased along storage, the increase being higher in ASA-treated fruit than in control pomegranates with final levels of ≈110 and ≈130 mg 100 g^{-1} for control and treated fruits, respectively (Fig. 1). For H-TAA (Fig. 1), a significant reduction was observed in control fruit over storage, from levels at harvest of 85.08±4.96 to 46.06±0.82 mg 100 g^{-1} at the end of the experiment, while significant lower decreases were found in treated fruits. On the contrary, the levels at harvest of L-TAA were lower and did not change along storage irrespective of treatments. Taking into account the change in H-TAA, it could be confirmed that both total phenolics and ascorbic acid are the main compounds contributing to the antioxidant capacity of the pomegranate arils, in agreement with previous reports (Gil *et al*., 2000; Mirdehghan *et al*., 2006, 2007c).

In conclusion, the data presented here unequivocally suggest that ASA reduced CI symptoms in pomegranates, delayed postharvest ripening processes and increased antioxidant potential by enhancing or maintaining bioactive compounds such as total phenolics, total anthocyanins and ascorbic acid. Hence, the application of ASA treatments could be considered as a natural postharvest tool to extend the commercialization and marketability of pomegranate, even at low temperatures which usually induce CI occurrence. In future, determination of endogenous SA after ASA treatment would answer the question of whether these effects are due to ASA per se or its conversion to SA.

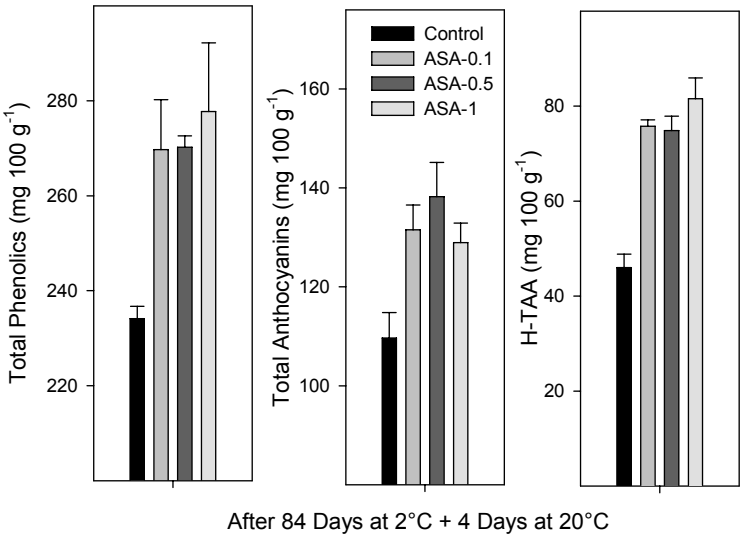

Fig. 1. Total phenolics, total anthocyanins and total antioxidant activity in the hidrophylic fraction of pomegranate arils treated with acetyl salicylic acid after 84 days of cold storage plus 4 days at 20ºC.

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