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Abstract. Pomegranate fruits (*Punica granatum* L. cv. 'Mollar de Elche') were treated with putrescine (Put) or spermdine (Spd) at 1 mM under pressure-infiltration or immersion prior cold storage at 2℃. Non-treated fruit developed rapidly chilling injury (CI), with main symptoms being skin browning, increased electrolyte leakage and weight loss. During storage losses of firmness and colour and increases in °Brix/acidity ratio and respiration rate were observed. All these changes were significantly delayed by polyamine treatments, with effectiveness being found similar either by pressure-infiltration or by immersion. The reduction of CI severity was correlated to the increased levels of free endogenous Put and Spd in the skin, which could induce acclimation of pomegranate to cold temperature and also a mechanism of protection to CI, with a net increase of the shelf-life. In conclusion, in this paper a novel technology based on the exogenous application of Put or Spd under pressure-infiltration or immersion could induce acclimation of pomegranate to low temperature, and in turn protect the fruit to CI by increasing the levels of endogenous Put and Spd.

Keywords. Browning – Chilling injury – Firmness – Putrescine – Spermidine.

I – Introduction

Storage at low temperature is necessary to avoid excessive desiccation and decay and to prolong pomegranate storability. However, pomegranates stored below 5℃ develop chilling injury (CI), the most common symptoms being surface pitting, husk scald and skin browning. Increases in polyamine concentration (putrescine, Put; spermidine, Spd; spermine, Spm) have been reported in fruits stored under CI conditions, although whether this polyamine enhancement is a result of the cold stress or a protective mechanism against CI is still unclear (Valero and Serrano, 2010). Thus, the aim of this paper was to study the role of exogenous Put or Spd application, either by pressure-infiltration or immersion, on pomegranate fruit quality stored at chilling temperatures.

II – Materials and methods

Mature pomegranates (*Punica granatum* L. cv. Mollar de Elche) were picked, randomized and divided into six lots of 125 fruit for the following treatments in 5 replicates (each replicate contained 25 individual fruit). Half lots were treated by pressure (in 20 l of solution, containing Tween-20 (2 g l^{-1}), and applying a pressure of 0.05 bar for 4 min) with 1 mM Put, 1 mM Spd or with distilled water, which served as the control. The other half was treated with the same concentration of polyamines and water by dipping at 25℃ for 4 min. Following treatments, fruit were placed on Kraft paper and allowed to dry before storage the next day at 2℃ (considered as day 0) in a temperature-controlled chamber, in permanent darkness and with a relative humidity of 90%. After 0, 30 and 60 days, 25 fruit for each treatment (5 from each replicate) were sampled and further stored at 20℃ for 3 days (shelf-life, SL). External browning, weight loss, fruit firmness, respiration rate and colour were measured in intact fruit, electrolyte leakage and polyamines were analysed in the skins, and soluble solid content (SSC), and titratable acidity were determined from the arils as described by Mirdehghan *et al.* (2007).

III – Results and discussion

In pomegranate fruit occurrence of CI developed from the first sampling date, as shown by the results of skin browning and electrolyte leakage (Fig. 1). However, these CI symptoms were significantly decreased by Put and Spd treatments, the efficacy of these treatments being similar whether applied by pressure-infiltration or immersion. This effect could be attributed to the role of polyamines on inducing cold acclimation, which would lead to maintenance of membrane fluidity at low temperatures, and could be responsible for reduced electrolyte leakage and skin browning, and thus for decreasing the severity of CI symptoms. Accordingly, during senescence of melons, polyamine treatment resulted in less membrane peroxidation and higher retention of chlorophyll (Lester, 2000). During storage of control pomegranates, decreases in fruit firmness and colour L* and increases in weight loss, respiration rate and ratio SSC/acidity (maturity index) were found. These changes, which are associated with acceleration of ripening, were significantly delayed after exogenous application of Put or Spd either by pressure or immersion (data not shown). Thus Put and Spd treatments, due to their anti-senescence properties, were able to retard the maturation process of pomegranates, as has been observed in a wide range of climacteric and non-climacteric fruit (Valero and Serrano, 2010).

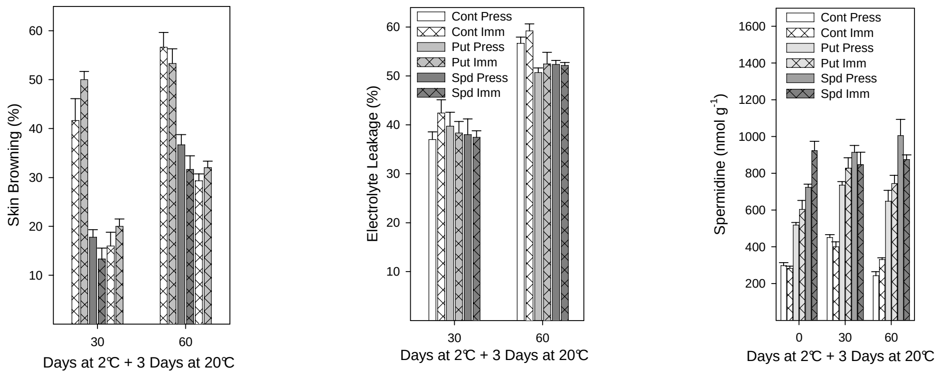

Fig. 1. Sking browning, electrolyte leakage and spermidine concentration along storage in control and treated pomegranates. Data are de mean ± SE.

Endogenous Put showed slight but non-significant increases while greater increases in Spd occurred (Fig. 1). This polyamine response supports the proposal that accumulation of Put or Spd in tissues seems to be a general response of fruit to CI (Martínez-Romero *et al.*, 2003). Skin browning was negatively correlated with endogenous Put concentration in Put or Spd-treated fruit under pressure, with the same correlation being found for endogenous Spd concentration when Put or Spd were applied under immersion. These results suggest an activation of the polyamine biosynthesis pathway. Part of the exogenous Put appears as free endogenous Put and part is likely to have transformed to Spd using decarboxylate S–adenosylmethione as an aminopropyl donor catalysed by Spd-synthase, while the conversion of Spd to Spm did not occur, since no significant increases in Spm were shown. Finally, the increased levels of endogenous Put and Spd after polyamine treatments could be responsible for the lower rate of softening, lower increase in SSC/acidity and weight loss and in turn a net increase in the shelf-life, since acceleration of ripening process and senescence has been associated with decreases in the content of endogenous polyamines (Valero *et al.*, 2002).

In conclusion, the exogenous application of Put or Spd under pressure-infiltration or immersion could induce acclimation of pomegranate to low temperature, and in turn protect the fruit from CI by increasing the levels of endogenous Put and Spd, since the normal levels would not be high enough to induce this adaptation to cold storage. In addition, the polyamine treatment retarded the maturation process by reducing softening and the increase of the ratio SSC/acidity, as well as the loss of weight. Thus storability and shelf-life could be extended in pomegranate stored at low temperatures that usually develop CI.

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