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Reduction of chilling injury of pomegranate by heat treatment before cold storage

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Abstract. Pomegranates are highly perishable fruit and sensitive to chilling injury (CI) when stored below 5ºC, manifested as skin browning, surface pitting, higher susceptibility to decay and reduction in both internal and external fruit quality. Thus, the aim of this experiment was to study the effect of hot water dip at 45 ºC for 4 min on reducing CI in pomegranates after storage at 2ºC for 15-90 days plus 3 days at 20ºC. Pomegranates developed CI, manifested as increases in skin browning and electrolyte leakage, which were highly correlated. The severity of damage in control fruit was related to loss of fatty acids with a concomitant reduction in the ratio of unsaturated/saturated fatty acids during storage. These CI symptoms were slightly, but significantly reduced in heat-treated pomegranates. In addition, the heat treatment induced increases in free putrescine and spermidine during storage. These higher polyamine levels as well as the maintenance of the unsaturated/saturated fatty acid ratio during storage could account for the maintenance of membrane integrity and fluidity and the reduction of CI.

Keywords. Browning – Electrolyte leakage – Fatty acids – Firmness – Putrescine – Spermidine.

I – Introduction

Pomegranates are highly perishable fruit due to problems of desiccation and especially chilling injury (CI) symptoms when stored below 5°C, manifested as skin browning, surface pitting and higher susceptibility to decay. These symptoms can reach the arils, leading to a reduction in both internal and external fruit quality (Sayyari *et al.*, 2009; 2011). Intermittent warming has been tested with satisfactory results in maintaining pomegranate quality during storage, in terms of retention of anthocyanin and titratable acidity, reduction of decay and alleviation of chilling injury (Fallik, 2004). Since heat treatments, which showed beneficial effects in alleviating chilling injury, were accompanied by increases in polyamines, a particular role for endogenous polyamines in increasing fruit tolerance to cold stress has been proposed (González-Aguilar *et al.*, 2000; Xu *et al.*, 2005). However, no information is available about the use of heat treatments (temperature over 35°C during short periods. Thus, the aim of this work was to study the effect of prestorage heat treatments on reducing CI in pomegranates evaluating browning, electrolyte leakage fatty acid composition of the skin. In addition, the mediation of polyamines in the reduction of CI caused by heat treatments before cold storage will be discussed.

II – Materials and methods

Pomegranates (*Punica granatum* L. cv. Mollar de Elche) were picked when fully mature according to commercial practice and randomized and divided into two lots of 175 fruits for the following treatments in quintuplicate (each replicate contained 35 individual fruits): control (distilled water at 25°C for 4 minutes) and heat treatment (hot water dip at 45°C for 4 minutes). Following treatments, fruits were placed on Kraft paper and allowed to dry before storage the next day at 2°C (considered as day 0) in a temperature-controlled chamber, in permanent

darkness and with relative humidity of 90%. After 15, 30, 45, 60, 75 and 90 days, 25 fruits for each treatment (5 from each replicate) were sampled and further stored at 20°C for 3 days (shelf life, SL). Fruit firmness, external browning and colour were measured in intact fruit. Then, each husk was carefully cut at the equatorial zone and peels were manually extracted. Some of the peel tissue of each replicate was used for electrolyte analysis, and the remaining was combined and frozen in liquid N_2, milled and stored at -20°C until analytical determinations of polyamines and fatty acid composition were made, as described in Valero *et al.* (1990), Mirdehghan *et al.* (2007) and Sayyari *et al.* (2010; 2011).

III – Results and discussion

All pomegranate husks developed CI from the first sampling date, which increased with storage time, as could be observed by skin browning (Fig. 1a) and ion leakage (Fig. 1b). However, the occurrence was significantly reduced in heat-treated fruit. The increased skin browning in control fruit was also observed by a reduction in Hue angle, which was retarded in heat-treated fruit. In addition, browning was positively correlated with electrolyte leakage, both in control ($y=1.17\ x - 24.65$, $r^2=0.80$) and heat-treated fruit ($y=1.09\ x - 27.82$, $r^2=0.80$), and negatively correlated to Hue angle ($y=-2.31\ x + 145$, $r^2=0.88$ and $y=-1.95\ x + 127$, $r^2=0.73$, for control and heat-treated fruit, respectively. However, no symptoms of decay were observed during storage, neither control nor heat-treated pomegranates.

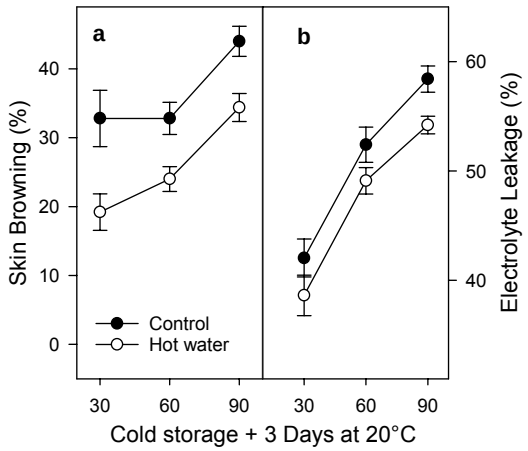

Fig. 1. Skin browning and electrolyte leakage during storage of control and treated pomegranates. Data are the mean±SE.

In pomegranate skin 10 fatty acids were identified and quantified, five saturated (C10, C12, C14, C16 and C18), two mono-unsaturated (C16:1, C18:1), and three poly-unsaturated (C18:2 cis, C18:2 trans, and C18:3). Among the saturated, palmitic fatty acid (C16) was predominant (≈ 33%), while linolenic acid (C18:3) was the major unsaturated fatty acid (≈25%). During storage, all fatty acid significantly decreased in control fruit (Fig. 2), with losses of 53% in saturated fatty acids, and 70 and 76% for mono-unsaturated and poly-unsaturated fatty acids, respectively. However, the concentrations of all fatty acids in heat-treated pomegranates remained significantly higher than in control fruit over storage, and did not show significant losses from day 0 to day 90. Thus, the ratio of unsaturated/saturated fatty acids decreased in control fruit from an initial value of 1.27±0.15 to 0.72±0.06 at the end of the experiment, while no significant changes and higher ratios were found in heat-treated fruit for all sampling dates. It is interesting

to point out that in control fruit, the decrease in the unsaturated/saturated fatty acid ratio was highly correlated with the increase in electrolyte leakage (y= -0.02 x +1.79 r^2= 0.859).

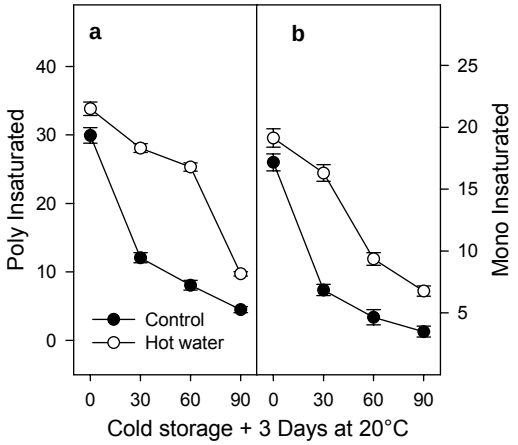

Fig. 2. Poly and mono-insaturated fatty acids (mg 100 g^{-1}) during storage of control and treated pomegranates. Data are the mean±SE.

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), 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 CI 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. However, heat-treatment could induce this response by maintenance of unsaturated fatty acids during cold storage, and thus the severity of chilling injury symptoms was reduced.

The application of heat treatment led to an increase in putrescine concentration during storage compared to control fruit, for which a reduction of this polyamine was observed. However, the main change was shown for spermidine, which increased during storage, for both control and treated fruit, although their levels were always higher in heat-treated than in control pomegranates. This effect could be a defense mechanism against this stress involving protection of cell membrane lipids, and could be responsible for the lower electrolyte leakage and browning found in heat-treated pomegranates. In addition, the higher polyamine concentration could account for the greater firmness retention in heat-treated fruit, since exogenous polyamine treatments have been shown to reduce softening of a wide range of fruit through reduction in hydrolytic cell-wall enzymes or rigidification of cell-wall by cross-linking to pectic substances (Valero and Serrano, 2010).

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