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*in*

Melgarejo P. (ed.), Valero D. (ed.).  
II International Symposium on the Pomegranate

Zaragoza : CIHEAM / Universidad Miguel Hernández  
Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 103

2012  
pages 233-236

Article available on line / Article disponible en ligne à l'adresse :

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To cite this article / Pour citer cet article

Zapata P.J., Guillén F., Martínez-Romero D., Sayyari M., Castillo S. **Chilling injury is reduced and the content of bioactive compounds enhanced by methyl salicylate treatment.** In : Melgarejo P. (ed.), Valero D. (ed.). *II International Symposium on the Pomegranate*. Zaragoza : CIHEAM / Universidad Miguel Hernández, 2012. p. 233-236 (Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 103)



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# Chilling injury is reduced and the content of bioactive compounds enhanced by methyl salicylate treatment

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**Abstract.** Methyl salicylate (MeSa) is a volatile plant compound having a role in plant defense-mechanism as signal molecule. Some evidences have shown that MeSa could indirectly provide protection against chilling damage (CI). Thus, given than pomegranate fruits are sensitive to CI, the effect of MeSa treatments (at 0.01 and 0.1 mM) on reducing the incidence of CI was analyzed. Control pomegranates exhibited CI symptoms manifested by pitting and browning when fruits were stored at 20°C after cold storage, the severity being enhanced as storage time advanced. The CI symptoms, as well as softening and increase in electrolyte leakage (EL), were significantly reduced by MeSa treatments, without significant differences among 0.01 and 0.1 mM doses. In addition, total phenolic and anthocyanin concentration and antioxidant activity were higher in arils from MeSa treated fruits than in those from controls. In conclusion in this paper we report the positive effect of MeSa on reducing CI of pomegranate fruits stored at chilling temperatures, which was accompanied by reduced softening and EL, showing a protective role on cell membranes and for increases in bioactive compounds and their related antioxidant activity have been found.

**Keywords.** *Punica granatum* L. – Quality – Anthocyanins – Phenolics – Antioxidant activity.

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## I – Introduction

Methyl salicylate (MeSa) is a volatile plant compound synthesised from salicylic acid having a role in the defense plant mechanism, including wounding, pathogens/insects, mechanical, drought and chilling injury (CI), among others (Hayat and Ahmad, 2007). As a result of CI damage, dysfunction of cell membrane occurs, affecting its permeability and being considered the primary molecular leading to the development of CI, which is strongly influenced by its lipid composition (Mirdehghan *et al.*, 2007a). Recent research has shown that MeSa treatment increased resistance of tomato (Fung *et al.*, 2006) and peach fruit (Han *et al.*, 2006) to low temperature stress due to the enhancement of antioxidant enzymes, which protects fruit cell membranes from lipid peroxidative injury. Thus, the main objective of this paper was to study the effect of MeSa on pomegranate fruit quality attributes, bioactive compounds and the antioxidant capacity during storage under CI conditions.

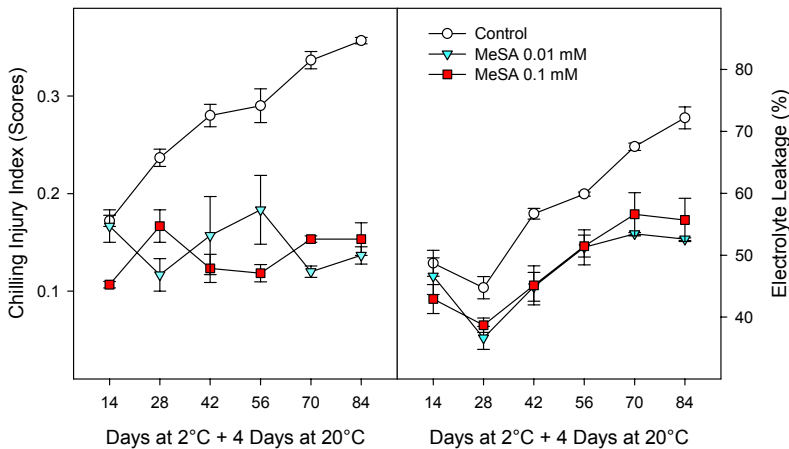
## II – Materials and methods

Pomegranates (*Punica granatum* L. cv. Mollar de Elche) were picked at mature stage (Melgarejo *et al.*, 1997), treated in triplicate with methyl jasmonate (MeSa) at 0.1 and 0.01 mM concentration for 16 h in 120-l container and stored at 2°C, in permanent darkness and with relative humidity of 90%. Sampling schedule was as follows: every 2 weeks 1 lot (5 fruits) from each replicate and treatment was transferred to a chamber at 20°C for 4 days and the following analytical determinations were performed: Chilling injury (CI), electrolyte leakage (EL), fruit

firmness, total soluble solids (TSS), total acidity (TA), total phenolics, total anthocyanins and total antioxidant activity as described by Mirdehghan *et al.* (2007a,b).

### III – Results and discussion

During postharvest of pomegranate fruits CI appeared after 14 days of storage manifested by pitting, browning and desiccation, the severity being enhanced as did storage time (Fig. 1). A similar behaviour was observed in EL, that is, increases along storage. However, the application of MeSa led to significantly lower CI and EL index (2-3 fold) than in control fruits, without significant differences among concentrations used (Fig. 1).



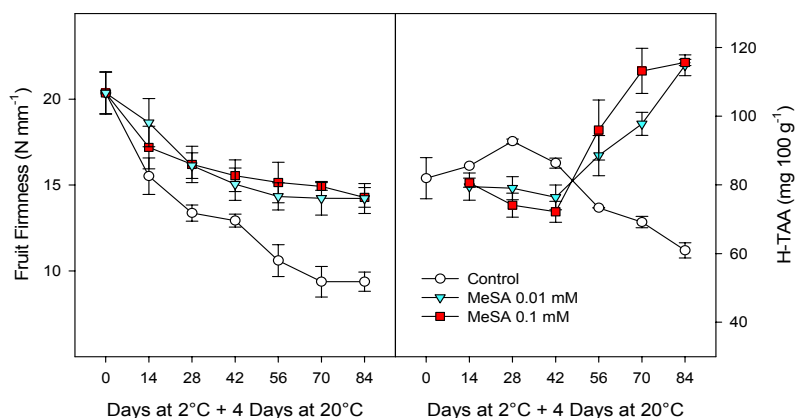
**Fig. 1. Chilling injury and electrolyte leakage during storage of control and methylsalicylate treated pomegranates. Data are the mean  $\pm$  SE.**

In general, CI primarily occurs in cell membrane and then the membrane damage initiates a cascade of secondary reactions finally leading to disruption of cellular and sub-cellular structures Mirdehghan *et al.* (2007a). In tomato, MeSA could induce some defence-mechanism responses that indirectly provide protection against chilling damage, rather than the compound itself producing a direct effect. Specifically, MeSA could inhibit catalase activity and in turn increase the amount of cellular  $H_2O_2$ , which would increase the expression of pathogenesis-protein genes, although accumulation of heat shock proteins (Ding *et al.*, 2002) or expression of alternative oxidase (Fung *et al.*, 2006) could not be discharged. Fruit firmness decreased gradually along storage, the softening process being retarded by MeSa treatments (Fig. 2), according to previous results in mango (Han *et al.*, 2006). The mechanism by which MeSa may affect the cell wall structure and maintain fruit firmness is not clear yet, and no research has been carried out in pomegranate specifically. TSS at harvest was  $16.1 \pm 0.1$  °Brix and increased during storage in both control and treated arils, reaching final values of  $\approx 17.5$  °Brix, while the values of TA at harvest ( $0.30 \pm 0.01$  g  $100$  g<sup>-1</sup> FW) decreased along storage, the final levels being  $\approx 0.24$  g  $100$  g<sup>-1</sup>, but without significant differences among treatments (data not shown). These changes are the result of the normal ripening process that occurs in these non-climacteric fruits, as previously reported (Mirdehghan *et al.*, 2007a; Sayyari *et al.*, 2009), which was delayed by the use of MeSa.

The content of total phenolics and total anthocyanin in the arils increased along storage in both

control and treated fruits, although concentrations were significantly higher in treated pomegranates than in control ones (data not shown). Total antioxidant activity due to hydrophilic compounds (H-TAA) decreased in control fruits after 56 days of storage, while in treated pomegranate an increase was found until the end of the experiment (Fig. 2).

Phenolics including anthocyanins are phytochemical compounds with antioxidant activity that impart beneficial health effects together with C and E plant vitamins (Valero and Serrano, 2010). 'Mollar de Elche' pomegranate cultivar have higher concentrations of ascorbic acid and total phenolics than 'Taifi', 'Wonderful' and 'Ganesh' cultivars (Gil *et al.*, 2000; Al-Maiman and Ahmad, 2002; Mirdehghan *et al.*, 2006). Thus, MeSa treatment could increase the health benefit of pomegranate fruit consumption, since concentration of bioactive compounds and antioxidant potential was higher, after long temp storage, in treated than in control pomegranates. In addition, application of MeSa treatments were effective on reducing CI and maintain quality attributes during storage.



**Fig. 2. Fruit firmness and hydrophilic total antioxidant activity (H-TAA) during storage of control and methylsalicylate treated pomegranates. Data are the mean  $\pm$  SE.**

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