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Preharvest or a combination of preharvest and postharvest treatments with methyl jasmonate reduced chilling injury, by maintaining higher unsaturated fatty acids, and increased aril colour and phenolics content in pomegranate

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ABSTRACT

In the present research the effects of preharvest 5 mM methyl jasmonate (MeJa) treatments, alone (Pre) or in combination with postharvest 5 mM MeJa treatment (Pre + Post), on reducing chilling injury (CI) of pomegranate during 90 d of storage at 2 °C plus 3 d at 20 °C and its relationship with changes in fatty acid composition of cell membranes were assayed. In addition, fruit and aril quality traits, total content in phenolics and anthocyanins and antioxidant activity of the arils were evaluated. Both, external and internal CI symptoms and the increase in ion leakage (IL) were reduced by Pre and Pre + Post MeJa treatments. The major fatty acids in pomegranate husk were palmitic, oleic, linoleic and linolenic acids. MeJa treatments led to higher concentration of unsaturated fatty acids (UFA) at harvest, which wasmaintained at higher levels during storage, while saturated fatty acid (SFA) concentration was lower in treated fruit than in controls. The concentration of total phenolics and anthocyanins were lower in the arils from control fruit than in arils of Pre and Pre + Post treated fruit during the whole storage period. In general, there were no significant differences between Pre and Pre + Post MeJa treatments on their effects on reducing CI, maintaining membrane stability and bioactive compounds with antioxidant activity. Thus, preharvest MeJa treatments may be sufficient to increase the cold storage potential of pomegranate fruit by reducing CI symptoms and enhancing the content bioactive compounds with antioxidant activity.

1. Introduction

Pomegranate fruit (*Punica granatum* L.) is one of the oldest known edible fruit and very appreciated by consumers, due to its high organoleptic properties. Also, the content of a wide range of phytochemical compounds with antioxidant potential has been associated with health beneficial properties, including protection against cardiovascular diseases, inflammations, infarct brain ischemia, Alzheimer, diabetes and cancers, among others (Faria and Calhau, 2011; Asgary et al., 2017; Panth et al., 2017). There are more than 1000 ornamental and edible cultivars of pomegranate described worldwide (Hasnaoui et al., 2011; Pareek et al., 2015). Among these, the Spanish cultivar 'Mollar de Elche' has high quality attributes, such as high sugar and low acid concentration, small and soft seeds and pleasant flavour (Melgarejo et al., 2000; Nuncio-Jáuregui et al., 2014; Fernandes et al., 2017). Recently, the 'Mollar de Elche' cultivar has been safeguarded by a Protected Designation of Origin (DOP) since 2016 [R (UE), 2016/83].

Important quality losses including husk desiccation and browning, decay and loss of firmness occur during postharvest storage of pomegranate fruit. In addition, decreases in ascorbic acid, acidity and colour of the arils led to reduction of consumers' acceptability in terms of freshness, juiciness and taste (Pareek et al., 2015). In order to avoid these undesirable changes and to prolong storability the best postharvest tool has been cold storage. However, pomegranate fruit is sensitive to chilling injury (CI) when stored at temperatures below 5 °C, the main symptoms being skin desiccation, browning and pitting, depression of the fruit surface and higher susceptibility to decay. Thus, recent studies are focused to find out postharvest treatments to be applied in combination with cold storage in order to reduce CI and increase storability. In this sense, positive results have been obtained by postharvest treatments of pomegranates with polyamines (Mirdehghan et al., 2007a), oxalic acid (Sayyari et al., 2010), salicylic acid (Sayyari

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et al., 2009, 2017), acetyl salicylic acid (Sayyari et al., 2011b), methyl salicylate (Sayyari et al., 2011a), and heat treatments (Mirdehghan et al., 2007b). More recently, carboxymethyl cellulose and chitosan edible coatings combined with oxalic or malic acids (Ehteshami et al., 2019), salicyloyl chitosan treatment (Sayyari et al., 2016) and controlled atmosphere storage (Sidhu et al., 2019) were also effective on reducing pomegranate CI.

On the other hand, methyl jasmonate (MeJa), an endogenous plant hormone derived from the jasmonic acid (JA) playing important roles in plant development, such as inducing systemic acquired resistance, providing plant tolerance against different kinds of stress, flowering, seedling germination and regulation of fruit growth and ripening (Wasternack and Strnad, 2016; Serrano et al., 2018) has been reported to reduce CI in a wide range of fruit (Aghdam and Bodbodak, 2013; Glowacz and Ree, 2016). For instance, postharvest MeJa treatment before storage at chilling temperature reduced CI in cherry tomato (Zhang et al., 2012), carambola (Mustafa et al., 2016), kiwifruit (Li et al., 2017), papaya (González-Aguilar et al., 2003), peach (Meng et al., 2009), and mangosteen (Mustafa et al., 2018). Specifically, in pomegranate, only in one previous paper the effect of postharvest MeJa treatment on reducing CI has been reported, with additional effects on delaying the postharvest ripening process and maintaining higher levels of antioxidant compounds (Sayyari et al., 2011a). Accordingly, in a recent paper, it has been reported that MeJa treatments (at 1, 5 and 10 mM) applied during on-tree 'Mollar de Elche' pomegranate fruit growth delayed the postharvest ripening during storage at 10 °C, manifested by lower losses in fruit weight, firmness and organic acids, leading to fruit quality maintenance. In addition, the concentration of antioxidant compounds (phenolics, individual anthocyanins and ascorbic acid) and total antioxidant activity were higher in arils from pomegranate treated fruit than in controls, at harvest and during storage at 10 °C, a non-chilling temperature (García-Pastor et al., 2020). The effects of preharvest MeJa treatment on reducing CI of pomegranate has been assayed only in a recent paper on 'Malas' cultivar (Koushesh Saba and Zarei, 2019), although the mechanism involved in this effect has not been elucidated yet and deserves further research. Thus, the main goal of the present research was to evaluate for the first time the effects of preharvest MeJa treatments, alone or in combination with postharvest MeJa treatment, on reducing CI of pomegranate and its relationship with changes in fatty acid composition of cell membranes during storage. In addition, pomegranate and arils quality and their content in bioactive compounds were also evaluated.

2. Materials and methods

2.1. Plant material and experimental design

The experiment was performed in a commercial orchard located in Elche (south of Alicante, Spain), in 2018 by using 9 years-old trees (planted at 6 x 5 m) of the cultivar 'Mollar de Elche'. Three blocks or replicates of 3 trees each one were randomly selected for 5 mM MeJa treatment and control. Trees were sprayed with 3 L of a freshly prepared 5 mM MeJa (Sigma Aldrich, Madrid) solution, containing Tween-20 (1 mL L^{-1}), or distilled water containing 1 mL L^{-1} Tween-20 at 80, 110, 140 and 170 days after full blossom, the last one being performed 4 d before harvesting. The concentration of MeJa and dates of application were selected based on previous experiments carried out in 2016 and 2017 seasons on this cultivar, in which 1, 5 and 10 mM MeJa doses were applied and the best results in term of yield and fruit quality attributes, at harvest and during storage at 10 °C, were obtained with 5 mM dose (García-Pastor et al., 2020). The fruit were harvested at commercial ripening stage characteristic of this cultivar based on fruit size (≈360 g) and total soluble solids (≈16 g L^{-1}) content. Immediately after harvest about 20 fruit were taken from each tree, that is, 60 fruit from each block or replicate and were transferred to the laboratory. Pomegranates with defects (sunburn, crack, bruise and cut in the husk) were discarded.

The following scheme was used for experimental design and sampling dates: All fruit were stored and analysed after 0, 30, 60, and 90 d of storage at 2 °C (85–90 % RH) plus 3 d at 20 °C (55–60 % RH). From each replicate of control trees, 4 lots of 15 fruit (5 per replicate) were taken, while from MeJa-treated trees 8 lots of 15 fruit, 4 of them being stored as indicated above for control fruit (Pre treatment) and the remaining being treated with 5 mM MeJa as postharvest treatment (Pre + Post treatment). Postharvest treatment was performed by dipping the fruit in 15 L of MeJa solution containing 1 mL L^{-1} Tween-20 for 15 min, and then they were left to dry at room temperature. The following parameters were measured for all fruit: respiration rate, fruit firmness, weight loss and external chilling injury (CI) index were assayed in the whole fruit. Then, each fruit was carefully cut at the equatorial zone with sharpened knives and the arils were manually extracted. One half of the skin was used to determine internal CI index individually and thereafter to measure ion leakage (IL). The other half was immediately cut in slices of 1 x 0.5 cm to obtain a homogeneous skin sample from fruit of each replicate. These samples were frozen in liquid N_2 and freeze-dried in an Alpha 2–4 freeze drier (Christ Alpha 2–4; Braum Biotech) for 1 d under reduced pressure, 2.2 MPa. The temperature in the drying chamber was −25 °C, while the heating plate reached 15 °C. Later, samples were milled until reach a fine powder and vacuum-packed to be used for measuring fatty acid composition. The arils from the 5 fruit of each replicate were also combined. Samples of 50 g were used to measure colour, total soluble solids (TSS) and titratable acidity (TA) and another 100 g sample of each replicate was frozen in liquid N_2, milled and stored at -20 °C for total phenolics, total anthocyanins and total antioxidant activity determinations. For these determinations results were expressed on a fresh weight basis.

2.2. Respiration rate and quality parameters

To measure fruit respiration rate, the 5 fruit of each replicate were hermetically sealed in a 3 L jar for 3600 s. After that, 1 mL from the holder atmosphere was withdrawn and used for CO_2 quantification in a gas chromatograph, equipped with thermal conductivity detector and under the chromatographic conditions previously described (Sayyari et al., 2011b). Respiration rate was expressed as μg kg^{-1} s^{-1} CO_2 and was the mean ± SE. The determination of fruit firmness was carried out individually in each of the 5 fruit of each replicate by using a TX-XT2i Texture Analyzer (Stable Mycrosystems, Godalming, UK) which applied a force to achieve a 5% deformation of the fruit diameter. Results were expressed as the relation between the applied force and the distance travelled (kN m^{-1}) and were the mean ± SE. Fruit were weighed at harvest and at each sampling date during storage and weight loss was expressed as percentage (%) with respect to the initial weight. TSS were determined in duplicate in the juice obtained from the aril sample of each replicate by using a digital refractometer Atago PR-101 (Atago Co. Ltd., Japan) at 20 °C, and results were the mean ± SE, expressed as g L^{-1}. TA was determined in duplicate in 1 mL of the same juice diluted in 25 mL distilled H_2O by potentiometric titration with 0.1 N NaOH up to pH 8.1, and results were the mean ± SE expressed as g of malic acid equivalent L^{-1}.

2.3. Chilling injury (CI) index and ion leakage

External and internal chilling injury index (CI) were individually evaluated in each fruit according to a 6-point hedonic scale (Fig. 1) based on the percentage of husk surface affected by CI symptoms (dehydration, browning and pitting): 0 (no symptoms), 1 (1–20 %), 2 (21–40 %), 3 (41–60 %), 4 (61–80 %) and 5 (> 81%). These scales were performed with own photographs made by the authors from previous studies and the degree of CI symptoms was assessed according to them. CI index was calculated as: Σ (value of hedonic scale) x (number of fruit with the corresponding score) / (total number of fruit in the sample).

Fig. 1. Reference scale to evaluate external e internal chilling injury (CI) index of 'Mollar de Elche' pomegranate during storage at 2 °C + 3 d at 20 °C.

Results were expressed as the mean ± SE of three replicates of 5 fruit.

To measure ion leakage, 16 disks (5 mm diameter ×5 mm thickness) were cut with a cork borer from the husks of the 5 fruit of each replicate and steeped in a glass vial containing 0.3 M mannitol. Conductivity was measured after 3 h under constant shaking (initial conductivity), using a conductivity meter (XS COND51 +). After that, the disks were frozen overnight, autoclaved for 900 s at 120 °C and cooled to room temperature, and then, conductivity was again measured. Ion leakage was expressed as percentage of the total and calculated as follow (Mirdehghan et al., 2007a):

(Initial conductivity × 100)/(total conductivity)

2.4. Fatty acid profile and quantification

Pomegranate husk fat was directly methylated according to Trigueros and Sendra (2015). Fatty acid profile and quantification were determined by high resolution gas chromatography, analysing the fatty acid methyl esters obtained by trans-esterification of 25 mg of sample with 2 mL of 0.5 M sodium methoxide. Methyl esters were separated on a Shimadzu GC17A gas chromatography unit coupled to a mass spectrometry detector GC–MS QP5050, Shimadzu (Kyoto, Japan) with a SupraWax-280 column, filled with 100% polyethylene glycol (Teknokroma S. Co. Ltd., 165 Barcelona, Spain; 30 m length, 0.25 mm internal diameter, 0.25 μm film thickness). The following conditions were applied (Ferrara et al., 2014): Helium was used as carrier gas, injector and FID-detector temperatures were 220 and 250 °C, respectively, and oven temperatures were 140 °C for 2 min, which increased to 165 °C at 6 °C/min and thereafter from 165 °C to 225 °C at 2.8 °C/min and was held at 225 °C for 25 min. Volume of injected sample was 1 μL with split 1:20. Fatty acid methyl esters were identified by comparison of their retention times with Supelco 37-component FAME Mix reference standard (Sigma-Aldrich Co., St. Louis, MO, USA). Quantification was carried out in duplicate in each sample on the basis of peak areas using nonadecanoic acid (19:0 c-19) as internal standard and results (mean ± SE) were expressed as milligram nonadecanoic acid equivalent per kg on a dry weight basis (mg kg^{-1}).

2.5. Aril colour, total anthocyanins, total phenolics and total antioxidant activity determination

The arils colour was measured on the surface of a Petri dish filled with arils, using the CIE Lab system in a colorimeter (CRC200, Minolta Camera Co., Tokyo, Japan). After recording L*, a* and b* parameters, arils colour was expressed as Hue angle (arctg b*/a*). For total anthocyanin quantification, 5 g of arils were homogenised in 15 mL of methanol/formic acid/water (25:1:24, v/v/v) and then, centrifuged at 10,000 *g* for 10 min at 4 °C. Total anthocyanin content (TAC) was quantified in the supernatant by reading absorbance at 520 nm and was expressed as mg kg^{-1} of cyanidin 3-*O*-glucoside equivalents (Cy 3-glu, molar absorption coefficient of 23,900 L cm^{-1} mol^{-1} and molecular weight of 449.2 g mol^{-1}).

Total phenolics were extracted by homogenizing 5 g of aril sample with 10 mL of water: methanol (2:8) containing 2 mM NaF (to inactivate polyphenol oxidase activity and prevent phenolic degradation). The extracts were centrifuged at 10,000 *g* for 10 min at 4 °C and total phenolic concentration was quantified in the supernatant by using the Folin-Ciocalteu reagent as previously described by Sayyari et al. (2011a). Results were expressed as g gallic acid equivalent (GAE) kg^{-1} and are the mean ± SE of three replicates. Total antioxidant activity (TAA) was measured according to Sayyari et al. (2011b), which enables to determine hydrophilic (H-TAA) and lipophilic (L-TAA) antioxidant activity in the same extraction. Briefly, for each sample, 5 g of arils were homogenised in 10 mL of 50 mM phosphate buffer pH = 7.8 and 5 mL of ethyl acetate, and then centrifuged at 10,000 *g* for 15 min at 4 °C. The upper fraction was used to quantify L-TAA and the lower to quantify H-TAA, respectively. In both cases, TAA was determined in a reaction mixture containing 2,20-azino-bis-(3- ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), horse radish peroxidase enzyme and its oxidant substrate (hydrogen peroxide), in which $ABTS^{+}$ radicals are generated and monitored at 730 nm. Then, pomegranate extract was added and the decrease in absorbance after 90 s was calculated, which 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 Aldrich (Madrid, Spain), and results are expressed as g of trolox equivalent (TE) kg^{-1} and are the mean ± SE of three replicates.

2.6. Statistical analysis

Results are expressed as mean ± SE of three replicates. Data for the analytical determinations were subjected to analysis of variance (ANOVA). Sources of variation were storage time and treatment. Mean comparisons were performed using HSD Tukey's test to examine if differences between control and treated fruit were significant at $P < 0.05$. All analyses were performed with SPSS software package v. 17.0 for Windows. Correlations were performed between internal or external CI indexes and IL and between arils Hue angle and their content in total anthocyanins.

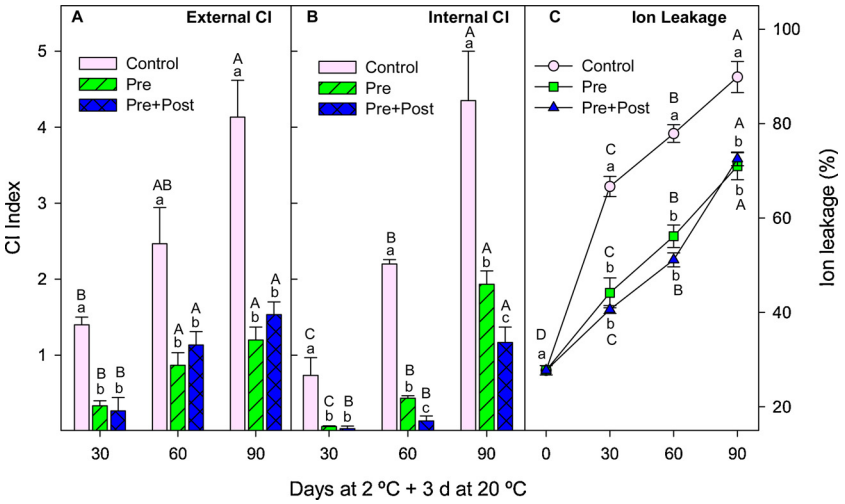

Fig. 2. External (A) and internal (B) chilling injury (CI) index and ion leakage (C) in the husk of control, preharvest (Pre) and preharvest plus postharvest (Pre + Post) 5 mM methyl jasmonate treated 'Mollar de Elche' pomegranate fruit during storage at 2 °C + 3 d at 20 °C. Data are the mean ± SE of three replicates of five fruit. Different capital letters show significant differences for each treatment during storage and different lowercase letters show significant differences among treatments for each sampling date at $P < 0.05$.

3. Results

3.1. Chilling injury and ion leakage

External chilling (CI) injury symptoms were manifested by depression of the fruit surface, browning and pitting, while internal CI symptoms appeared as brown spots in internal husk surface and locular septa (Fig. 1). Both, external and internal CI index increased during storage reaching final values of 4.13 ± 0.48 and 4.35 ± 0.65, respectively, in control fruit (Fig. 2A and B). However, in Pre and Pre + Post MeJa treated fruit the increases in external and internal CI indexes were significantly lower ($P < 0.05$) than in controls. However, differences between Pre and Pre + Post treatments were only significant for internal CI after 60 and 90 days of storage. Ion leakage (IL) in husk tissue also increased during storage in control and treated fruit, although this increase was significantly ($P < 0.05$) delayed in treated fruit with respect to controls, without significant differences between Pre and Pre + Post treatments (Fig. 2C).

3.2. Changes in fatty acid composition

Twenty-five fatty acids were identified and quantified in pomegranate skin, the major ones being C16:0 (palmitic), C18:1 (oleic), C18:2 (linoleic) and C18:3 (linolenic) acids, followed by C10:0 (capric), C14:0 (myristic), and C18:0 (stearic) acids (Fig. S1). In addition, other seventeen minor fatty acids were also identified, twelve of them for the first time in pomegranate: myristoleic acid (C14:1), heneicosanoic acid (C21:0), pentadecanoic acid (C15:0), pentadecenoic acid (C15:1), margaric acid (C17:0), margaroleic acid (C17:1), vaccenic acid (C18:1), arachidic acid (C20:0), methyl linolenate (C18:3), eicosadienoic acid (C20:2), behenic acid (C22:0), lignoceric acid (C24:0) and nervonic acid (C24:1). This fatty acid profile was similar in control and treated fruit, either at harvest or during postharvest storage, and thus chromatogram from control fruit at harvest is shown (Fig. S1).

Palmitic acid concentration in the skin of control fruit at harvest was significantly ($P < 0.05$) higher (191.6 ± 12.4 mg kg^{-1}) than in those of Pre and Pre + Post MeJa treated ones (160.9 ± 10.5 and 14.34 ± 0.63 mg kg^{-1}, respectively). These differences were observed until the last sampling date in spite of the fact that palmitic acid decreased in skin of control and treated fruit during storage (Fig. 4). C10:0 and C18:0 concentrations also decreased during storage although no significant differences were observed between control and treated fruit, while no changes were observed in C14:0 either during storage or attributed to MeJa treatments (Fig. 3). All the UFA fatty acids were found at significant ($P < 0.05$) higher concentration in treated fruit than in controls at harvest, the highest enhancement being found in linolenic

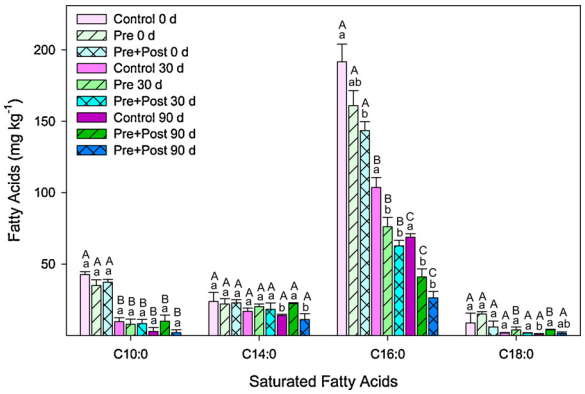

Fig. 3. Effect of preharvest (Pre) and preharvest plus postharvest (Pre + Post) 5 mM methyl jasmonate treatments on saturated fatty acid content in pomegranate husk at harvest (0 d) and after 30 and 90 d of storage at 2 °C + 3 d at 20 °C. Data are the mean ± SE of three replicates of five fruit. For each saturated fatty acid, different capital letters show significant differences for each treatment during storage and different lowercase letters show significant differences among treatments for each sampling date at $P < 0.05$.

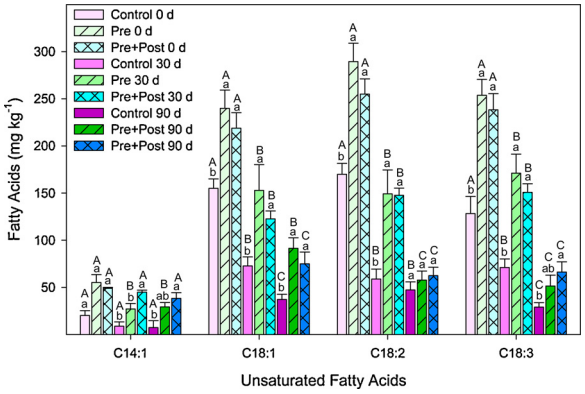

Fig. 4. Effect of preharvest (Pre) and preharvest plus postharvest (Pre + Post) 5 mM methyl jasmonate treatments on unsaturated fatty acid content in pomegranate husk at harvest (0 d) and after 30 and 90 d of storage at 2 °C + 3 d at 20 °C. Data are the mean ± SE of three replicates of five fruit. For each unsaturated fatty acid, different capital letters show significant differences for each treatment during storage and different lowercase letters show significant differences among treatments for each sampling date at $P < 0.05$.

Table 1
Respiration rate (RR) and quality parameters, firmness, weight loss, total soluble solids (TSS) and titratable acidity (TA) in pomegranate fruits from control and preharvest (Pre) and pre- plus postharvest methyl jasmonate (MeJA)-treated (Pre + Post) fruit during postharvest storage at 2 °C + 3 d at 20 °C.

Parameter	Days	Control	MeJA 5 mM	
			Pre	Pre + Post
RR (μg kg^{-1} s^{-1})	0	5.96 ± 0.41 ^{aA}	5.14 ± 0.43 ^{bA}	5.26 ± 0.82 ^{bA}
	30	6.61 ± 0.61 ^{aB}	6.00 ± 0.58 ^{bB}	5.77 ± 0.64 ^{bB}
	60	6.14 ± 0.60 ^{aAB}	5.61 ± 0.34 ^{bB}	5.36 ± 0.41 ^{bAB}
	90	4.48 ± 0.39 ^{aC}	4.06 ± 0.29 ^{bC}	3.68 ± 0.75 ^{bC}
Firmness (kN m^{-1})	0	26.10 ± 0.41 ^{bA}	28.08 ± 0.62 ^{aA}	27.93 ± 0.55 ^{aA}
	30	18.94 ± 0.46 ^{bB}	25.19 ± 0.83 ^{aB}	24.95 ± 0.76 ^{aB}
	60	18.54 ± 0.72 ^{bB}	21.83 ± 0.89 ^{aC}	21.48 ± 0.69 ^{aC}
	90	18.01 ± 0.52 ^{bB}	19.98 ± 0.48 ^{aC}	19.77 ± 0.39 ^{aC}
Weight loss (%)	0	–	–	–
	30	10.28 ± 0.40 ^{aA}	7.13 ± 0.13 ^{bA}	7.27 ± 0.29 ^{bA}
	60	17.39 ± 0.55 ^{aB}	12.57 ± 0.54 ^{bB}	13.13 ± 0.24 ^{bB}
	90	18.98 ± 0.27 ^{aC}	16.04 ± 0.40 ^{bC}	16.44 ± 0.55 ^{bC}
TSS (g L^{-1})	0	157.70 ± 2.10 ^{aA}	166.80 ± 3.70 ^{aA}	169.50 ± 4.20 ^{aA}
	30	167.00 ± 2.20 ^{aB}	170.70 ± 1.90 ^{aA}	171.20 ± 2.70 ^{aA}
	60	168.30 ± 1.40 ^{aB}	171.5 ± 1.40 ^{aA}	172.20 ± 2.00 ^{aA}
	90	169.50 ± 1.30 ^{aB}	172.3 ± 1.70 ^{aA}	172.50 ± 1.40 ^{aA}
TA (g L^{-1})	0	4.10 ± 0.30 ^{aA}	4.30 ± 0.20 ^{aA}	4.20 ± 0.20 ^{aA}
	30	5.30 ± 0.40 ^{aAC}	5.60 ± 0.30 ^{aB}	5.50 ± 0.40 ^{aB}
	60	8.40 ± 0.50 ^{aB}	8.90 ± 0.50 ^{aC}	8.80 ± 0.60 ^{aC}
	90	6.50 ± 0.30 ^{aC}	6.60 ± 0.20 ^{aD}	6.60 ± 0.30 ^{aB}

*Data are the mean ± SE. Different capital letters show significant differences for each treatment during storage and different lowercase letters show significant differences among treatments for each sampling date at $P < 0.05$.

acid, 1.98 and 1.86-fold increases in Pre and Pre + Post MeJa treated fruit, respectively, with respect to control fruit (Fig. 4). All the UFA decreased during storage, although their concentrations were maintained at significant ($P < 0.05$) higher levels in treated than in control fruit, without significant differences between Pre and Pre + Post treatments.

3.3. Respiration rate, fruit and aril quality parameters, bioactive compounds and total antioxidant activity

Pre and Pre + Post MeJa treatment significantly inhibited the fruit respiration rate ($P < 0.05$) at harvest and during storage, although no significant differences were found between both treatments (Table 1). Fruit firmness decreased during storage in control and treated fruit, but were significantly ($P < 0.05$) delayed in Pre and Pre+Post MeJa treated fruit, without significant differences between both treatments. Fruit weight loss increased during storage reaching final values 18.98 ± 0.27 % in control fruit and significantly ($P < 0.05$) lower (16.04 ± 0.40 %) in treated ones. The major effects of MeJa treatments on delaying weight losses and fruit softening were found after 30 and 60 d of storage (Table 1). However, TSS and TA were not affected by MeJa treatments, with values of TSS ranging between 160 and 170 g L^{-1} and TA increasing from 4.0 to 6.5 g L^{-1} during the whole storage period (Table 1). On the other hand, colour Hue angle at 0 d was significantly ($P < 0.05$) higher in control fruit than in treated ones and these differences were maintained during storage, although decreases during storage occurred in all fruit (Fig. 5A). On the contrary, total anthocyanin content was increased by MeJa treatments, either at harvest and during storage, the major effect being found in Pre + Post MeJa treated fruit (Fig. 5B). Similarly, total phenolic concentration and antioxidant activity of the hydrophilic extracts (H-TAA) were significantly ($P < 0.05$) higher in treated than in control fruit from harvest until the last sampling date (Fig. 5C and D).

4. Discussion

The first cell structures affected by CI are cell membranes, which change from a flexible liquid-crystalline phase to a solid-gel structure when fruit are exposed to chilling temperatures, leading to losses of semi-permeability and functionality of cytoplasmic and intracellular cell membranes (Rui et al., 2010; Valero and Serrano, 2010). These impairments on cell membrane structure and function leads to decompartmentalization of the substrates and enzymes in fruit organelles resulting in enzymatic oxidation of phenols to *o*-quinones by peroxidase and polyphenol oxidase (Zhang et al., 2015) which could be responsible for the brown spots in external and internal pomegranate husk surfaces. Then, Pre and Pre + Post MeJa treatments would have an effect on maintaining membrane structure since lower CI symptoms and IL values were observed in treated fruit in comparison to the control (Figs. 1 and 2). It was found that both, external and internal CI indexes were correlated with IL ($r^2 = 0.823$ and 0.733, respectively) by taking into account data of control and treated fruit for all sampling dates. Thus, IL could be used as an indicator of CI and cell membrane integrity, according to previous reports in a wide range of fruit species including pomegranate (Sayyari et al., 2011a; Ehteshami et al., 2019). However, significant differences between Pre and Pre + Post MeJa treatments were observed only in internal CI while external CI and ion leakage were similar in both treatments. Thus, for practical purposes in order to reduce CI, pre-harvest MeJa treatments could be sufficient, with similar effects on reducing CI than the postharvest treatments previously reported (Sayyari el al., 2011a).

It is well known that damage of the membrane structure and subsequent changes in lipid constituents is correlated with the occurrence of CI. These changes in lipid composition show mainly decrease in the ratio of unsaturated to saturated fatty acids. This could be affecting the phase transition of membrane lipids from a liquid-crystalline to a solid-gel state, and in turn leading to membrane peroxidation and damage with accelerating the occurrence of CI (Wongsheree et al., 2009).

Fatty acid profile in 'Mollar de Elche' pomegranate husk addressed in the present results agrees with previous published papers. Thus, Mirdehghan et al. (2007b) identified and quantified 10 fatty acids in this pomegranate cultivar, 5 saturated (C10:0, C12:0, C14:0, C16:0 and C18:0), 2 mono-unsaturated (C16:1 and C18:1) and 3 poly-unsaturated (C18:2 *cis*, C18:2 *trans* and C18:3). Sayyari et al. (2017) also identified C20:1 (gadoleic acid) and C22:1 (erucic acid). However, twelve new fatty acids (Fig. S1) were identified and quantified for the first time in pomegranate husk in the present research. Among these new fatty acids, myristoleic acid (C14:1) accounted for ca. 2 % of the total fatty acid content, while the remaining fatty acids, heneicosanoic acid (C21:0), pentadecanoic acid (C15:0), pentadecenoic acid (C15:1), margaric acid (C17:0), margaroleic acid (C17:1), vaccenic acid (C18:1), arachidic acid (C20:0), methyl linolenate (C18:3), eicosadienoic acid (C20:2), behenic acid (C22:0), lignoceric acid (C24:0) and nervonic acid (C24:1) were found at very low concentrations, accounting for 0.5–0.1% of total fatty acid composition. In addition, in these previous papers, it was reported that heat (Mirdehghan et al., 2007b) or salicylic acid (Sayyari et al., 2017) treatments decreased CI damages and delayed the losses in the major SFA and UFA occurring during storage at chilling temperatures. Accordingly, 'Mallas Saveh' pomegranate fruit treated with salicyloyl chitosan showed reduced CI symptoms and exhibited higher membrane UFA/SFA ratio (Sayyari et al., 2016). The destructive process of cellular membranes during fruit storage at chilling temperatures has been ascribed to phospholipids hydrolysis into free fatty acids and peroxidation of UFA due to the coordinated action of lipid metabolizing enzymes, such as phospholipase, lipase and lipoxygenase (Wang et al., 2018; Zhang et al., 2018; Lin et al., 2017). Accordingly, glycine betaine treatment reduced CI in peach fruit, throughout reduction of lipoxygenase, phospholipase D and lipase activities and their gene expression and increasing the expression of genes related to fatty acid biosynthesis and desaturation (Wang et al., 2019).

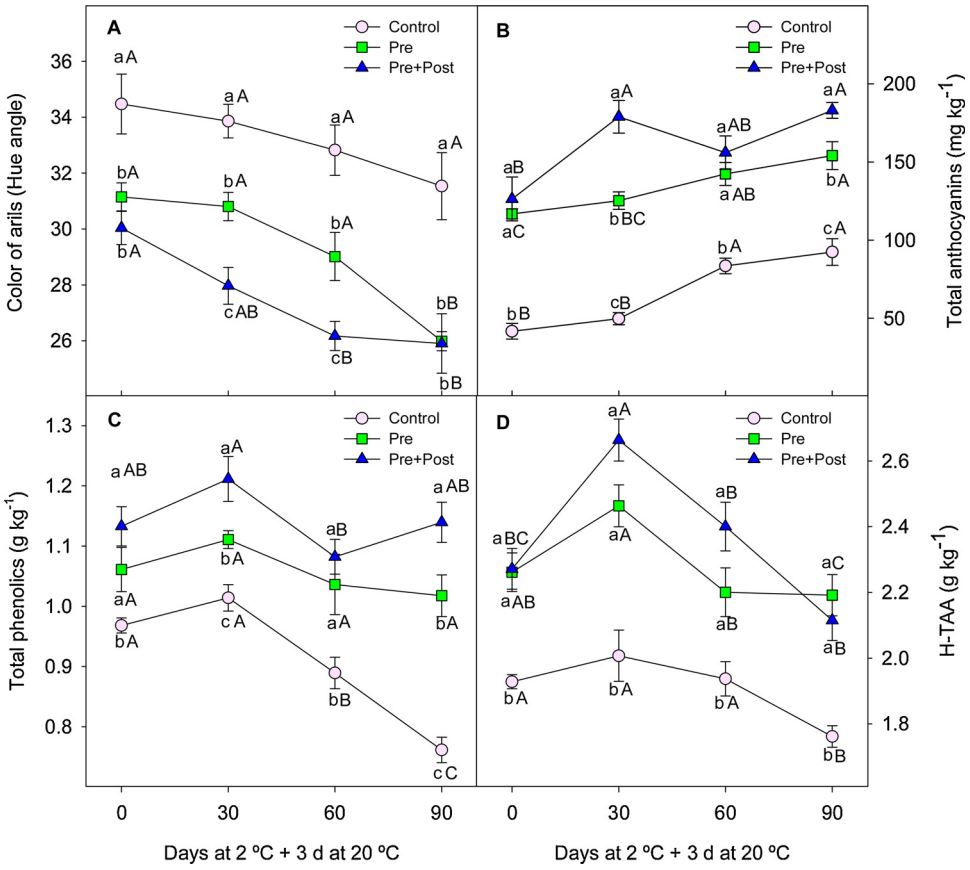

Fig. 5. Colour (A), total anthocyanin (B), total phenolics (C) and hydrophilic total antioxidant activity (H-TAA, D) in the arils of control, preharvest (Pre) and preharvest plus postharvest (Pre + Post) 5 mM methyl jasmonate treated 'Mollar de Elche' pomegranate fruit during storage at 2 °C + 3 d at 20 °C. Data are the mean ± SE of three replicates of five fruit. Different capital letters show significant differences for each treatment during storage and different lowercase letters show significant differences among treatments for each sampling date at $P < 0.05$.

These effects resulted in higher levels of unsaturated/saturated fatty acid ratio and maintenance of normal cell membrane structure and function. Thus, MeJa treatments might reduce peroxidation of the cell membrane lipids leading to maintenance of high fatty acid content and particularly, higher UFA/SFA ratio during storage and in turn, higher membrane integrity and lower CI and IL.

The effect of MeJa treatment on decreasing CI in pomegranate has been reported in a recently published paper in 'Malas' cultivar (Koushesh Saba and Zarei, 2019), although fatty acids were not evaluated in this study. Moreover, to the best of our knowledge, just in one previous paper the effect of MeJa treatment on fatty acid composition of cell membranes has been reported. In this study, Cao et al. (2009) showed that postharvest treatment with MeJa of loquat fruit significantly reduced CI and maintained higher UFA/SFA ratio during storage, suggesting that MeJa induced CI tolerance in fruit tissues by reducing losses in UFA and maintaining a high UFA/SFA ratio. The results of the present study are in agreement with this previous report, and show that MeJa reduced CI throughout maintenance of cell membrane stability. Thus, the sharp decrease in individual and total UFA concentration observed in control fruit from 0 to 30 d of storage were significantly ($P < 0.05$) delayed in all MeJa treated fruit (Figs. 4 and 2S). Moreover, UFA/SFA ratio was significantly ($P < 0.05$) increased as a consequence of Pre and Pre + Post MeJa treatments at 0 d and maintained at higher levels during the whole storage period, the effect being significantly ($P < 0.05$) higher when MeJa was applied as Pre +Post treatment than as Pre treatment alone (Fig. 3S). These effects could be described as a mechanism of acclimation to low temperature and would account for maintenance of membrane semi-permeability, leading to lower losses of intracellular water, ions and metabolites and, in turn, being responsible for the lower weight loss, IL and CI index scores found in treated fruit.

Fruit and aril quality parameters and antioxidant compounds were increased in pomegranate fruit as a consequence of Pre and Pre + Post MeJa treatments. Thus, weight loss and softening were delayed in treated fruit, without significant differences between Pre and Pre + Post treatments, as commented above for reduction of CI damages and IL. Both treatments were also effective on decreasing colour Hue angle of the arils showing they had a deeper red colour, due to an increase in the biosynthesis of anthocyanins whose concentration was higher in treated than in control fruit from 0 d until the last sampling date, especially in the arils of fruit receiving Pre + Post MeJa treatments. In fact, a negative correlation ($y = -14.4x + 553$, $r^2 = 0.879$) was found between arils Hue angle and their content in anthocyanins considering data of all treatments and sampling dates. With respect to total phenolics and H-TAA, significant ($P < 0.05$) higher levels were also found in treated than in control fruit during the whole storage period. These results are supported by previous experiments in which concentration of anthocyanins and other phenolic compounds were increased by preharvest MeJa treatments in plums (Martínez-Esplá et al., 2014), blackcurrants (Flores and Ruiz Del Castillo, 2016), mangoes (Muengkaew et al., 2016), apples (Ozturk et al., 2015), table grape (García-Pastor et al., 2019) or lemon (Serna-Escolano et al., 2019), as a consequence of stimulation of the expression of genes involved in their biosynthesis (Jia et al., 2016; Wei et al., 2017). Total phenolic contents were also maintained at higher levels in MeJa treated peaches due to increases in PAL and SOD activities and decreases in PPO and POD activities, which were related to reduction of CI symptoms (Jin et al., 2009). On the contrary, carambola exposed to postharvest MeJa treatments led to a reduction in both total phenolics and antioxidant activity (Mustafa et al., 2016). Increases in phenolic content as a consequence of preharvest MeJa treatment in pomegranates stored at 10 °C have been also reported in our previous paper (García-Pastor et al., 2020). In the present paper, these effects of preharvest MeJa treatment were also observed during storage at 2 °C, confirming a positive role of MeJa treatments on increasing health properties of pomegranate fruit, which have been attributed to anthocyanins and other phenolic

compounds (Reyes-Díaz et al., 2016; Asgary et al., 2017; Serrano et al., 2018; Bassiri-Jahromi, 2018). It is worth noting that total phenolic and anthocyanin concentrations were higher in Pre + Post MeJa treated fruit than in Pre-treated ones during the whole storage time, showing an accumulative effect of Pre + Post MeJa treatments. In this sense, it has been reported in apple fruit that the eff ;ect of postharvest MeJa treatments on promoting anthocyanin accumulation was dose-dependent since higher up-regulation in the expression of the genes involved in anthocyanin biosynthesis occurred for 1 than for 0.1 mM doses (Feng et al., 2017).

5. Conclusions

The results from this study demonstrate that Pre and Pre + Post MeJa treatments reduced external and internal CI symptoms in pomegranate husk, likely by better maintaining the cell membrane structure through reducing UFA losses and enhancing the UFA/SFA ratio. We identify twelve new fatty acids in the pomegranate husk. In addition, fruit and aril quality parameters and their content in antioxidant compounds were increased in pomegranate fruit as a consequence of Pre and Pre + Post MeJa treatments. For most of the analysed parameters, there were no significant differences between Pre and Pre + Post MeJa treatments. For this reason, preharvest MeJa treatments is recommended over postharvest application to increase storability of pomegranate fruit during cold storage, with reduction of CI symptoms and increasing quality traits and bioactive compounds with antioxidant potential, and in turn its health beneficial effects.

Declaration of Competing Interest

The authors declare that the study was conducted in absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.postharvbio.2020.111226.

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