Scientia Horticulturae 247 (2019) 247–253

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

# Reducing incidence of peel physiopathies and increasing antioxidant activity in pomegranate fruit under different irrigation conditions by preharvest application of chitosan

I. Griñán[a,1], D. Morales[b,1], J. Collado-González[c,1], A.B. Falcón-Rodríguez[b,2], A. Torrecillas[d,2], M.J. Martín-Palomo[e,2], A. Centeno[f,2], M. Corell[e,2], A.A. Carbonell-Barrachina[c,2], F. Hernández[a,2], A. Galindo[e,g,*,1]

[a] Dpto. Producción Vegetal y Microbiología, Grupo de Investigación de Producción Vegetal y Tecnología, Universidad Miguel Hernández de Elche, Ctra. de Beniel, km 3,2, E-03312 Orihuela, Alicante, Spain
[b] Dpto. Fisiología y Bioquímica, Instituto Nacional de Ciencias Agrícolas (INCA), Ctra. de Tapaste, km 3.5, San José de Las Lajas, Mayabeque, Cuba
[c] Universidad Miguel Hernández de Elche, Department of Agrofood Technology, Food Quality and Safety Research Group, Ctra. de Beniel, km 3,2, E-03312 Orihuela, Alicante, Spain
[d] Dpto. Riego, Centro de Edafología y Biología Aplicada del Segura (CSIC), P.O. Box 164, E-30100 Espinardo, Murcia, Spain
[e] Dpto. Ciencias Agroforestales, ETSIA, Universidad de Sevilla, Crta de Utrera km 1, E-41013 Sevilla, Spain
[f] Dpto. Producción Vegetal, Fitotecnia, ETSIAAB, Universidad Politécnica de Madrid, Ciudad Universitaria s/n, E-28040 Madrid, Spain
[g] Dept. of Water Engineering & Management, Faculty of Engineering Technology, University of Twente, P.O. Box, 217, 7500 AE Enschede, the Netherlands

ARTICLE INFO

*Keywords:*
Fruit cracking
Fruit splitting
Fruit sunburn
Water relations
Water stress

ABSTRACT

No previous information exits on the effect of preharvest chitosan, a plant biostimulant and antitranspirant, which has been considered as a food additive by the USFDA, spraying on pomegranate trees. Then, the effect of chitosan spraying in fully irrigated and water stressed trees on yield, fruit quality and the occurrence of fruit peel physiopathies was studied. Some of these effects were negative such as the reduction in fruit weight and the less reddish and duller appearance of the arils. However, these negative aspects could be regarded as being compensated by other very important positive effects, such as the increase in the antioxidant activity and the significant reduction in fruit peel cracking or splitting and fruit sunburn physiopathies occurrence, which would considerably improve the returns of pomegranate growers.

## 1. Introduction

Although pomegranate (*Punica granatum* L.) is frequently considered a crop of minor importance, it is one of the oldest known edible fruits. Mainly grown in semi-arid mild-temperate to subtropical climates (Blumenfeld et al., 2000), pomegranate confronts water deficit by developing stress avoidance and stress tolerance mechanisms (Rodríguez et al., 2012), which endow it with the capacity to support heat and to thrive in arid and semiarid areas, even under desert conditions (Aseri et al., 2008).

In recent years, pomegranate fruit consumption has been increasing due to its perceived health-related characteristics such as its anti-atherosclerotic effects, which are able to reduce blood pressure (Aviram et al., 2008), its high antioxidant activity (Gil et al., 2000; Seeram et al., 2006) and the anticarcinogenic compounds it contains (Malik et al., 2005; Malik and Mukhtar, 2006; Adhami and Mukhtar, 2006). To fulfil consumer satisfaction, its health-related properties and pleasing taste need to be accompanied by an attractive appearance in terms of size and redness, and the absence of pesticide residues, insect attack injuries and mechanical damage.

Under Mediterranean culture conditions, the incidence of some pomegranate physiopathies, mainly sunburn, cracking and splitting, is frequent, making fruits unmarketable and causing substantial economic losses to farmers, who may lose half of their crop yield (Blumenfeld et al., 2000; Melgarejo et al., 2004; Yazici and Kaynak, 2009). Pomegranates are terminal-bearing plants with thin branches, which bend

* Corresponding author at: Dept. of Water Engineering & Management, Faculty of Engineering Technology, University of Twente. P.O. Box, 217, 7500 AE Enschede, the Netherlands.
*E-mail address:* a.galindoegea@utwente.nl (A. Galindo).
[1] These authors contributed equally to this work.
[2] These authors contributed equally to this work.

https://doi.org/10.1016/j.scienta.2018.12.017
Received 7 June 2018; Received in revised form 10 December 2018; Accepted 12 December 2018
Available online 24 December 2018
0304-4238/ 

under the fruit's weight, and peel sunburn occurs mainly in fruits that previously developed in the shade (Melgarejo et al., 2004). For their part, fruit cracking and splitting are the result of changes in fruit water relations and fruit skin properties (Rodríguez et al., 2018). In fact, when previously stressed fruits are rehydrated, an asymmetric increase in turgor pressure takes place. So, aril turgor increases to a much greater extent than peel turgor. This increase in aril pressure puts pressure on the peel and make it susceptible to cracking and/or splitting, especially bearing in mind that, under water stress, the mechanical properties of peel change, peel elasticity tending to decline, so that the peel becomes thicker and stiffer (Galindo et al., 2014b; Rodríguez et al., 2018).

Chitin is a naturalpolysaccharide consisting of a copolymer of *N*-acetyl-D-glucosamine and D-glucosamine residues, linked by β-1,4 glycosidic bonds (Nge et al., 2006). It is found in various sources in nature such as the shells of crustaceans, cuticles of insects and the cell wall of fungi and some algae, where it is produced by demineralization and deproteinization (Rinaudo, 2006; Pichyangkura and Chadchawan, 2015; Younes and Rinaudo, 2015).

Chitosan is the deacetylated (water soluble) form of chitin and is the overall name of a group of heteropolysaccharides differing in structure, molecular weight, degree of acetylation and properties such as pKa, solubility, viscosity, etc. (Falcón-Rodríguez et al., 2012; Pichyangkura and Chadchawan, 2015). Chitosan is considered a biostimulant because it has broad applications in crop culture for its significant antimicrobial activity (Palma-Guerrero et al., 2008; Badawy and Rabea, 2012; Falcón-Rodríguez et al., 2012), and through (i) promoting the growth of roots, shoots and leaves of various plants (Chibu and Shibayama, 2001; Wanichpongpan et al., 2001), (ii) increasing crop yield (Katiyar et al., 2014), (iii) conserving water use due to its effective antitranspirant effect (Bittelli et al., 2001), (iv) mitigating the effects of water stress (Yang et al., 2009) and (v) decreasing postharvest table grape cracking (Zoffoli et al., 2008; Shiri et al., 2013).

Despite the above mentioned beneficial characteristics, there have been no reports on the effect of chitosan spray on pomegranate yield and fruit quality, or on the interaction of this factor and plant water status. Consequently, the main objective in the current study was to analyse the interaction between preharvest pomegranate fruit chitosan spraying and plant water status on yield and the occurrence of fruit cracking or splitting and fruit sunburn physiopathies. In addition, the effect of both factors and their interaction on fruit quality attributes was studied as a complementary objective.

## 2. Materials and methods

### 2.1. Plant material, experimental conditions and treatments

The experiment was carried out in the summer of 2017 in the Tres Caminos Experimental Station near the city of Santomera (Murcia, Spain) (38°6′ N; 1°2′ W). The plot soil was stony (33%, w/w) and shallow, with a clay-loam texture. The plant material consisted of own rooted 7-year old pomegranate trees (*P. granatum* (L.) cv. Mollar de Elche) in a 3 × 5 m spacing pattern.

Irrigation was performed daily and during the night using a drip irrigation system with one lateral pipe per tree row and four emitters (each delivering 4 L $h^{-1}$) per plant. Fully irrigated plants (FI) were irrigated above the estimated crop water requirements (115% crop reference evapotranspiration, ETo) while irrigation was withheld from the water stressed plants (WS) from the day of the year (DOY) 221 to DOY 269 (48 days), after which, irrigation was resumed at FI level until harvest (DOY 286, 13 October). In addition, on day of the year, DOY, 221 (10 days after the end of fruit thinning) and on DOY 254, plants from both irrigation treatments were sprayed with a Quitomax® solution at 45 g of active ingredient per ha. This active ingredient consists of chitosan polymers of medium molecular weight (≥ 100 kDa), obtained with basic deacetylation from chitin. Plants treated with Quitomax® comprised treatment Q, while treatment NQ consisted of plants that were not sprayed with chitosan. Pomegranate fruits from each replicate were manually harvested on DOY 286, when commercial maturity (colour and size sufficiently attractive for consumers) was reached and 18 fruits from each replicate were immediately transported under ventilated conditions to the laboratory and stored under controlled conditions (5 °C and 90% relative humidity, RH) for less than a week, until analysis.

The design of this experiment was completely randomized with four replications. Three adjacent eleven tree rows were used per each replication. The inner plants of the central row of each replicate were used for measurements, whereas the other plants served as border plants.

### 2.2. Measurements

#### 2.2.1. Weather, plant water status, yield, fruit cracking or splitting and sunburn

Wind speed 2 m above the soil surface, rainfall, solar radiation, air temperature and air relative humidity data were collected from an automatic weather station placed near the experimental plot. Daily values of ETo were calculated using the Penman-Monteith equation (Allen et al., 1998) and mean daily air vapour pressure deficit (VPDm) was calculated according to Allen et al. (1998).

Stem water potential ($\Psi_{stem}$, MPa) was measured at midday (12 h solar time), using a pressure chamber (PMS 600-EXP, PMS Instruments Company, Albany, USA), in two fully expanded leaves from the south-facing side and middle third of the tree of four plants per treatment, which were enclosed in a small black plastic bag and covered with aluminium foil for at least 2 h before the measurements.

In order to estimate the marketable yield (kg $tree^{-1}$), the total number of harvested fruits from each replicate were counted, along with the number of fruits rejected because of sunburn, cracking and/or splitting disorder, the mean fruit weight of the marketable fruit yield was determined according to the weight and number of healthy fruits per box in two randomly selected boxes per replicate.

#### 2.2.2. Morphological fruit characteristics, arils and peel colour

A digital calliper was used to measure the equatorial diameter of the fruit (mm), which were then emptied and the arils were weighed with a precision balance in order to calculate the arils percentage content.

Pomegranate peel colour was estimated with a Minolta CR 2000 colorimeter (Osaka, Japan), measuring the colour at four equidistant points of the equatorial region of each individual fruit. Arils in each fruit were spread on a white plate and their colour was assessed in ten different places of the plate, expressing the results in the CIE$L^*a^*b^*$ system. The mean values for lightness ($L^*$), green-red ($a^*$), and blue-yellow ($b^*$) coordinates for each fruit were calculated. The objective colour was calculated as chromaticity, colour saturation or chroma ($C^* = (a^{*2} + b^{*2})^{1/2}$) and hue angle or tone ($H^o$ = arctan ($b^*/a^*$)) (Galindo et al., 2015).

#### 2.2.3. Fruit chemical characteristics

Pomegranates were squeezed with a manual squeezer to obtain the juice in order to analyze different chemical parameters. An acid-based potentiometer (877 Titrino plus; Metrohm ion analyses CH9101, Herisau, Switzerland) was used to measure the titratable acidity (Galindo et al., 2015) and a digital refractometer Atago (model N-20; Atago, Bellevue, WA) to measure the total soluble solids (ºBrix). The maturity index was calculated as the ratio between both parameters.

Organic acids and sugars (citric acid (CA), succinic acid (SA), glucose (Glu) and fructose (Fru), g 100 m $L^{-1}$) were quantified according to Melgarejo-Sánchez et al. (2015). For this, 20 mL of juice obtained by squeezing the arils was centrifuged at 15,000 × g for 20 min (Sigma 3–18 K, Osterode & Harz, Germany). Then, 1 mL of supernatant was filtered through a 0.45 µm cellulose nitrate membrane filter and the samples (10 µL) were injected onto a heated (30 °C) Supelcogel TM C-

610H column (30 cm × 7.8 mm i.d., Supelco, Bellefonte, PA, USA) protected with a Supelcogel C610H guard column (5 cm × 4.6 mm, Supelco, Inc.). The HPLC system used was a Hewlett-Packard 1100 series model (Wilmington Del., USA) with autosampler and UV detector, set at 210 nm, coupled to a refractive index detector (HP 1100, G1362 A). The elution system consisted of 0.1% phosphoric acid at a flow rate of 0.5 mL/min. Standard curves of pure organic acids and sugars were used for the quantification. Sugar and organic acid standards were supplied by Supelco analysis (Bellefonte, PA, USA).

The total phenol content (TPC, mg GAE 100 $g^{-1}$) of pomegranate fruits was estimated using the Foling-Ciocalteu reagent following the recommendations of Singleton et al. (1999).

A methanol extract of each sample was prepared in order to analyze the antioxidant activity (AA) by mixing 1 mL juice with 10 mL of MeOH/water (80:20, v/v) + 1% HCl, before sonicating at 20 °C for 15 min and leaving for 24 h at 4 °C. Then the extract was sonicated again for 15 min, and centrifuged at 15,000 x g for 10 min. The $ABTS^+$ [2,2-azinobis-(3-ethylbenzothiazoline- 6-sulfonic acid)] radical cation method was used according to Re et al. (1999). Briefly, 10 µL of the supernatant were mixed with 990 µL of $ABTS^+$ and after allowing the reaction to proceed for 10 min, the absorbance was measured at 734 µm. The absorbance was measured by UV–vis Uvikon XS spectrophotometer (Bio-Tek Instruments, Saint Quentin Yvelines, France). The calibration curves, in the range 0.01–5.00 mmol Trolox $L^{-1}$ were used for the quantification of antioxidant activity (mmol Trolox $kg^{-1}$ dw) and showed good linearity ($r^2 \geq 0.998$).

### 2.3. Statistical analysis

The statistical analysis was a two-way ANOVA considering two independent variables or factors (factor A: irrigation and factor B: chitosan application), each one having two different levels (FI and WS for irrigation factor and NQ and Q for chitosan factor) and the software utilized was SPSS (2012). Mean values were compared by Tukey's multiple range test at $p < 0.05$. $\Psi_{stem}$ values for each replicate were averaged before the mean and the standard error of each treatment were calculated. AW, SPI and SUI percentage values were arc-sin-transformed before statistical analysis because they were not normally distributed.

## 3. Results

Throughout the experimental period, VPDm ranged from 0.23 to 2.01 kPa and ETo amounted to 244 mm. Moreover, average daily maximum and minimum air temperatures were 30 and 18 °C, respectively. Total rainfall amounted to 51 mm, which fell mainly on DOY 241 (21 mm), 242 (7 mm), 245 (18 mm) and 250 (4 mm) (Fig. 1).

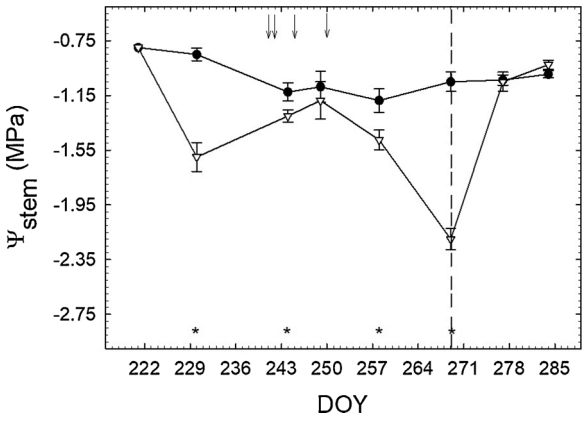


**Fig. 2.** Midday stem water potential ($\Psi_{stem}$) values for pomegranate trees in FI (closed circles) and WS (open triangles) treatments during the experimental period. Vertical bars on data points are ± s.e. of the mean (not shown when smaller than the symbols). Vertical medium-medium line indicates the end of the period for which irrigation was withheld. Arrows indicate daily rainfall events.

Similar $\Psi_{stem}$ values were found in treated (Q) and non-treated (NQ) plants under FI and WS conditions (data not shown). Nevertheless, $\Psi_{stem}$ values in FI and WS plants behaved differently, remaining high and almost constant (average -1.02 MPa) in FI plants, and gradually decreasing in WS plants to reach minimum values of −1.60 MPa on DOY 230. These minimum $\Psi_{stem}$ values in WS plants increased up to reach values similar to those of FI plants on DOY 249, when it rained. After this day, as a result of the water withholding effect, $\Psi_{stem}$ values in WS plants decreased once again, reaching minimum values of -2.20 MPa at the end of the water withholding period (DOY 269). When irrigation was restarted in WS plants, their $\Psi_{stem}$ value rapidly recovered (Fig. 2).

The total yield was lower in WS than in FI trees because of the smaller size of the WS fruit (Table 1). In addition, WS fruits showed a higher incidence of fruit cracking and/or splitting than FI fruits, which increased the difference between the marketable yield values of FI and WS (Table 1). Chitosan did not affect total yield, but induced smaller fruits. However, fruits from chitosan sprayed trees showed a lower incidence of sunburn and cracking and splitting, so that the marketable yield of treated plants was significantly higher than that observed in non-treated plants (Table 1). As regards the interaction between irrigation and chitosan spraying, it is important to note that the chitosan spraying effect on fruit cracking and/or splitting incidence was only

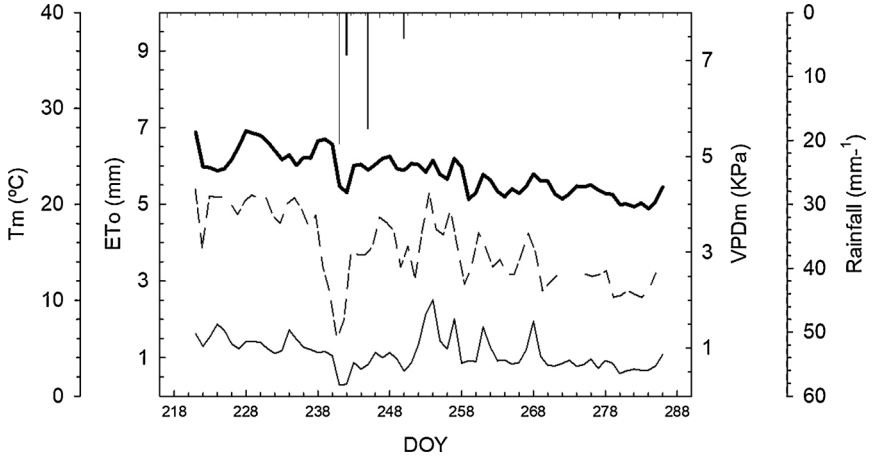


**Fig. 1.** Daily crop reference evapotranspiration (ETo, medium-medium line), daily mean air temperature (Tm, solid line), mean daily air vapour pressure deficit (VPDm, thin line) and daily rainfall (vertical bars) during the experimental period.

**Table 1**
Effect of irrigation and chitosan treatments on pomegranate total yield (TY, kg tree$^{-1}$), marketable yield (MY, kg tree$^{-1}$), average fruit weight (FW, g), fruit equatorial diameter (ED, mm), arils weight (AW, %), and fruit peel physiopathies incidence (cracking and/or splitting (SPI) and sunburn (SUI), %). FI = full irrigation, NQ = fruits non sprayed with chitosan, Q = chitosan sprayed fruits, WS = water stress. Means within a column for each simple factor or the interaction that do not have a common letter are significantly different at $P \leq 0.05$ (*) or $P \leq 0.001$ (***). n.s. = not significant.

| Treatment | TY | MY | FW | ED | AW | SPI | SUI |
|---|---|---|---|---|---|---|---|
| ANOVA | | | | | | | |
| Irrigation | *** | *** | *** | *** | n.s. | *** | n.s. |
| Chitosan | n.s. | *** | *** | n.s. | n.s. | *** | * |
| Irrigation | | | | | | | |
| FI | 60.26a | 49.97a | 506.52a | 98.57a | 52.49 | 3.17b | 14.79 |
| WS | 43.52b | 29.20b | 437.38b | 93.41b | 51.91 | 15.12a | 18.45 |
| Chitosan | | | | | | | |
| NQ | 51.90 | 35.43b | 485.67a | 96.85 | 52.18 | 11.63a | 21.39a |
| Q | 51.88 | 43.74a | 453.66b | 94.82 | 52.24 | 6.54b | 10.25b |
| Tukey`s multiple range test | | | | | | | |
| Irrigation x Chitosan | *. | *** | *** | *** | n.s. | *** | * |
| FINQ | 58.33a | 44.72b | 512.88a | 99.09a | 50.43 | 4.36c | 18.97a |
| FIQ | 62.18a | 55.22a | 498.05b | 97.86a | 53.89 | 1.98c | 9.21b |
| WSNQ | 45.46b | 26.13c | 458.47ab | 94.62ab | 53.94 | 18.90a | 23.82a |
| WSQ | 41.57b | 32.27c | 409.26b | 91.79b | 50.58 | 11.09b | 11.29b |

significant under WS conditions, whereas the effect on fruit sunburn was significant under both FI and WS conditions (Table 1). In this sense, the effect of chitosan spraying on marketable yield was only significant in FI trees.

The effects of irrigation water withholding and chitosan spraying on pomegranate peel colour were characterized by an increase in $C^*$ values and a decrease in $b^*$ and $H^o$ values, respectively (Table 2). The interaction between both factors was significant only when peel $b^*$ and $C^*$ values were considered. In this sense, the effect of chitosan on $b^*$ values was significant only in FI fruits, while only peel $C^*$ values in fruits from the FINQ and WSQ treatments differed significantly, the FINQ values being significantly higher (Table 2).

The effect of water withholding and chitosan spraying on pomegranate arils colour differed from that observed for pomegranate peel (Table 3). Irrigation water withholding did not affect aril the colour characteristics, whereas chitosan decreased $a^*$ and $C^*$ aril values and increased $H^o$ aril values. The interaction between both factors showed

**Table 2**
Effect of irrigation and chitosan treatments on pomegranate peel lightness (CIE $L^*$), red/greenness (CIE $a^*$), yellow/blueness (CIE $b^*$), chroma ($C^*$) and hue angle ($H^o$) values. FI = full irrigation, NQ = fruits non sprayed with chitosan, Q = chitosan sprayed fruits, WS = water stress. Means within a column for each simple factor or the interaction that do not have a common letter are significantly different at $P \leq 0.05$ (*) or $P \leq 0.001$ (***). n.s. = not significant.

| Treatment | $L^*$ | $a^*$ | $b^*$ | $C^*$ | $H^o$ |
|---|---|---|---|---|---|
| ANOVA | | | | | |
| Irrigation | n.s. | n.s. | n.s. | *** | n.s. |
| Chitosan | n.s. | n.s. | *** | n.s. | *** |
| Irrigation | | | | | |
| FI | 60.36 | 24.37 | 28.96 | 37.51b | 51.50 |
| WS | 59.17 | 23.07 | 28.33 | 38.88a | 50.53 |
| Chitosan | | | | | |
| NQ | 59.42 | 23.18 | 29.31a | 38.36 | 52.26a |
| Q | 60.23 | 24.44 | 27.75b | 37.97 | 49.36b |
| Tukey`s multiple range test | | | | | |
| Irrigation x Chitosan | n.s. | n.s. | * | * | n.s. |
| FINQ | 60.33 | 23.48 | 29.94a | 39.08a | 52.41 |
| FIQ | 60.39 | 25.54 | 27.65b | 38.62ab | 48.03 |
| WSNQ | 58.49 | 22.87 | 28.68ab | 37.65ab | 52.11 |
| WSQ | 60.07 | 23.34 | 27.86b | 37.32b | 50.68 |

**Table 3**
Effect of irrigation and chitosan treatments on pomegranate aril lightness (CIE $L^*$), red/greenness (CIE $a^*$). yellow/blueness (CIE $b^*$), chroma ($C^*$) and hue angle ($H^o$) values. FI = full irrigation, NQ = fruits non sprayed with chitosan, Q = chitosan sprayed fruits, WS = water stress. Means within a column for each simple factor or the interaction that do not have a common letter are significantly different at $P \leq 0.05$ (*) or $P \leq 0.001$ (***). n.s. = not significant.

| Treatment | $L^*$ | $a^*$ | $b^*$ | $C^*$ | $H^o$ |
|---|---|---|---|---|---|
| ANOVA test | | | | | |
| Irrigation | n.s. | n.s. | n.s. | n.s. | n.s. |
| Chitosan | n.s. | *** | n.s. | *** | *** |
| Irrigation | | | | | |
| FI | 34.60 | 17.37 | 8.88 | 19.01 | 27.61 |
| WS | 34.25 | 17.13 | 7.85 | 19.74 | 24.75 |
| Chitosan | | | | | |
| NQ | 33.22 | 18.40a | 8.29 | 20.33a | 24.35b |
| Q | 36.02 | 15.71b | 8.46 | 18.10b | 28.64a |
| Tukey`s multiple range test | | | | | |
| Irrigation x Chitosan | * | * | n.s. | n.s. | * |
| FINQ | 31.95b | 19.06a | 8.54 | 20.98 | 24.03b |
| FIQ | 38.12a | 15.12b | 9.33 | 18.09 | 32.39a |
| WSNQ | 34.48ab | 17.74ab | 8.04 | 19.69 | 24.66b |
| WSQ | 33.93ab | 16.30ab | 7.59 | 18.12 | 24.87b |

that the chitosan effect on $L^*$, $a^*$ and $H^o$ values was significant only in FI arils. In this sense, FIQ arils showed higher $L^*$ and $H^o$ values, and lower $a^*$ values than those in FINQ arils (Table 3).

The effects of irrigation water withholding and chitosan spraying on the chemical characteristics of pomegranate fruit were very scarce (Table 4). The chitosan effect was significant only on the AA-ABTS$^+$ values, which increased in treated (Q) fruits. The effect of withholding irrigation water increased total soluble solids and titratable acidity and decreased the succinic acid content. In this respect, the interaction between these two factors was significant not only for total soluble solids, titratable acidity, succinic acid and antioxidant activity levels but also for the citric acid content (Table 4). The decrease in succinic acid through a water withholding effect was significant only when chitosan sprayed fruits were considered (FIQ and WSQ); chitosan induced a decrease in the citric acid content only in WSQ fruits and antioxidant activity increased through a chitosan effect in FIQ fruits (Table 4). Despite irrigation water withholding effect increased fruit titratable acidity, the interaction with chitosan spraying factor induced similar values in FIQ and WSNQ fruits, whereas these values in WSQ fruits were higher than those in FIQ and FINQ fruits. Even though the interaction between irrigation water withholding and chitosan spraying was significant, the chitosan effect was not significant in fruits from both plant water status considered (FI and WS) (Table 4).

## 4. Discussion

The high $\Psi_{stem}$ values in FI plants throughout the experimental period (Fig. 2) suggested that control plants were under non-limiting soil water conditions (Galindo et al., 2013, 2014a). In contrast, at the end of the water withholding period, the low $\Psi_{stem}$ values indicated that WS plants were experiencing a relatively strong water stress situation (Galindo et al., 2013) (Fig. 2), even though these minimum $\Psi_{stem}$ values were not reached progressively due to the rain that fell during the experimental period. The absence of a chitosan effect on $\Psi_{stem}$ values in FI and WS at any time point examined (data not shown), agrees with the results of Yang et al. (2009), who concluded that spraying chitosan, at any of the concentrations tested, had no effect on apple predawn leaf water potential changes induced by drought.

As expected, WS fruits were smaller than those in FI plants because of pomegranate fruit growth and fruit ripening are critical phenological periods from the yield point of view since water deficit affects total yield and fruit size (Mellisho et al., 2012; Laribi et al., 2013; Galindo

**Table 4**
Effect of irrigation and chitosan treatments on pomegranate fruit total soluble solids (TSS, ºBrix). titratable acidity (TA, g citric acid $L^{-1}$). maturity index (MI, TSS/TA), citric acid (CA, g 100 m $L^{-1}$), succinic acid (SA, g 100 m $L^{-1}$), glucose (Glu, g 100 m $L^{-1}$), fructose (Fru, g 100 m $L^{-1}$), total polyphenols content (TPC, mg GAE 100 $g^{-1}$) and total antioxidant activity measured according to $ABTS^+$ assay (AA-$ABTS^+$, mmol Trolox $kg^{-1}$ dw) content. FI = full irrigation, NQ = fruits non sprayed with chitosan, Q = chitosan sprayed fruits, WS = water stress. Means within a column for each simple factor or the interaction that do not have a common letter are significantly different at $P \leq 0.05$ (*), $P \leq 0.01$ (**) or $P \leq 0.001$ (***). n.s. = not significant.

| Treatment | TSS | TA | MI | CA | SA | Glu | Fru | TPC | AA-$ABTS^+$ |
|---|---|---|---|---|---|---|---|---|---|
| ANOVA test | | | | | | | | | |
| Irrigation | *** | *** | n.s. | n.s. | *** | n.s. | n.s. | n.s. | n.s. |
| Chitosan | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | *** |
| Irrigation | | | | | | | | | |
| FI | 15.64b | 2.35b | 66.62 | 0.46 | 0.27a | 3.20 | 2.52 | 425.90 | 17.84 |
| WS | 17.46a | 2.63a | 66.70 | 0.38 | 0.20b | 2.01 | 3.85 | 461.12 | 18.07 |
| Chitosan | | | | | | | | | |
| NQ | 16.66 | 2.48 | 67.37 | 0.45 | 0.24 | 2.04 | 2.50 | 482.74 | 14.44b |
| Q | 16.40 | 2.51 | 65.70 | 0.38 | 0.23 | 3.36 | 4.08 | 391.19 | 22.65a |
| Tukey`s multiple range test | | | | | | | | | |
| Irrigation x Chitosan | *** | * | n.s. | * | ** | n.s. | n.s. | n.s. | * |
| FINQ | 15.52b | 2.33c | 66.70 | 0.45a | 0.25ab | 1.09 | 1.41 | 433.89 | 13.82b |
| FIQ | 15.78b | 2.38bc | 66.49 | 0.48a | 0.29a | 3.24 | 3.98 | 415.25 | 23.74a |
| WSNQ | 17.80a | 2.63ab | 68.04 | 0.46a | 0.22ab | 2.99 | 3.60 | 531.60 | 15.05b |
| WSQ | 17.01a | 2.64a | 64.91 | 0.27b | 0.17b | 3.48 | 4.19 | 367.13 | 21.56ab |

et al., 2017). Moreover, the fact that withholding irrigation water effect was larger on marketable yield than total yield was due to the high fruit cracking and/or splitting incidence in WS treatment fruits, which is directly linked to the fruit water status at the end of fruit growth and ripening phase. When previously water stressed pomegranate fruits are rehydrated, the increase in turgor pressure is higher in the arils than in the fruit peel, leading to fruit incidence of cracking and/or splitting physiopathies (Galindo et al., 2014b; Rodríguez et al., 2018).

It is well known that chitosan is an ideal fruit preservative coating due to its film-forming and physical and biochemical properties (Park et al., 2002; Romanazzi et al., 2002; Shiri et al., 2013). The plant response to exogenous chitosan application depends not only on its chemical characteristics and the concentration of the chitosan molecules (Lin et al., 2005; Limpanavech et al., 2008; Kananont et al., 2010) but also on the plant material (Ohta et al., 2004) and their developmental stage (Pornpienpakdee et al., 2010). Whatever the case, the semi-permeable layer formed by polysaccharides like chitosan coating modifies the internal atmosphere of the fruit, and due to their hygroscopic properties enable the formation of a water barrier and consequently reduce external water transfer and decrease the rate of respiration, among other effects (Zhang and Quantick, 1997; Zhang et al., 2011). For these reasons, the reduction of fruit cracking and/or splitting incidence in WS fruits as a result of chitosan spraying may have been due to an effective antitranspirant effect of the chitosan coating, which led to a more conservative water use in treated fruits (WSQ) than in non-treated fruits (WSNQ) (Table 1). Hence, when irrigation was resumed, the increase in aril turgor pressure in WSQ fruits was lower than in WSNQ, so that the pressure of the arils on the peel (which favours cracking and/or splitting) was lower in WSQ fruits than in WSNQ fruits. On the other hand, the fact that sunburn incidence decreased in FIQ and WSQ fruits in relation to that observed in FINQ and WSNQ, respectively (Table 1), could be attributed to the characteristics of the chitosan film around the fruit, which would act as a physical barrier against overall heat stress, reflecting harmful UV and IR radiation away from plants and, consequently, preserving fruit peel from sunburn.

The effect of water withholding and chitosan spraying on pomegranate peel and arils colour were low (Tables 2 and 3). In this sense, the first factor did not affect arils colour, but induced an increase in peel brightness, whereas the second factor induced less reddish and duller arils and a less yellowish and more reddish peel. Furthermore, the interaction between chitosan spraying and water withholding treatments led to the peel from FIQ fruits being less yellow than in FINQ, while the peel in FINQ fruits was brighter than that of WSQ fruits (Table 2). This interaction also induced FI arils to be lighter and less red through a chitosan effect (Table 3). With regard to the effect of the first factor, in a comparison of different withholding irrigation water treatments during fruit growth and late ripening Galindo et al. (2017) described a significant effect of water stress on pomegranate peel colour because *L*, b* and H°* values of the peel tended to decrease with accumulated water stress effect. These different behaviours could be attributed to the fact that the response to water stress of fruit of a specific cultivar depends not only on the water stress level, but also of the phenological phase at which it takes place, its duration and its development rate (Galindo et al., 2018; Rodríguez et al., 2018). As regards the second factor, Munhuweyi et al. (2017) observed a significant effect of the pomegranate arils cultivar in response to chitosan treatment during post-harvest cold storage, because Wonderful cv. arils colour did not change, whereas Herskawitz cv. arils showed a less red and yellower colour, especially at the beginning of the storage period.

The increase in total soluble solids as a result of irrigation water withholding is in line with the results presented by Laribi et al. (2013) and can be attributed to the active hydrolysis of starch to sugars, whereas the increase in titratable acidity could be due to an increase in the conversion of soluble sugars into organic acids (Munhuweyi et al., 2017). The increases in total soluble solids and titratable acidity in pomegranate fruit can be considered as a positive characteristic from a consumer acceptance point of view (Martínez-Romero et al., 2013).

The increase in AA-$ABTS^+$ values through a chitosan effect agrees with the observations of Candir et al. (2018), who showed an increase in antioxidant capacity for pomegranate fruits treated with chitosan during cold storage. Similarly, Zahran et al. (2015) described an increase in antioxidant activity for pomegranate arils treated with irradiated chitosan during cold storage. However, the fact that chitosan increased AA-$ABTS^+$ values and did not affect TPC levels (Table 4) is not in line with the results of Borochov-Neori et al. (2009), who indicated that phenolic compounds are the main contributors to the antioxidant activity in pomegranate juice. In this respect, to fully understand the antioxidant capacity and bioactivity of pomegranate fruits treated with chitosan, it is important to take into consideration that antioxidant activity of pomegranate arils is due to anthocyanin content, ascorbic acid and phenolic acids, either or in combination, are responsible for antioxidant activity of pomegranate arils (Sarkhosh et al., 2009). So, complementary analysis of fatty acids (Alcaraz-Mármol et al., 2015), organic acids such as gallagic acid, ellagic acid and gallic acids (Kulkarni et al., 2004; Calín-Sánchez et al., 2013), punicalin and punicalagin (Kulkarni et al., 2004) should be conducted.

## 5. Conclusions

Overall, the above-mentioned observations suggest that preharvest pomegranate fruit spraying with chitosan had little effect on peel and aril colour and the chemical characteristics of the arils. Some of these effects were negative such as the reduction in fruit weight and the less reddish and duller appearance of the arils. However, these negative aspects could be regarded as being compensated by other very important positive effects, such as the increase in the antioxidant activity and the significant reduction in fruit peel physiopathies, which would considerably improve the returns of pomegranate growers. In addition, it is important to consider that chitosan spraying constitutes a suitable and reliable cultural practice because chitosan is considered a food additive by the USFDA (United States Food and Drug Administration) and it has passed all the toxicological tests to which it has been submitted (Hirano et al., 1990).

## Acknowledgements

We are grateful to the Ministerio de Economía y Competitividad (MINECO) for funding this research through the project AGL2016-75794-C4-1-R. IG is a predoctoral student at the Miguel Hernández University. AG acknowledge the financial support received from the Ramón Areces Foundation and VI PPIT-US. JC-G is beneficiary of a Juan de la Cierva post-doctoral fellowship. This work is the result of the internships of DM (20127/IV/17) funded by the Seneca Foundation-Agency for Science and Technology in the Region of Murcia under the Jiménez de la Espada Program for Mobility, Cooperation and Internationalization.

## References

Adhami, V.M., Mukhtar, H., 2006. Polyphenols from green tea and pomegranate for prevention of prostate cancer. Free Radic. Res. 40, 1095–1104.
Alcaraz-Mármol, F., Nuncio-Jáuregui, N., Calín-Sánchez, A., Carbonell-Barrachina, A.A., Martínez, J.J., Hernández, F., 2015. Determination of fatty acid composition in arils of 20 pomegranates cultivars grown in Spain. Sci. Hortic. 197, 712–718.
Allen, R.G., Pereira, L.S., Raes, D., Smith, M., 1998. Crop Evapotranspiration. Guidelines for Computing Crop Water Requirements. FAO Irrigation and Drainage Paper 56. FAO, Roma.
Aseri, G.K., Jain, N., Panwar, J., Rao, A.V., Meghwal, P.R., 2008. Biofertilizers improve plant growth fruit yield, nutrition, metabolism and rhizosphere enzyme activities of pomegranate (*Punica granatum* L.) in Indian Thar Desert. Sci. Hortic. 117, 130–135.
Aviram, M., Coleman, R., Dreher, M., Reddy, M.K., Ferreira, D., Rosenblat, M., 2008. Pomegranate phenolics from the peels, arils, and flowers are antiatherogenic: studies in vivo in atherosclerotic apolipoprotein E-deficient (E 0) mice and in vitro in cultured macrophages and lipoproteins. J. Agric. Food Chem. 56, 1148–1157.
Badawy, M.E.I., Rabea, E.I., 2012. Characterization and antimicrobial activity of water-soluble N-(4-carboxybutyroyl) chitosans against some plant pathogenic bacteria and fungi. Carbohydr. Polym. 87, 250–256.
Bittelli, M., Flury, M., Campbell, G.S., Nichols, E.J., 2001. Reduction of transpiration through foliar application of chitosan. Agric. For. Meteorol. 107, 167–175.
Blumenfeld, A., Shaya, F., Hillel, R., 2000. Cultivation of pomegranate. Options Méditerranées 42, 143–147.
Borochov-Neori, H., Judeinstein, S., Tripler, E., Harari, M., Greenberg, A., Shomer, I., Holland, D., 2009. Seasonal and cultivar variations in antioxidant and sensory quality of pomegranate (*Punica granatum* L.) fruit. J. Food Anal. 22, 189–195.
Calín-Sánchez, A., Figiel, A., Hernández, F., Melgarejo, P., Lech, K., Carbonell-Barrachina, A.A., 2013. Chemical composition, antioxidant capacity, and sensory quality of pomegranate (*Punica granatum* L.) arils and rind as affected by drying method. Food Bioprocess Technol. 6, 1644–1654.
Candir, E., Ozdemir, A.E., Aksoy, M.C., 2018. Effects of chitosan coating and modified atmosphere packaging on postharvest quality and bioactive compounds of pomegranate fruit cv. 'Hicaznar'. Sci. Hortic. 235, 235–243.
Chibu, H., Shibayama, H., 2001. Effects of chitosan application on the growth of several crops. Kodansha Scientific LTD (Ed.) Chitin and Chitosan-chitin and Chitosan in Life Science 235–237 Tokyo.
Falcón-Rodríguez, A.B., Wegria, G., Cabrera, J.C., 2012. Exploiting plant innate immunity to protect crops against biotic stress: chitosaccharides as natural and suitable candidates for this purpose. In: Bandani, A.R. (Ed.), New Perspectives in Plant Protection. INTECH, Croatia, pp. 139–166.
Galindo, A., Rodríguez, P., Mellisho, C.D., Torrecillas, E., Moriana, A., Cruz, Z.N., Conejero, W., Moreno, F., Torrecillas, A., 2013. Assessment of discretely measured indicators and maximum dailytrunk shrinkage for detecting water stress in pomegranate trees. Agric. For. Meteorol. 180, 58–65.
Galindo, A., Calín-Sánchez, A., Collado-González, J., Ondoño, S., Hernández, F., Torrecillas, A., Carbonell-Barrachina, A.A., 2014a. Phytochemical and quality attributes of pomegranate fruits for juice consumption as affected by ripening stage and deficit irrigation. J. Sci. Food Agric. 94, 2259–2265.
Galindo, A., Rodríguez, P., Collado-González, J., Cruz, Z.N., Torrecillas, E., Ondoño, S., Corell, M., Moriana, A., Torrecillas, A., 2014b. Rainfall intensifies fruit peel cracking in water stressed pomegranate trees. Agric. For. Meteorol. 194, 29–35.
Galindo, A., Noguera-Artiaga, L., Cruz, Z.N., Burló, F., Hernández, F., Torrecillas, A., Carbonell-Barrachina, A., 2015. Sensory and physico-chemical quality attributes of jujube fruits as affected by crop load. LWT 63, 899–905.
Galindo, A., Calín-Sánchez, A., Griñán, I., Rodríguez, P., Cruz, Z.N., Girón, I.F., Corell, M., Martínez-Font, R., Moriana, A., Carbonell-Barrachina, A.A., Torrecillas, A., Hernández, F., 2017. Water stress at the end of the pomegranate fruit ripening stage produces earlier harvest and improves fruit quality. Sci. Hortic. 226, 68–74.
Galindo, A., Collado-González, J., Griñán, I., Corell, M., Centeno, A., Martín-Palomo, M.J., Girón, I.F., Rodríguez, P., Cruz, Z.N., Memmi, H., Carbonell-Barrachina, A.A., Hernández, F., Torrecillas, A., Moriana, A., López-Pérez, D., 2018. Deficit irrigation and emerging fruit crops as a strategy to save water in Mediterranean semiarid agrosystems. Agric. Water Manag. 202, 311–324.
Gil, M.I., Tomas-Barberán, F.A., Hess-Pierce, B., Holcroft, D.M., Kader, A.A., 2000. Antioxidant activity of pomegranate juice and its relationship with phenolic composition and processing. J. Agric. Food Chem. 48, 4581–4589.
Hirano, S., Itakura, C., Seino, H., Akiyama, Y., Nonata, I., Kanbara, N., Kawakami, T., 1990. Chitosan as an ingredient for domestic animal feeds. J. Agric. Food Chem. 38, 1214–1217.
Kananont, N., Pichyangkura, R., Chanprame, S., Chadchawan, S., Limpanavech, P., 2010. Chitosan specificity for the *in vitro* seed germination of two *Dendrobium* orchids (Asparagales: Orchidaceae). Sci. Hortic. 124, 239–247.
Katiyar, D., Hemantaranjan, A., Singh, B., Bhanu, A.N., 2014. A future perspective in crop protection: chitosan and its oligosaccharides. Adv. Plants Agric. Res. 1, 00006.
Kulkarni, A.P., Aradhya, S.M., Divakar, S., 2004. Isolation and identification of a radical scavenging antioxidant punicalagin from pith and carpellary membrane of pomegranate fruit. Food Chem. 87, 551–557.
Laribi, A.I., Palou, L., Intrigliolo, D.S., Nortes, P.A., Rojas-Argudo, C., Taberner, V., Bartual, J., Pérez-Gago, M.B., 2013. Effect of sustained and regulated deficit irrigation on fruit quality of pomegranate cv. 'Mollar de Elche' at harvest and during cold storage. Agric. Water Manag. 125, 61–70.
Limpanavech, P., Chaiyasuta, S., Vongpromek, R., Pichyangkura, R., Khunwasi, C., Chadchawan, S., Lotrakul, P., Bunjongrat, R., Chaidee, A., Bangyeekhun, T., 2008. Chitosan effects on floral production, gene expression, and anatomical changes in the *Dendrobium* orchid. Sci. Hortic. 116, 65–72.
Lin, W., Hu, X., Zhang, W., Rogers, W.J., Cai, W., 2005. Hydrogen peroxide mediates defence responses induced by chitosans of different molecular weights in rice. J. Plant Physiol. 162, 937–944.
Malik, A., Mukhtar, H., 2006. Prostate cancer prevention through pomegranate fruit. Cell Cycle 5, 371–373.
Malik, A., Afaq, F., Sarfaraz, S., Adhami, V.M., Syed, D.N., Mukhtar, H., 2005. Pomegranate fruit juice for chemoprevention and chemotherapy of prostate cancer. Proc. Natl. Acad. Sci. U. S. A. 102, 14813–14818.
Martínez-Romero, D., Castillo, S., Guillén, S., Díaz-Mula, H.M., Zapata, P.J., Valero, D., Serrano, M., 2013. *Aloe vera* gel coating maintains quality and safety of ready-to-eat pomegranate arils. Postharvest Biol. Technol. 86, 107–112.
Melgarejo, P., Martínez, J.J., Hernández, F., Martínez-Font, R., Barrows, P., Erez, A., 2004. Kaolin treatment to reduce pomegranate sunburn. Sci. Hortic. 100, 349–353.
Melgarejo-Sánchez, P., Martínez, J.J., Legua, P., Martínez, R., Hernández, F., Melgarejo, P., 2015. Quality, antioxidant activity and total phenols of six Spanish pomegranates clones. Sci. Hortic. 182, 65–72.
Mellisho, C.D., Egea, I., Galindo, A., Rodríguez, P., Rodríguez, J., Conejero, W., Rodríguez, P., Rodríguez, J., Romojaro, F., Torrecillas, A., 2012. Pomegranate (*Punica granatum* L.) fruit response to different deficit irrigation conditions. Agric. Water Manag. 114, 30–36.
Munhuweyi, K., Lennox, C.L., Meitz-Hopkins, J.C., Caleb, O.J., Sigged, G.O., Opara, U.L., 2017. Investigating the effects of crab shell chitosan on fungal mycelial growth and postharvest quality attributes of pomegranate whole fruit and arils. Sci. Hortic. 220, 78–89.
Nge, K.L., New, N., Chandrkrachang, S., Stevens, W.F., 2006. Chitosan as a growth stimulator in orchid tissue culture. Plant Sci. 170, 1185–1190.
Ohta, K., Morishita, S., Suda, K., Kobayashi, N., Hosoki, T., 2004. Effect of chitosan soil mixture treatment in the seedling stage and flowering of several ornamental plants. J. Jpn. Soc. Hortic. Sci. 73, 66–68.
Palma-Guerrero, J., Jansson, H.B., Salinas, J., Lopez-Llorca, L.V., 2008. Effect of chitosan on hyphal growth and spore germination of plant pathogenic and biocontrol fungi. J. Appl. Microbiol. 104, 541–553.
Park, S.Y., Marsh, K.S., Rhim, J.W., 2002. Characteristics of different molecular weight chitosan films affected by the type of organic solvents. J. Food Sci. 67, 194–197.
Pichyangkura, R., Chadchawan, S., 2015. Biostimulant activity of chitosan in horticulture. Sci. Hortic. 196, 49–65.
Pornpienpakdee, P., Singhasurasak, R., Chaiyasap, P., Pichyangkura, R., Bunjongrat, R., Chadchawan, S., Limpanavech, P., 2010. Improving the micropropagation efficiency of hybrid *Dendrobium* orchids with chitosan. Sci. Hortic. 124, 490–499.
Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Rice-Evans, C., 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic. Biol. Med. 26, 1231–1237.
Rinaudo, M., 2006. Chitin and chitosan: properties and applications. Prog. Polym. Sci. 31, 603–632.
Rodríguez, P., Mellisho, C.D., Conejero, W., Cruz, Z.N., Ortuño, M.F., Galindo, A., Torrecillas, A., 2012. Plant water relations of leaves of pomegranate trees under

different irrigation conditions. Environ. Exp. Bot. 77, 19–24.
Rodríguez, P., Galindo, A., Collado-González, J., Medina, S., Corell, M., Memmi, H., Girón, I.F., Centeno, A., Martín-Palomo, M.J., Cruz, Z.N., Carbonell-Barrachina, A.A., Hernandez, F., Torrecillas, A., Moriana, A., Pérez-López, D., 2018. Fruit response to water-scarcity scenarios. Water relations and biochemical changes. In: García-Tejero, I.F., Durán-Zuazo, V.H. (Eds.), Water Scarcity and Sustainable Agriculture in Semiarid Environment: Tools, Strategies and Challenges for Woody Crops. Elsevier - Academic Press, pp. 349–375.
Romanazzi, G., Nigro, F., Ippolito, A., DiVenere, D., Salerno, M., 2002. Effects of pre- and postharvest chitosan treatments to control storage grey mold of table grapes. J. Food Sci. 67, 1862–1867.
Sarkhosh, A., Zamani, Z., Fatahi, R., Sayyari, M., 2009. Antioxidant activity, total phenols, anthocyanin, ascorbic acid content and woody portion index (wpi) in Iranian soft-seed pomegranate fruits. Food 3, 68–79.
Seeram, N.P., Henning, S.M., Zhang, Y., Suchard, M., Li, Z., Heber, D., 2006. Pomegranate juice ellagitannin metabolites are present in human plasma and some persist in urine for up to 48 hours. J. Nutr. 136, 2481–2485.
Shiri, M.A., Bakhshi, D., Ghasemnezhad, M., Dadi, M., Papachatzis, A., Kalorizou, H., 2013. Chitosan coating improves the shelf life and postharvest quality of table grape (*Vitis vinifera*) cultivar Shahroudi. Turk. J. Agric. For. 37, 148–156.
Singleton, V.L., Orthofer, R., Lamuela-Raventos, R.M., 1999. Analysis of total phenols and other oxidation substrates andantioxidants by means of folin-ciocalteu reagent. Methods Enzymol. 299, 152–178.
SPSS, Inc., 2012. SPSS Professional Statistics. Business Intelligence Division, v. 12, Chicago.
Wanichpongpan, P., Suriyachan, K., Chandrkrachang, S., 2001. Effects of chitosan on the growth of Gerbera flower plant (*Gerbera jamesonii*). In: Uragami, T., Kurita, K., Fukamizo, T. (Eds.), Chitin and Chitosan in Life Science, pp. 198–201 Yamaguchi.
Yang, F., Hu, J., Li, J., Wu, X., Qian, Y., 2009. Chitosan enhances leaf membrane stability and antioxidant enzyme activities in apple seedlings under drought stress. Plant Growth Regul. 58, 131–136.
Yazici, K., Kaynak, L., 2009. Effects of air temperature, relative humidity and solar radiation on fruit surface temperatures and sunburn damage in pomegranate (*Punica granatum* L. cv. Hicaznar). Acta Hortic. 818, 181–186.
Younes, I., Rinaudo, M., 2015. Chitin and chitosan preparation from marine sources structure, properties and applications. Mar. Drugs 13, 1133–1174.
Zahran, A.A., Hassanein, R.A., AbdelWahab, A.T., 2015. Effect of chitosan on biochemical composition and antioxidant activity of minimally processed 'Wonderful' pomegranate arils during cold storage. J. Appl. Bot. Food Qual. 88, 241–248.
Zhang, D., Quantick, P.C., 1997. Effects of chitosan coating on enzymatic browning and decay during postharvest storage of litchi (*Litchi chinensis* Sonn.) fruit. Postharvest Biol. Technol. 12, 195–202.
Zhang, H., Zhao, X., Yang, J., Yin, H., Wang, W., Lu, H., Du, Y., 2011. Nitric oxide production and its functional link with OIPK in tobacco defense response elicited by chito oligosaccharide. Plant Cell Rep. 30, 1153–1162.
Zoffoli, J.P., Latorre, B.A., Naranjo, P., 2008. Hairline, a postharvest cracking disorder in table grapes induced by sulfur dioxide. Postharvest Biol. Technol. 47, 90–97.