

Effect of preharvest fruit bagging on fruit quality characteristics and incidence of fruit physiopathies in fully irrigated and water stressed pomegranate trees

Isabel Griñán,^{a,†} Donaldo Morales,^{b,†} Alejandro Galindo,^{c*,†} Arturo Torrecillas,^d David Pérez-López,^e Alfonso Moriana,^f Jacinta Collado-González,^g Ángel A Carbonell-Barrachina^g and Francisca Hernández^{a*}

Abstract

BACKGROUND: This report studied the response of pomegranate fruit under full irrigation (FI) and water stress conditions to bagging with externally glossy, single-layer, cellulosic paper bags, open at the bottom, from the end of fruit thinning to harvest time.

RESULTS: Bagging decreased fruit size and the maturity index, and increased antioxidant activity in FI conditions. Moreover, fruit bagging substantially reduced the incidence of peel sunburn in both irrigation conditions.

CONCLUSION: The delay in fruit growth and ripening as a result of pomegranate fruit bagging is outweighed by the very important commercial benefit in terms of the reduced incidence of peel sunburn and the increase in fruit antioxidant activity.

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Keywords: fruit splitting; fruit sunburn; *Punica granatum*; water deficit

INTRODUCTION

Pomegranate (*Punica granatum* L.) is one of the oldest known edible fruits and, along with the species *Punica protopunica*, it constitutes the Punicaceae family.¹ Pomegranate fruit has been extensively used in medicine by many traditional cultures because it is one of the healthiest fruits in terms of antioxidant activity^{2–4} with high anticarcinogenic compound content,^{5–7} and antiatherosclerotic effects that are able to reduce blood pressure.⁴

Pomegranate is considered a drought-resistant crop due to its efficient stress avoidance and stress-tolerance mechanisms,^{8,9} which permit it to thrive in arid and semiarid areas, even under desert conditions.¹⁰ Nevertheless, despite its toughness, pomegranate grown for commercial production in these conditions requires regular irrigation throughout the season to reach optimal growth, a marketable yield, and acceptable fruit quality,^{11,12} and to reduce the incidence of fruit physiopathies^{13,14} (e.g., fruit cracking and fruit splitting).

To cover fully the demands of modern consumers, in addition to pomegranates' health-related properties, the fruits need to be pesticide-free and excel in terms of attractiveness, mainly regarding size, skin redness, and the absence of physiopathies (e.g. sunburning, fruit cracking, fruit splitting or internal breakdown),

insect attack injuries, or mechanical damage, while maintaining a pleasant taste. To this end, a fruit physical protection technique (preharvest fruit bagging) is sometimes used, alongside other

* Correspondence to: A Galindo, Department of Water Engineering & Management, Faculty of Engineering Technology, University of Twente, P.O. Box 217, 7500 AE, Enschede, The Netherlands, E-mail: a.galindoegea@utwente.nl; or F Hernández, Departamento de 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, E-mail: francisca.hernandez@umh.es

† These authors contributed equally to this work.

a Departamento de 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 Departamento de Fisiología y Bioquímica, Instituto Nacional de Ciencias Agrícolas (INCA), Ctra. de Tapaste, km 3.5, San José de Las Lajas, Mayabeque, Cuba

c Department of Water Engineering & Management, Faculty of Engineering Technology, University of Twente, 7500 AE, Enschede, The Netherlands

d Departamento de Riego, Centro de Edafología y Biología Aplicada del Segura (CSIC), E-30100 Espinardo, Murcia, Spain

farming practices, to improve fruit quality and to protect fruit from pathogens and pests, reducing the presence of pesticide residues. Indeed, this technique is very frequently used in peach, apple, pear, grape, and loquat farming in countries such as Japan, Australia, China, and the USA.^{15,16} Nevertheless, the literature on the beneficial effects of fruit bagging is not unanimous and some contradictory results have been obtained.^{16–18}

To the best of our knowledge, reports on the response of pomegranate fruit to bagging are very scarce, although Yuan *et al.*¹⁹ showed that bagging is an effective cultivation measure to prevent pomegranate fruit cracking. Shlomo¹⁸ recommended the use of bags open at the bottom as a good mechanical protection against important pest infestations. Meena *et al.*²⁰ demonstrated the potency of photoselective netting for improving the agro-economic performance of pomegranate crops, especially in harsh climates and arid zones.

While pomegranate fruit quality parameters have been studied under different water-deficit conditions,^{9,13,21–23} the literature on the effect of bagging on fruit quality is very limited and there are no reports on the interaction of both factors applied simultaneously. The main objective of the current work was therefore to study the interaction between preharvest pomegranate fruit bagging and plant water status on the sensory and quality attributes of the fruit. In addition, the effect of both factors on fruit sunburn and fruit splitting was studied as a complementary objective.

MATERIAL AND METHODS

Plant material, experimental conditions, and treatments

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

Fully irrigated plants (treatment FI) were irrigated during the night to above crop water requirements – 115% evapotranspiration (ET_o) – using a drip irrigation system with one lateral pipe per tree row and four emitters (each delivering 4 L h⁻¹) per plant. Irrigation in water-stressed plants (treatment WS) was withheld for 60 days (from day of the year, DOY, 209 to 269), when evaporative demand is very high and water availability for irrigation is very scarce. To guarantee the recovery of WS plants, re-irrigation was performed at the levels used in FI from DOY 269 to 286 (harvest time). From DOY 209 (the end of fruit thinning) to harvest, pomegranate fruits from both irrigation treatments were submitted to bagging with Pantone® 1205C colored bags (262 mm × 397 mm) open at the bottom, made from externally glossy single-layer cellulosic paper (grade: 50 g m⁻², Bendtsen porosity: 373 mL min⁻¹) and stapled tightly around the fruit peduncle. All fruits from the treatment trees were bagged, and formed treatment B, while treatment NB consisted of fruits that

were not bagged. For prophylactic purposes, plants were sprayed with fungicide (containing 80% sulfur) and insecticide (10% of 4-phenoxyphenyl (RS)-2-(2-pyridyloxy)propyl ether) a week before the bagging practice.

Pomegranate fruits from each treatment were manually harvested on DOY 286 (13 October), when commercial maturity was reached. Twenty fruits from each replicate were immediately transported in ventilated plastic pallet boxes to the laboratory (a 15 min trip) and stored under controlled conditions (5 °C and 90% relative humidity, RH) for less than a week, until analysis.

The experiment had completely randomized design, with four replications, each replication consisting of three adjacent tree rows, each with 11 trees. Measurements were taken on the inner plants of the central row of each replicate, which were very similar in appearance, while the other plants served as border plants.

Measurements

Weather, plant water status, yield, morphology, fruit splitting, sunburn and color

Using an automatic weather station (Adcon Telemetry GmbH, Vienna, Austria), placed near the experimental plot, the following parameters were measured: wind speed 2 m above the soil surface, rainfall, solar radiation, air temperature and air relative humidity. Daily values of crop reference ET_o were calculated using the Penman–Monteith equation.²⁴ Mean daily air vapor pressure deficit (VPD_m) was calculated according to Allen *et al.*²⁴

Midday (12 h solar time) stem water potential (Ψ_{stem}) was measured in two fully expanded leaves from the south-facing side and the middle third of the canopy of four plants per treatment. To allow the water tension in the leaf to come to equilibrium with the water tension in the stem, leaves were enclosed in a small black plastic bag and covered with aluminum foil for at least 2 h before measurements, which were made using a pressure chamber (PMS 600-EXP, PMS Instruments Company, Albany, USA). Detached leaves were placed into the pressure chamber (lined with damp filter paper) and slowly pressurized (0.025 MPa s⁻¹) until the balance pressure was reached (when the leaf sap appeared through the cut petiole protruding from the chamber).²⁵

At harvest, the incidence of each disorder in pomegranate fruit was determined by counting the number of healthy or specific disorder-affected fruit – splitting (SPI) or sunburn (SUI) – in all fruits from each replicate. The mean fruit weight (FW) of marketable fruit yield (MY) was determined according to the weight and number of fruits per box in two randomly selected boxes per replicate.

Equatorial diameter (ED) and peel thickness (PT) were measured in each fruit with a digital caliper on 12 fruits per replicate. To measure PT, pomegranate fruits were cut in half and the measurements were performed on two opposite points in the equatorial zone. After PT measurement, each fruit was emptied by hand. The arils were weighted to calculate their weight and the ratio arils: whole fruit weights (AW, %), and they were used for further analyses.

Pomegranate peel color was assessed at four equidistant points of the equatorial region of individual fruit (the same 12 used for the measurement of ED and PT) using a Minolta CR 2000 colorimeter (Osaka, Japan). The arils obtained to calculate aril weight in each fruit were extended on a white plate and their color was assessed in ten different places on the plate, expressing the results using the CIE L*a*b* system.²⁶ The mean values for lightness (L*), green-red (a*) and blue-yellow (b*) coordinates for each fruit were calculated.

e Departamento de Producción Vegetal, Fitotecnia, ETSIAAB, Universidad Politécnica de Madrid, Ciudad Universitaria s/n, E-28040 Madrid, Spain

f Departamento de Ciencias Agroforestales, ETSIA, Universidad de Sevilla, Crta de Utrera km 1, E-41013 Sevilla, Spain

g Department of Agrofood Technology, Food Quality and Safety Research Group, Universidad Miguel Hernández de Elche, Ctra. de Beniel, km 3,2, E-03312 Orihuela, Alicante, Spain

The objective color was calculated as chromaticity or chroma ($C^* = (a^{*2} + b^{*2})^{1/2}$) and hue angle ($H^\circ = \arctan(b^*/a^*)$).

Fruit total soluble solids, acidity, maturity index and moisture content

Using six pomegranate fruits cut in half per replicate, juice was obtained by a squeezer (Braun model 3050, Barcelona, Spain). The total soluble solid (TSS) content of the juice was measured using a digital Atago refractometer (model N-20; Atago, Bellevue, WA, USA) and the titratable acidity was measured using acid-based potentiometry (877 Titrimo plus; Metrohm ion analyses CH9101, Herisau, Switzerland). The maturity index was calculated as the ratio between the TSS and the titratable acidity.

Total phenolics content and total antioxidant activity of the fruit

The total phenol content of pomegranate aril juice was estimated using the Folin–Ciocalteu reagent following the recommendations of Singleton *et al.*²⁷ Absorption was measured at 760 nm using a UV-Vis Uvikon XS spectrophotometer (Bio-Tek Instruments, Saint Quentin Yvelines, France). Calibration curves, with a concentration range between 0 and 0.25 g GAE L⁻¹, were used for the quantification of TPC, and showed good linearity ($r^2 \geq 0.996$).

According to Nuncio-Jáuregui *et al.*,²⁸ a methanol extract from each sample was prepared, 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×g for 10 min. The ABTS⁺ [2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)] radical cation method was measured according to Re *et al.*²⁹ Briefly, 10 µL of the supernatant was mixed with 990 µL of ABTS⁺ and, after allowing the reaction to proceed for 10 min, absorbance was measured at 734 µm. The absorbance was measured using a UV-Vis Uvikon XS spectrophotometer (Bio-Tek Instruments, Saint Quentin Yvelines, France). Calibration curves in the range of 0.01–5.00 mmol Trolox L⁻¹ were used for the quantification of antioxidant activity and showed good linearity ($r^2 \geq 0.998$).

Organic acids and sugars

Organic acids and sugars were quantified according to Melgarejo-Sánchez *et al.*³⁰ For this, 20 mL of pomegranate juice, obtained by squeezing the arils, was centrifuged at 15 000×g for 20 min (Sigma 3-18K, Osterode and 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™ 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 DE, USA) with an autosampler and an UV detector, set at 210 nm, coupled to a refractive index detector (HP 1100, G1362A). The elution system consisted of 0.1% phosphoric acid with a flow rate of 0.5 mL min⁻¹. Standard curves of pure organic acids (oxalic, citric, tartaric, malic, quinic, shikimic, succinic, and fumaric acids) and sugars (glucose, fructose and sucrose) were used for quantification. Calibration curves, with a concentration range between 1 and 10 g L⁻¹, were used for the quantification of organic acids and sugars, and showed good linearity ($r^2 \geq 0.999$). Sugar and organic acid standards were supplied by Supelco analysis (Bellefonte, PA, USA).

Fruit sensory analysis

Eleven trained panelists (four men and seven women aged between 24 and 70 years), all with lengthy experience in testing pomegranates, described pomegranate arils based on their expertise and training. The samples (codified using three-digit numbers) were served on odor-free disposable plastic plates at room temperature, and water was provided to clean panelists' palates between samples. The panelists evaluated samples according to the lexicon reported by Vázquez-Araújo *et al.*³¹ using a numerical scale to quantify the intensity of the attributes of the arils, where 0 represents no intensity and 10 extremely high intensity, with 0.5 increments.

Statistical analysis

Data were analyzed using SPSS software.³² Two-way analysis of variance was performed considering two independent variables or factors (irrigation and bagging), each one having two different levels (FI and WS for irrigation factor and B and NB for bagging factor). Mean values were compared by Tukey's multiple range test at $P < 0.05$. AW, SPI and SUI percentage values were arc-sin-transformed before statistical analysis because they were not normally distributed. Ψ_{stem} values for each replicate were averaged before the mean and the standard error of each treatment was calculated.

RESULTS

During the experiment, the meteorological characteristics were those of a classic Mediterranean climate. Average daily maximum and minimum air temperatures were 30 and 18 °C, respectively. The VPDm ranged from 0.23 to 2.06 kPa, the ETo amounted to 313 mm and the rainfall was 51 mm, which fell mainly on DOY 241, 242, 245, and 250 (21, 7, 18 and 4 mm, respectively) (Fig. 1).

Throughout the experimental period, there were no differences in Ψ_{stem} values between B and NB plants under FI and WS conditions (data not shown). However, the Ψ_{stem} values in FI and WS plants showed significant differences (Fig. 2). Ψ_{stem} values in FI plants remained high and near stable during this period, showing mean values of -0.99 MPa. By contrast, during the water withholding period, Ψ_{stem} values in WS plants gradually fell, to reach minimum values of -2.40 MPa on DOY 230. Rainfall increased Ψ_{stem} values in WS plants up to values similar to those in FI plants on DOY 244, and after this Ψ_{stem} values in WS plants once again decreased gradually as a result of the water withholding effect, reaching minimum values of -2.20 MPa before irrigation was resumed (DOY 269) (Fig. 2). Moreover, when irrigation was resumed, a very rapid recovery of Ψ_{stem} values in WS plants was observed (Fig. 2).

Withholding irrigation water induced a decrease in fruit size and a substantial increase in the number of pomegranate fruits affected by fruit splitting. Consequently, FI and WS plants showed greater differences in marketable yield than in total yield (Table 1). Marketable yield in the B treatment plants was significantly higher than in the NB treatment plants due to the high incidence of sunburn in NB fruits. In addition, pomegranate B fruits were significantly smaller and had a significantly thinner peel than NB fruits (Table 1). Considering the interaction between irrigation and bagging, it is important to note that the bagging effect on marketable yield was significant only under WS conditions, whereas the bagging effect on fruit weight, fruit equatorial diameter, and peel thickness was significant only under FI conditions. Moreover, despite the absence of any effect of irrigation or bagging on arils

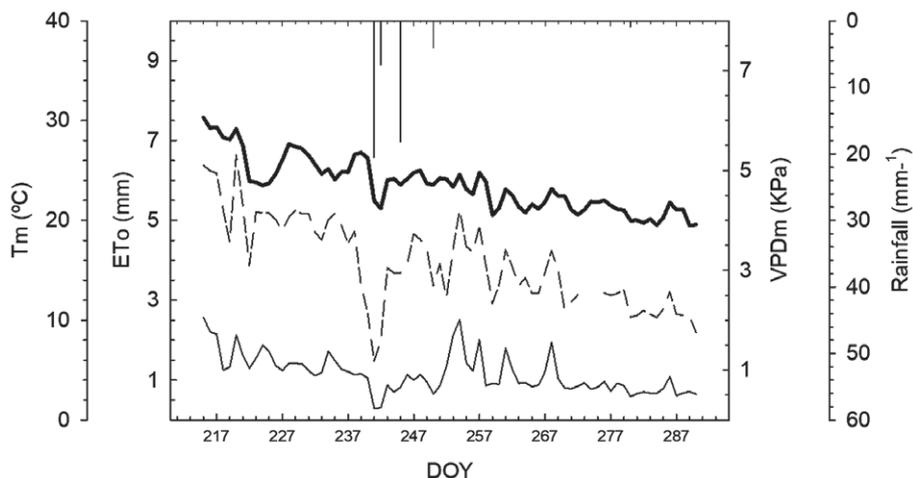


Figure 1. Daily crop reference evapotranspiration (ETo, dashed line), daily mean air temperature (Tm, solid bold line), mean daily air vapor pressure deficit (VPDm, solid thin line) and daily rainfall (vertical bars) during the experimental period (DOY 209–286).

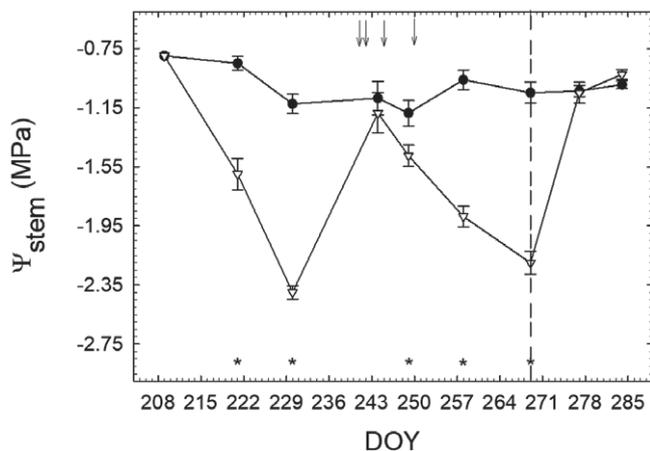


Figure 2. Midday stem water potential (Ψ_{stem}) values for pomegranate trees in FI (closed circles) and WS (open triangles) treatments during the experimental period. Vertical bars on data points are \pm standard error of the mean (not shown when smaller than the symbols). Asterisks indicate significant differences between treatments. Vertical dashed line indicates the end of the period for which irrigation was withheld. Arrows indicate daily rainfall events.

weight, the interaction of both factors showed that aril weight values in B fruits were significantly higher than in NB fruits, but only under FI conditions (Table 1).

Water withholding and bagging affected some chromatic characteristics of the pomegranate fruit peel. For example, L^* , b^* , C^* and H° values were significantly lower (darker and with a more intense blue color) in WS than in FI fruit peel, whereas bagging increased b^* and C^* values (more objective yellow color) compared to those in NB fruit peels. However, a^* values were not affected by the water deficit effect or by bagging (Table 2). As regards the interaction between the two factors considered here, the effect of irrigation on L^* values was significant only when FIB and WSB fruits were considered (irrigation was more important than bagging). The b^* and C^* values in FIB fruits were also higher (more objective yellow color) than in WSNB but similar to those in FINB and WSB fruits, and H° values in FIB fruits were significantly higher (more yellowish in color) than those in WSB and WSNB but similar to those in FINB fruits (Table 2). Conversely, the effect of both

treatments (bagging and irrigation) on pomegranate aril color was different from that observed in pomegranate peel (Table 3). In this sense, FI arils showed higher b^* and C^* values (more objective yellow color) than WS arils, as was also observed in pomegranate peel, but this did not affect L^* and H° values, whereas B arils had significantly higher b^* and H° values (more yellowish and less reddish color) than NB arils although the C^* values remained constant. Like pomegranate peel, differences in b^* values between treatments were significant only when FIB and WSNB fruit were considered (Table 3).

The succinic acid, glucose, and fructose content of pomegranate fruit was not affected by bagging or by water withholding effect (Table 4). However, bagging decreased the citric acid and TSS content significantly and increased the titratable acidity content significantly, which led to a significant decrease in maturity index values. By contrast, WS increased TSS, leading to significantly higher maturity index values (Table 4). Regarding the interaction between bagging and water withholding, the bagging effect on titratable acidity and TSS was significant only in FI and WS plants, respectively, whereas the bagging effect on maturity index values was significant only in FI plants. The bagging effect on the citric acid content was significant only in WS plants (Table 4).

Neither WS nor B significantly affected the total polyphenolic contents. However, WS fruits showed significantly lower AA-ABTS values than FI fruits, and bagging induced significantly higher AA-ABTS values than NB fruits (Table 4). The WS effect on AA-ABTS values was significant only in bagged plants (FIB and WSB), while B only affected FI fruits (Table 4).

Only three of the 33 sensory attributes of pomegranate fruits evaluated were affected by WS, whereas bagging did not affect any sensory attribute level (Fig. 3). Specifically, apple, pomegranate, and fruity flavors significantly increased in WS fruits; in this way, the treatments WSNB (red line) and WSB (yellow line) were the treatments having significantly ($P < 0.05$) higher intensities of key sensory attributes (pomegranate and fruity flavors) for the quality of pomegranate arils.

DISCUSSION

The high and near constant Ψ_{stem} values in the FI plants (Fig. 2) suggest that their water requirements were covered.¹³ The very low minimum Ψ_{stem} values before and after the rainfall episodes

Table 1. Effects of different irrigation and bagging 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), peel thickness (PT, mm), arils weight ratio (AW, %) and fruit physiopathies incidence (splitting (SPI, %) and sunburn (SUI, %), B = bagged fruits, FI = full irrigation, NB = no bagged fruits, WS = water stress. Values followed by the same letter, within the same column and factor, were not significantly different at $P \leq 0.05$ (*), $P \leq 0.01$ (**) or $P \leq 0.001$ (***). n.s. = not significant

Treatment	TY	MY	FW	ED	PT	AW	SPI	SUI
ANOVA								
Irrigation	***	***	***	***	n.s.	n.s.	***	n.s.
Bagging	n.s.	**	***	***	***	n.s.	n.s.	***
Irrigation × bagging	**	***	***	***	***	**	**	***
Irrigation								
FI	63.38a	50.83a	454.83a	95.80a	5.29a	52.95a	7.22b	12.23a
WS	39.70b	26.53b	429.87b	93.76b	5.41a	53.96a	20.82a	12.74a
Bagging								
B	50.79a	42.19a	399.07b	92.70b	4.98b	53.80a	14.48a	3.57b
NB	52.29a	35.18b	485.63a	96.86a	5.72a	53.11a	13.55a	21.40a
Tukey's multiple range test								
Irrigation × bagging								
FIB	60.30a	48.68a	396.85b	92.50b	4.51b	55.48a	7.79b	5.49b
FINB	66.46a	52.99a	512.80a	99.09a	6.07a	50.42b	6.64b	18.97a
WSB	41.29b	31.39b	401.28b	92.90b	5.45a	52.11ab	22.32a	1.65b
WSNB	38.12b	21.68c	458.46ab	94.62b	5.36a	55.80a	19.31a	23.82a

Table 2. Effects of different irrigation and bagging treatments on pomegranate peel lightness (CIE L^*), red/greenness (CIE a^*), yellow/blueness (CIE b^*), chroma (C^*), and hue angle (H°) values. B = bagged fruits, FI = full irrigation, NB = not-bagged fruits, WS = water stress. Values followed by the same letter, within the same column and factor, were not significantly different at $P \leq 0.05$ (*), $P \leq 0.01$ (**) or $P \leq 0.001$ (***). n.s. = not significant

Treatment	L^*	a^*	b^*	C^*	H°
ANOVA					
Irrigation	***	n.s.	***	***	***
Bagging	n.s.	n.s.	***	***	n.s.
Irrigation × bagging	*	n.s.	***	**	*
Irrigation					
FI	60.71a	22.73a	30.88a	39.68a	54.60a
WS	58.09b	23.58a	29.34b	38.66b	52.01b
Bagging					
B	59.39a	23.13a	30.91a	39.98a	54.35a
NB	59.41a	23.18a	29.31b	38.36b	52.25a
Tukey's multiple range test					
Irrigation × bagging					
FIB	61.09a	21.98a	31.83a	40.28a	56.79a
FINB	60.33ab	23.48a	29.94ab	39.07ab	52.40ab
WSB	57.70b	24.29a	30.00ab	39.67ab	51.91b
WSNB	58.49ab	22.87a	28.68b	37.64b	52.11b

(DOY 241–250) indicated that WS plants reached a severe WS level¹³ (Figs 1 and 2), although these minimum Ψ_{stem} values were reached at a low rate of around 0.08 MPa day⁻¹ and 0.04 MPa day⁻¹, before and after the rainfall episodes, respectively.¹³

In addition to the very high fruit-splitting incidence in WS fruits, the marketable yield was lower than in FI plants due to the lower WS fruit size (Table 1). In this sense, it is known that water deficit during pomegranate fruit growth and fruit ripening affects yield and fruit size.^{9,21,33,34} For this, these two phenological periods are considered as critical from the total yield point of view.

The decrease in peel thickness values in FI fruits as a result of bagging (FIB) could be due to the low light intensity and high humidity inside the bag, which can affect the cell structure and peel thickness.^{17,35} However, despite this effect on peel thickness,

and in contrast to the results obtained by Yuan *et al.*,¹⁹ fruit bagging did not affect fruit-splitting in FI plants or in WS plants. This behavior could be attributed to the fruit peel characteristics of each cultivar, such as peel elasticity or tannic contents in the peel, which can lead to different behaviors under bagging conditions.¹⁹ Whatever the case, this changing behavior suggested that the asymmetric rehydration of previously water-stressed fruits is the main factor leading to pomegranate fruit-splitting.¹³

The higher marketable yield in WSB than in WSNB was due to the high incidence of unmarketable fruits in WSNB plants caused by peel sunburn (Table 1). This physiological disorder arises from the fruit peel being directly exposed to high sunlight, which burns the fruit surface, decreasing its appeal and leading to unmarketable fruits, with inevitable economic losses.^{36,37} Rabinowitch

Table 3. Effect of different irrigation and bagging treatments on pomegranate aril lightness (CIE L^*), red/greenness (CIE a^*), yellow/blueness (CIE b^*), chroma (C^*), and hue angle (H°) values. B = bagged fruits, FI = full irrigation, NB = no bagged fruits, WS = water stress. Values followed by the same letter, within the same column and factor, were not significantly different at $P \leq 0.05$ (*), $P \leq 0.01$ (**) or $P \leq 0.001$ (***). n.s. = not significant

Treatment	L^*	a^*	b^*	C^*	H°
ANOVA					
Irrigation	n.s.	n.s.	***	***	n.s.
Bagging	n.s.	n.s.	***	n.s.	***
Irrigation \times bagging	n.s.	n.s.	**	n.s.	n.s.
Irrigation					
FI	33.32a	19.51a	9.55a	21.93a	26.26a
WS	33.85a	17.79a	8.43b	19.88b	25.59a
Bagging					
B	33.96a	18.90a	9.69a	21.48a	27.49a
NB	33.22a	18.40a	8.29b	20.33a	24.35b
Tukey's multiple range test					
Irrigation \times bagging					
FIB	34.69a	19.96a	10.56a	22.89a	28.47a
FINB	31.95a	19.06a	8.54ab	20.97a	24.03a
WSB	33.22a	17.83a	8.81ab	20.07a	26.50a
WSNB	34.48a	17.74a	8.04b	19.69a	24.66a

Table 4. Effect of different irrigation and bagging treatments on pomegranate fruit total soluble solids (TSS, °Brix), titrable acidity (TA, g anhydrous citric acid L^{-1}), maturity index (MI, TSS/TA), citric acid (CA, g $100 mL^{-1}$), succinic acid (SA, g $100 mL^{-1}$), glucose (Glu, g $100 mL^{-1}$), fructose (Fru, g $100 mL^{-1}$), 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. B = bagged fruits, FI = full irrigation, NB = no bagged fruits, WS = water stress. Values followed by the same letter, within the same column and factor, were not significantly different at $P \leq 0.01$ (**) or $P \leq 0.001$ (***). n.s. = not significant

Treatment	TSS	TA	MI	CA	SA	Glu	Fru	TPC	AA-ABTS ⁺
ANOVA									
Irrigation	***	n.s.	***	n.s.	n.s.	n.s.	n.s.	n.s.	***
Bagging	***	***	***	***	n.s.	n.s.	n.s.	n.s.	***
Irrigation \times bagging	***	**	***	***	n.s.	n.s.	n.s.	n.s.	***
Irrigation									
FI	15.51b	2.51a	62.15b	0.40a	0.29a	2.33a	2.83a	460.73a	19.20a
WS	17.19a	2.60a	66.26a	0.31a	0.20a	2.66a	3.21a	485.80a	14.12b
Bagging									
B	16.05b	2.64a	61.04b	0.26b	0.26a	2.96a	3.53a	463.82a	18.89a
NB	16.66a	2.47b	67.37a	0.45a	0.24a	2.04a	2.50a	428.70a	14.44b
Tukey's multiple range test									
Irrigation \times bagging									
FIB	15.51c	2.71a	57.59b	0.26ab	0.26a	3.52a	4.27a	487.58a	24.60a
FINB	15.52c	2.32b	66.71a	0.50a	0.32a	1.63a	1.96a	433.89a	13.81b
WSB	16.58b	2.58ab	64.50a	0.16b	0.18a	2.34a	2.82a	440.00a	13.19b
WSNB	17.80a	2.63ab	68.03a	0.46a	0.22a	2.99a	3.60a	531.60a	15.05ab

*et al.*³⁸ indicated that peel sunburn seems to be caused mainly by the concurrence of two factors, heat and light, the threshold values of which are cultivar dependent. However, the amount of time that is necessary to induce sunburn has not been established. Yazici and Kaynak³⁹ showed that during July, August, and September, when air temperature and solar radiation are higher than 30 °C and 600 $W m^{-2}$, respectively, sunburn peel damage takes place. In this sense, it is important to emphasize that under our experimental conditions, daily maximum radiation values were above 600 $W m^{-2}$ every day and on 38 days the maximum daily air temperature was above 30 °C, which could explain the high sunburn incidence in NB fruits (Table 1).

From the statistical point of view, bagging induced a significant overall tendency for peel and arils to turn more yellow (Tables 2

and 3), which supports the idea that bagging affected fruit growth and ripening. However, it is important to consider that chromatic changes observed in peel and arils were of limited real significance because changes of less than two units do not cause perceptible visual differences.^{40–42}

The decrease in TSS values due to bagging in WS plants (Table 4) would have been due to the partial protection of the bagged fruits, which would decrease fruit water loss and would have favored water accumulation within the fruit. Moreover, a higher fruit water content would favor the translocation of soluble solids.^{43,44} Similar results were obtained by Meena *et al.*²⁰ in pomegranate fruits under colored shade nets, by Amarante *et al.*¹⁷ in bagged pear fruits and by Seeley *et al.*⁴⁵ in shaded apple fruits. However, Bentley and Viveros,⁴⁶ described how TSS increased in bagged apple fruits

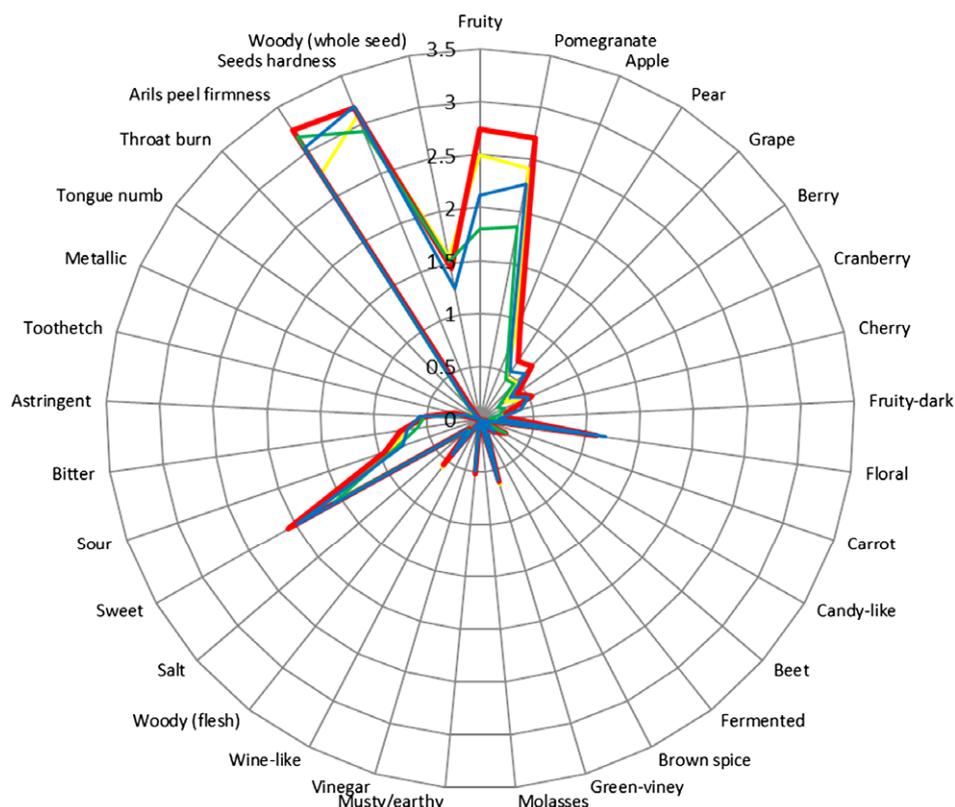


Figure 3. Descriptive sensory analysis in fully irrigated and not bagged (FINB, blue line), fully irrigated and bagged (FIB, green line), water stressed and not bagged (WSNB, red line) and water stressed and bagged (WSB, yellow line) pomegranate fruits.

in only one of the two years of the experimental period, and Hofman *et al.*⁴⁷ concluded that TSS values in mango fruits were not affected by bagging. In any case, the decrease in maturity index values by the bagging effect in FI fruits as a result of the maintenance of TSS and the increase in titratable acidity values (Table 4) can be considered an unfavorable aspect because the overall consumer perception of pomegranate fruit quality is related more to maturity index than to the soluble sugar content alone.⁴⁸

The decrease in antioxidant activity of pomegranate juice caused by the WS effect (Table 4) could be explained by the fact that WS inhibits the biosynthesis of punicalagin,²² which significantly contributes to the antioxidant activity of pomegranate juice.⁴⁹ The increase in antioxidant activity observed in FI fruits as a result of the bagging effect (Table 4) contrasted with the results of Meena *et al.*,²⁰ who indicated that pomegranate fruits cultivated in open conditions have higher antioxidant activity than those grown under shade net, and hypothesized that plants probably use the induction of antioxidants as a protective measure to avoid peel sunburn. Weerakkody *et al.*⁵⁰ showed that sunscreen did not affect the total polyphenols content or the antioxidant activity of pomegranate juice. These different responses of antioxidant activity to bagging may be related to the characteristics of the bags in question – mainly light transmittance.⁵¹

Only significant differences were detected in apple, pomegranate, and fruity flavor notes as a result of WS, but no significant differences were observed as result of the bagging. This finding indicated that the chemical changes occurring in the pomegranate fruits (Table 4) were not reflected in significant changes in the organoleptic attributes (Fig. 3). However, it is also

possible that the heterogeneity of the fruits can mask the small differences in the sensory attributes due to the bagging factor; this masking effect of fruit heterogeneity on quality parameters is quite frequent in fruits and vegetables. The increase in TSS and maturity index values as a result of WS and the decrease in the same values as a result of bagging were not related to changes in the corresponding sensory attributes (sweetness, pomegranate, apple, pear, fruity dark, grape, berry, cranberry, and floral notes).³¹ Finding no significant effect of bagging on sensory qualities is a positive result and indicated that the inner quality of the pomegranates was as good as that of the control samples.

CONCLUSIONS

In summary, the observations discussed above suggested that preharvest pomegranate fruit bagging may have certain negative effects in terms of slowing down fruit growth and ripening. However, such negative aspects may be outweighed by other very important commercial benefits such as the increase in antioxidant activity and the reduction in peel sunburn. Moreover, the chemical changes that occurred in pomegranate fruits as a result of bagging and WS were not perceived by the trained panellists. The fruits' response to bagging interacts with plant water status and probably with other environmental or farming practices. This would explain why the literature on fruit responses to preharvest bagging is not unanimous and contradictory results are frequent. Future research into fruit bagging should take into consideration that, in addition to the specific characteristics (physical and chemical characteristics, size, color, basal aperture or not, etc.) of the bags, other factors and environmental conditions may interact

and affect the fruit response to bagging. These factors would include the plant cultivar, the phenological period during which fruits are bagged, the material of the bags, the duration of fruit exposure to natural light from the time the bags are removed until fruit harvesting, and cultivation practices (irrigation, pruning and thinning characteristics, among others).

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