Food Chemistry: Molecular Sciences 4 (2022) 100112

Contents lists available at ScienceDirect

Food Chemistry: Molecular Sciences

journal homepage: www.sciencedirect.com/journal/food-chemistry-molecular-sciences

Influence of fruit bagging technique on the morphometric and biochemical characteristics of two pomegranate varieties (*Punica granatum* L.)

Pilar Legua, Juan José Martínez-Nicolás *, Pedro Guirao, Francisca Hernández, Dámaris Núñez-Gómez, Pablo Melgarejo

Centro de Investigación e Innovación Agroalimentaria y Agroambiental (CIAGRO-UMH), Miguel Hernandez University. Ctra. Beniel km 3.2 03312, Orihuela, Spain

ARTICLE INFO

Keywords:
Pomegranate variety
Weight fruit
Fruit bagging technique
Phenolic compounds
Organic acids
Sugars
Antioxidant activity

ABSTRACT

The pomegranate tree is cultivated and its fruits consumed since ancient times. This tree is typical of the Mediterranean climate, with high thermal demands to mature properly. The main objective of this work was to study the influence of the fruit bagging technique on the morphometric and biochemical characteristics of the pomegranate fruits of two new varieties that are currently cultivated in the Southeast of Spain. The results indicated that the fruit bagging presented a significant effect on the weight, equatorial diameter, height and shape of the fruit, however, it did not show any influence on the peel thickness. No significant differences have been observed in the number of healthy fruits with and without bagging, however, the number of cracked fruits with *Cryptoblabes gnidiella* damage was higher for the non-bagged fruits. The fruit bagging presented a significant effect on the total soluble solids, maturity index, glucose, α-punicalagin, α + β-punicalagin and ellagic acid, but it did not show influence on pH, acidity, ABTS, DPPH, FRAP, total phenols, fructose, citric, malic, and quinic acid, β-punicalagin and anthocyanins. The internal fruit color was not affected by the bagging, although it did affect the external color of the fruit, and unevenly depending on the variety. Based on the results, it can be said that bagging can improve the quality of the fruit by reducing damage from pests and pathophysiology, and this benefit compensates or even exceeds the negative effects of bagging on peel color.

1. Introduction

The pomegranate tree is a species cultivated since ancient times, it is native to Center IV of Vavilov (Middle East Center), belonging to the *Punicaceae* family that only presents one species cultivated for its fruits, *Punica granatum* L. (Pablo Melgarejo, Núñez-Gómez, Legua, Martínez-Nicolás, & Almansa, 2020; Melgarejo & Salazar, 2003). It is a typical species of Mediterranean climate, with high thermal requirements to fruit mature adequately, so the Southeast of Spain has a very appropriate climate to develop and produce high-quality fruits (Melgarejo, Martínez-Valero, Guillamón, Miró, & Amorós, 1997).

In recent years, the growing interest in the pomegranate and especially in its fruits is based not only on economic reasons but also on the benefits that its fruits have for human health, being considered a functional product rich in antioxidants, minerals, and vitamins, among other compounds useful for disease prevention (Melgarejo & Salazar, 2003; Melgarejo et al., 2020; Melgarejo-Sánchez et al., 2021; Rajaei & Yazdanpanah, 2015). Its world production has gone from 3,000,000 t in 2012 (Melgarejo & Valero, 2012) to 5,954,000 t in 2017 (Board, 2017), and its cultivation has spread to five continents and too many countries in both hemispheres.

As pomegranate cultivation has been expanding and acquiring greater economic importance, new cultivation techniques have been developed and improved to optimize the yield of this fruit and its quality. In this sense, progress has been made in irrigation techniques (Martínez-Nicolás et al., 2019), prevention of physiopathies such as the setting of fruits (Melgarejo et al., 2004) amount others. About the phytopaties fruit prevention, the moth *Cryptoblabes gnidiella* (Millière, 1867) (Lepidoptera: Pyralidae) have a special interest, since its larvae feed on the fruits, causing significant economic damage to the producers (IVIA, 2017). Although it is true that the traditional control and management of *C. gnidiella* has been carried out through the use of chemical treatments, the incipient social demand for the reduction of pesticides in agriculture urges the search, study and improvement of non-invasive

Abbreviations: V, represents the pomegranate variety; B, the fruit bagging; V x B, Interacción V x B.

* Corresponding author.
E-mail address: juanjose.martinez@umh.es (J. José Martínez-Nicolás).

https://doi.org/10.1016/j.fochms.2022.100112
Received 14 December 2021; Received in revised form 2 May 2022; Accepted 14 May 2022
Available online 17 May 2022

techniques for the C. gnidiella management, among which fruit bagging stands out (Cocuzza, Mazzeo, Russo, Giudice, & Bella, 2016). From a bibliography point of view, some published works were found, since 2012, focused on the fruit bagging technique, and frequently the results showed were not coincident in various fruit characteristics (Abou El-Wafa, 2014; Asrey, Kumar, Sharma, & Meena, 2020; Griñán et al., 2019).

In this context, the main objective of this work consisted in studying the influence of the fruit bagging technique on the morphometric and biochemical characteristics of the fruits of two new pomegranate varieties that are currently cultivated in the Southeast of Spain. The results obtained will be useful to know the influence of fruit bagging on the production and physical–chemical pomegranate quality produced with this cultivation technique.

2. Materials and methods

2.1. Plant material and experimental design

For this work, the fruits from two pomegranate varieties were used 'MR-Mix' and 'Purple Queen®'. For both varieties, the 5 years-old pomegranate trees, at the beginning of the study, were located in a commercial plantation in the Southeast of Spain (Ojós, Murcia, Spain).

The trees were planted with a 5 × 2 m frame, with a drip irrigation system (6 interline drippers per tree with a flow rate of 2 L h^{-1}). All the pomegranate trees were cultivated under homogeneous conditions and presented a good phytosanitary state at the time of harvesting the fruits.

The pomegranate fruits were collected manually during two years, 2020 and 2021, in order to identify and compare the effect of bagging on the quality of the fruits. In both experimental years, the pomegranate fruits collection began at the beginning of September, when the fruits presented a commercial maturity state.

Aiming to minimize the external impacts, for the experimental design were selected four tree rows (two tree rows for each pomegranate variety) located in the inner part of the cultivation area. For each pomegranate variety studied, a total of 18 pomegranate trees (9 trees per row) were selected and dived in two sub-groups, control and experimental group. For the control group trees, the pomegranate fruits were not bagged aiming to identify the normal fruit development and the real impact of the fruit bagging technique.

In the control group trees, the pomegranate fruits were not bagged, aiming to identify the normal fruit development and the real impact of the fruit bagging technique. On the other hand, into the experimental tree group, were selected and bagged three pomegranate fruits per orientation (12 fruits per tree). For each pomegranate variety, a total of 108 pomegranate fruits were bagged and morphological studied (3 fruits × 4 orientations × 9 trees).

The physical–chemical parameters were determined in pomegranate juice. For each pomegranate variety were selected two fruits per tree and group (with and without bagging). A total of 18 pomegranate fruits were used (2 fruits X 9 trees) to make the juices. The pomegranate juice was obtained by pressure from the pulpy seeds inside a nylon mesh (150 mesh, Dimoba, Spain). The juice samples were freezer −20 °C until used.

2.2. Morphometric measurements

The fruit morphology study was carried out according to the methodology described by Melgarejo-Sánchez et al. (2015), considering the following parameters: fruit weight (W) expressed in grams, equatorial diameter (ED) in mm, longitudinal height or fruit length (A) in mm, peel thickness (Pt) expressed in mm, and ED/A ratio. The ED, A and Pt parameters were measured with a Mitutoyo digital electronic caliper (model CD-15 DC, England, precision 0.01 mm). The fruit weight was measured using a Sartorius digital scale (model BL-600, with a precision of 0.01 g). The results are shown as mean value (n = 108) ± standard error.

2.3. Internal and external color fruit

The fruit color, internal and external, was determined by CM-700d spectrophotometer (Minolta Corp., Osaka, Japan), using a viewing angle of 10°, standard illuminant D65 and a CIE L * a * b * color space. Where, L* indicates the lightness of the color (L * = 0 and L * = 100 represent black and white, respectively), a* it is position between green and red (negative and positive values of a * indicate green and red, respectively) and b* its position between blue and yellow (negative and positive values of b * point towards blue and yellow, respectively). The target color $\left[C^{*} = \sqrt{(a^{*2} + b^{*2})}\right]$ and Hue angle ($H^{\circ} = arctan\frac{b^{*}}{a^{*}}$) were also determined.

The external color was carried out in situ immediately after collection in order to avoid possible variations. The pomegranate color peel data were collected from four opposite sides of the equatorial fruit zone. The internal pomegranate color was measured in 20 mL of fruit juice at constant room temperature (23 ± 3 °C). The results are presented as a mean value ± standard error.

2.4. Chemical characterization of pomegranate fruits

The chemical characterization of the pomegranate fruits, with and without bagging, was carried out using pomegranate juice, as described previously, and the parameters determined were: pH, Total Soluble Solids (TSS), Total Acidity (TA) and Maturity Index (IM). The pH, total soluble solids (TSS; ∘Brix) and titratable acidity (TA; g of citric acid L^{-1}) parameters were measured as previously reported by Legua et al. (2012). The maturity index (MI) was calculated as the relationship between TSS TA^{-1}. All analyzes were performed in triplicate, and the results were expressed as mean ± standard error.

2.5. Sugar and organic acids content

The sugars and organic acids content were determined according to the methodology described by Tozzi et al. (2020), briefly: a volume of 20 mL of filtered juice was centrifuged at 10,000 rpm for 20 min at 4 °C (Sigma 3–18 K, Osterode and Harz, Germany). After centrifugation, the supernatant was filtered through a nylon membrane filter (0.45 μm pore size) and disposed in chromatography vial. The determination was carried out using High performance liquid chromatography (HPLC) Agilent Technologies 1200/1100 model (Agilent Technologies Inc.) using a Supelcogel TM C-610H column (30 °C) (30 cm × 7.8 mm ID, Supelco, Bellefonte, PA, USA) which was protected with a Supelcogel C610H protection column (5 cm × 4.6 mm, Supelco, Inc.). The HPLC system used an autosampler and a UV detector, set at 210 nm, coupled with 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}. For the quantification, standard curves of pure organic acids and sugars were used. Sugar and organic acid standards were provided by Supelco (Bellefonte, PA, USA) analysis. The results were expressed as g 100 g^{-1} of juice.

2.6. Total antioxidant activity (TAA) and total phenolic compounds (TP)

The total antioxidant activity (TAA) of the pomegranate fruits, with and without bagging, was quantified according to the methodology described by Legua et al. (2012) using three different methods: DPPH (radical 2,2-diphenyl-1-picrylhydrazyl), FRAP and ABTS (2,2-azinobis-(3-ethylbenzothiazoline- 6-sulphonic acid). These methodologies have already been successfully applied by the authors (Legua et al., 2012; Legua, Forner-Giner, Nuncio-Jáuregui, & Hernández, 2016; Tozzi et al., 2020). All the tests were made per triplicate (n = 3) in a constant room temperature (23 ± 3 °C) and the results (mean ± standard error) were expressed as mg of Trolox equivalents per 100 g FW (fresh weight).

Total phenolics (TP) determination was also carried out following the methodology described by Legua et al. (2012), where a dilution of 5 mL of water:methanol (2:8), containing 2 mM NaF, was added to 5 mL of pomegranate juice. Folin–Ciocalteu reagent was used for TP quantification. Absorption was measured at 760 nm by spectrophotometer UV–VIS. All the analyses were performed in triplicate (n = 3) and results were expressed in mg of gallic acid (GA) per 100 mL of fresh juice (mean values ± standard error).

2.7. Anthocyanins and ellagic acid

Ellagic acid and anthocyanins content were identified and quantified in the pomegranate juice by high performance liquid chromatography (HPLC) following the methodology described by Calín-Sánchez et al. (2013) and Legua et al. (2016). For that, aliquots of pomegranate juice (5 mL) was mixed with 5 mL of MeOH and vortexed for 1 min. The extraction was carried out in an ultrasound bath for 10 min at room temperature. The extract was centrifuged at 4000 g for 4 min. and passed through a 0.45 μm PTFE filter (Waters, Milford, USA) prior to injection into the chromatographic system. Chromatographic analysis was performed on an Agilent 1100 series Ion Trap HPLC-ESI-DAD-MSn Ion Trap (Agilent Technologies, Waldbronn, Germany). An Agilent Pursuit XRs 5 C18 reverse phase column (250 × 4.6 mm i.d. and 5 μm particle size, Phenomenex, Macclesfield, UK) was used. The mobile phase consisted of two solvents: (1) waterformic acid (95: 5, v / v) and (2) acetonitrile, with a flow rate of 0.8 mL / min. The gradient started with 5% solvent B, reaching 60% solvent B at 37 min and 98% at 40 min, which was maintained for up to 2 min. The injection volume was 10 μL. The identification of the compounds was carried out by means of their fragmentation patterns obtained from mass spectra (MSn). The data provided by the reference standards and the information from the literature were also used for the comprehensive evaluation of the samples. Anthocyanins were monitored and quantified at 520 nm. The results are presented as mean value (n = 3) ± standard error, and were expressed as μM of cyanidin 3-O-glucoside.

2.8. Statistical analysis

Statistical analysis was applied to the experimental results obtained, analysis of variance (ANOVA), both one-way and multifactorial for mean comparison. A multivariate analysis was also performed, given the high number of variables measured, it was decided to perform a principal component analysis (PCA) in order to synthesize the results obtained and graphically visualize the behavior of the two varieties studied, as well as the influence of the bagging technique. The Principal Components procedure is designed to extract k principal components from a set of p quantitative variables X. Principal components are defined as the set of orthogonal linear combinations of X that have the maximum variance. Determining principal components is frequently used to reduce the dimensionality of a set of predictor variables. When the variables are highly correlated (as they are in this case), few of the first components may be sufficient to describe most of the variability present. One criterion selected to choose the number of principal components to extract has been to choose all the components for which the corresponding eigenvalue is at least 1 (Kayser's criterion), which implies that the component represents at least a fraction of 1/ p of the total population variance. When working with many variables measured in different units, as is the case here, it is best to base the analysis on the correlation matrix and standardize the variables, and we have done so.

The statistical analysis was carried out using the software package IMB® SPSS 25.0 for Windows (SPSS Science, Chicago, IL, USA).

3. Results and discussion

3.1. Morphometric results of pomegranate fruits cultivated with and without bagged

Although the pomegranate can be considered a "*superfruit*" and has the appreciation of consumers and markets, the improvement and study of its cultivation techniques, as well as its impacts on its physicochemical fruit characteristics, are poor (Yuan et al., 2012). In this sense, and given that the appearance of the pomegranate fruit is an extremely relevant factor for the consumer, the present work studied and identified the impacts produced by fruit bagging techniques on the characteristics and potentialities of fruits. Considering that the bagging technique is used mainly to protect and improve the appearance of the fruits by minimizing external imperfections, knowing its impact on the quality and morphology of the fruits should be essential for deciding whether the technique is viable for its application in pomegranate cultivation.

The results of morphological pomegranate characterization, with and without bagged, are shown in Table 1. The results indicated that, for the two pomegranate varieties studied, the fruits without bagging presented higher values for fruit weight (342.65 g for 'MR- Mix' and 320.51 g for 'Purple Queen'), equatorial diameter (86.77 mm for 'MR- Mix' and 84.68 mm for 'Purple Queen') and fruit length (74.36 mm for 'MR- Mix' and 75.04 mm for 'Purple Queen'). On the other hand, the bagged fruits were smaller, with an 11.97% lower fruit weight, 2.35% smaller equatorial diameter and 4.56% less length. Significant differences were only identified for the fruit results with or without bagging for each pomegranate variety. This confirms the influence of the bagging technique on fruit morphology independently of the pomegranate variety according to the bibliography (Ali, Anwar, Yousef, Li, Luvisi, De Bellis, & Chen, 2021; Hamedi Sarkomi, Moradinezhad, & Khayyat, 2019). The fruit bagging technique did not show an influence on the thickness of the shell (Pt), for all the pomegranate fruits the values were between 3.54 mm and 3.83 mm, which is shown in accordance with expected for these pomegranate varieties (Tozzi et al., 2020). Based on statistical analysis, the only pomegranate variety × bagging interaction significant identified was the ED/A ratio (Table 1). Analyzing the results in more detail, and despite that the studies on the pomegranate bagging are not very abundant, similar results were observed by El-Wafa (2014). In this study, El-Wafa was working with Wonderful cv. and his results also

Table 1
Morphological characterization of pomegranate fruits (var. 'MR-Mix' and 'Purple Queen®') cultivated with and without bagging technique. The parameter considered were the fruit weight (W, grams) equatorial diameter (ED, mm), fruit (A, mm), peel thickness (Pt, mm) and ED A^{-1} ratio. The results are presented as mean value ± standard error (n = 18).

	Pomegranate variety						
	'MR-Mix'		'Purple Queen®'		Statistical analysis		
Parameter	Without bagging	With bagging	Without bagging	With bagging	V	B	VxB
W (g)	342.65 ± 7.84^{a}	301.63 ± 13.89^{b}	320.51 ± 11.69^{a}	267.04 ± 9.20^{b}	*	**	ns
ED (mm)	86.77 ± 1.12^{a}	82.89 ± 1.24^{b}	84.68 ± 0.99^{a}	79.23 ± 0.98^{b}	*	*	ns
A (mm)	74.36 ± 0.85^{a}	69.38 ± 1.26^{b}	75.04 ± 1.12^{a}	71.62 ± 0.97^{b}	ns	***	ns
Pt (mm)	3.57 ± 0.22^{a}	3.61 ± 0.09^{a}	3.54 ± 0.11^{a}	3.83 ± 0.16^{a}	ns	ns	ns
ED/A ratio	1.17 ± 0.02^{a}	1.20 ± 0.01^{a}	1.13 ± 0.01^{a}	1.11 ± 0.01^{a}	*	*	*

Where ns: not significative; *: $p < 0.05$; **: $p < 0.01$; $p < 0.001$ according to the multifactorial ANOVA. The different letters within the rows for each pomegranate variety indicate significant differences. For the statistical analysis: V) represents the pomegranate variety; and B) the fruit bagging.

indicated that the non-bagged pomegranate fruits were higher in size when compared with bagged fruits. Our results agree with Griñán et al. (2019). In this work, the authors studied in Santomera (Murcia), the effect of pomegranate bagging on the quality characteristics and incidence of the fruit of pomegranate trees with and without water stress. It should be noted that this work was carried out in a region close to ours, and therefore, the environmental edaphoclimatic conditions are similar. 'MR-Mix' and 'Purple Queen' pomegranates, both with and without bagging, showed higher fruit size values than other varieties of pomegranates grown under commercial conditions, such as 'Wonderful', 'Bhagwa' and 'Ruby' grown in South Africa and the USA, but inferior to some varieties such as 'Barski slatki', 'Crveni rani', and 'Dividiš' grown in Croatia (Arendse, Fawole, Magwaza, & Opara, 2016; Fawole & Opara, 2013; Radunić et al., 2015; Wetzstein, Zhang, Ravid, & Wetzstein, 2011).

In addition to the external morphological parameters of the pomegranate fruits, the number of healthy pomegranates (without external damage), peel cracked fruits and pomegranates with peel damage caused by *Cryptoblabes gnidiella* were monitored and the results are shown in Table 2. Although no significant differences were observed in the number of healthy fruits obtained, the bagging technique did show relevance in the number of fruits with peel cracked and *C. gnidiella* damage, independently of the pomegranate variety. These two parameters were higher for the non-bagged fruits in both varieties, since in pomegranate bagged fruits these were not detected, however, the number of fruits affected by both damages was greater for the 'Purple Queen®' variety than for the 'MR-Mix' variety. Since *C. gnidiella* is considered a relevant pest in pomegranate crops (Ricciardi, Di Giovanni, Cosci, Ladurner, Savino, Iodice, & Lucchi, 2021; Zebitz, Salman, Lubin, & Gavish-Regev, 2022), the results obtained in this study can be considered promising and encouraging, once the appearance of damage caused by the arthropod is eliminated without additional treatment. To the knowledge of the authors, no specific bibliographical references have been found that allow the results obtained to be compared.

3.2. Chemical characterization of pomegranate fruits

The chemical characterization results of the pomegranate obtained with or without bagging technique are presented in Table 3. The parameters pH, SST, TA, ABTS, FRAP, total phenols, citric acid, quinic acid, β punicalagin and anthocyanins did not show significant differences related to the bagging and/or the pomegranate variety. In all cases, the values are consistent with those already reported for these varieties (Legua et al., 2012; Mouas, Kabouche, Benssuici, & Chaoui, 2021; Tozzi et al., 2020).

On the other hand, the IM, DPPH, glucose, fructose, malic acid, α punicalagin and ellagic acid parameters showed significant differences, generally associated with fruit bagging. In this sense, the IM results were only significant for the bagged 'Purple Queen' fruits, with slightly lower values than the other fruits. The bagged fruits of 'Purple Queen' also showed significant differences for the parameter's DPPH, glucose, fructose and malic acid when compared both in relation to the bagging technique and the pomegranate variety. In all the cases, the bagged 'Purple Queen' fruits results were lower than the values obtained for the other variety and crop technique. So, while non-bagged 'MR-Mix' pomegranates presents DPPH values of 30.37 42 mg of Trolox equivalents per 100 g FW, bagged 'Purple Queen®' fruits barely reaches 28.58 mg of Trolox equivalents per 100 g FW. However, the results obtained are higher than those reported for ten Israeli pomegranate varieties grown under homogeneous conditions, and similar to those reported for the 'Wonderful' and 'Ruby' varieties (Borochov-Neori et al., 2009; Fawole & Opara, 2013; Gil, Tomá S-Barberán, Hess-Pierce, Holcroft, & Kader, 2000). Related to the other parameters, bagged 'Purple Queen' fruits presented relevant reductions of around 37.57% less malic acid content, 10.97% less glucose and 10.21% less fructose. Although it has traditionally been indicated that bagging can increase fruit sugars and organic acid content (Islam et al., 2019; Sarker, Rahman, & Barman, 2009; Zhang et al., 2015), the response to bagging varies according to the fruits considered. In fact, a decrease in sugar and organic acid content has been reported in olives, apples and dates (Al-Obeed & Harhash, 2010; Jing, Feng, Zhao, Wu, & Chen, 2020; Zhou, Zhong, Lin, Xu, &

Table 2
Number of healthy, peel cracked and *Cryptoblabes gnidiella* damage pomegranate fruits of 'MR-Mix' and 'Purple Queen®' varieties cultivated with and without bagged. The results are presented as mean value (n = 6) ± standard error.

	Pomegranate variety			
	'MR-Mix'		'Purple Queen®'	
N° of pomegranate fruits	Without bagging	With bagging	Without bagging	With bagging
Healthy	43.17 ± 2.27[a]	49.0 ± 3.70[a]	78.67 ± 13.03[a]	68.67 ± 10.62[a]
Peel cracked	0.17 ± 0.17[a]	0.00 ± 0.00[b]	2.33 ± 0.67[a]	0.00 ± 0.00[b]
Damage by *Cryptoblabes gnidiella*	1.50 ± 0.81[a]	0.00 ± 0.00[b]	3.33 ± 0.88[a]	0.00 ± 0.00[b]

The different letters within the rows for each pomegranate variety indicate significant differences.

Table 3
Chemical parameters of pomegranate fruits, for each variety with and without bagging. The results indicate the mean (n = 3) ± standard error. The different letters within the rows for each pomegranate variety indicate significant differences.

	Pomegranate variety			
	'MR-Mix'		'Purple Queen®'	
Parameter	Without bagging	With bagging	Without bagging	With bagging
pH	3.63 ± 0.04[a]	3.61 ± 0.03[a]	3.63 ± 0.02[a]	3.62 ± 0.03[a]
TSS (°Brix)	16.35 ± 0.24[a]	15.82 ± 0.29[a]	15.70 ± 0.22[a]	15.05 ± 0.30[a]
TA (g citric acid L^{-1})	2.79 ± 0.087[a]	2.79 ± 0.09[a]	2.59 ± 0.07[a]	2.48 ± 0.07[a]
MI (SST/TA ratio)	19.97 ± 0.09[a]	19.42 ± 0.17[b]	19.57 ± 0.12[a]	19.11 ± 0.20[b]
ABTS	15.06 ± 0.51[a]	20.19 ± 2.97[a]	22.84 ± 2.30[a]	19.38 ± 2.00[a]
DPPH	30.37 ± 0.49[a]	29.49 ± 0.35[a]	28.42 ± 0.24[a]	29.58 ± 0.35[b]
FRAP	18.62 ± 1.24[a]	18.57 ± 1.64[a]	22.54 ± 1.63[a]	20.94 ± 2.09[a]
Total phenols (mg GA 100 mL^{-1})	223.19 ± 3.25[a]	216.15 ± 5.29[a]	233.76 ± 5.62[a]	227.60 ± 3.41[a]
Glucose (g L^{-1})	52.01 ± 1.37[a]	50.29 ± 1.08[a]	49.26 ± 0.85[a]	46.30 ± 0.94[b]
Fructose (g L^{-1})	59.92 ± 1.38[a]	58.66 ± 1.16[a]	56.80 ± 0.82[a]	53.80 ± 0.94[b]
Citric acid (g L^{-1})	6.59 ± 0.38[a]	6.84 ± 0.64[a]	6.29 ± 0.87[a]	4.11 ± 0.75[a]
Malic acid (g L^{-1})	5.62 ± 0.32[a]	5.60 ± 0.32[a]	5.55 ± 0.74[a]	3.51 ± 0.55[b]
Quinic acid (g L^{-1})	13.50 ± 1.20[a]	16.75 ± 1.91[a]	14.61 ± 1.59[a]	12.54 ± 1.80[a]
α punicalagin (g L^{-1})	0.30 ± 0.02[a]	0.59 ± 0.09[b]	0.47 ± 0.03[a]	0.46 ± 0.04[a]
β punicalagin (g L^{-1})	0.34 ± 0.03[a]	0.55 ± 0.11[a]	0.53 ± 0.02[a]	0.52 ± 0.03[a]
α + β punicalagin(g L^{-1})	0.64 ± 0.06[a]	1.14 ± 0.19[b]	1.00 ± 0.05[a]	0.98 ± 0.07[a]
Ellagic acid (g L^{-1})	0.02 ± 0.0006[a]	0.03 ± 0.0007[b]	0.025 ± 0.0003[a]	0.025 ± 0.0004[a]
Anthocyanins (μM)	32.20 ± 2.24[a]	32.17 ± 3.15[a]	25.54 ± 2.64[a]	25.49 ± 2.43[a]

The different letters within the rows for each pomegranate variety indicate significant differences.

Mathooko, 2010).

For 'Purple Queen®' pomegranate, the content of α punicalagin was the same with and without bagging (0.47 g L^{-1}), while for the 'MR-Mix' variety the content of α punicalagin with bagging is significantly higher (0.59 g L^{-1}) than that without bagging (0.30 g L^{-1}). Something very similar was identified for the ellagic acid content, where for 'Purple Queen' fruits no differences were observed (0.025 g L^{-1}), while that the bagging technique did affect its content in the 'MR-Mix' variety, with 0.02 and 0.03 g L^{-1} respectively. Yuan et al. (2012) evaluated pomegranate bagging in Shandong, China, and indicated that the phenol content decreased with bagging, nevertheless in this study no differences in the phenol content with and without bagging were found (Table 4). Although the bibliographical references of the impact of the cultivation techniques on the chemical characteristics of the pomegranate are limited and, therefore, their discussion, in all cases the results obtained in the present study were within the expected ranges for the pomegranate juices according to the available bibliography (Amri et al., 2017; El-Nemr, Is, & Ragab, 1990; Kar, Ferchichi, Attia, & Bouajila, 2011; Legua et al., 2012; Tozzi et al., 2020).

The chemical characterization results of the pomegranate fruits indicated that the bagging technique had a significant effect on total soluble solids (TSS), maturity index (MI), glucose, α punicalagin, α + β punicalagin and ellagic acid. However, it did not show an influence on pH, acidity, ABTS, DPPH, FRAP, total phenols (TP), fructose, citric, malic and quinic acids, β punicalagin and anthocyanins (Table 4). In addition, the interactions between the varieties of pomegranate (V) and bagging (B) were significant for DPPH, α punicalagin, and ellagic acid.

3.3. Internal and external color fruit

The external and internal color variation is shown in Table 5. Based on the results, it can be observed that the bagging did not affect the internal coloration of the pomegranate in both studied varieties. However, it does affect the external color of the fruit and unevenly depending on the variety. Thus, in the cultivar 'MR-Mix', the b * and H * color components presented significantly higher values in the bagged fruits than in the non-bagged ones, due to an increase in yellowish tones. This colorimetric behavior was also described by Tran, Yen, and Chen (2015) in a study carried out with red pitahaya fruits, var. 'Chuchi Liu', observing significant differences in the values of b * and H * compared to the control.

For 'Purple Queen®' pomegranate fruits, the parameters L *, b * and H * were significantly higher in the bagged fruits, which indicates that they showed greater luminosity and a greater yellow hue. This effect on the external fruit luminosity was also described by Zhang et al. (2015) in a bagging study carried out on late-maturing peach [*Prunus persica* (L.) Batsch] var. 'Guibao'.

Likewise, Chonhenchob et al. (2011), observed greater luminosity and yellow coloration in bagged mangoes (cv. *Nam Dok Mai # 4*) than in those not bagged. Hofman, Smith, Joyce, Johnson, and Meiburg (1997) also evaluated the effect of bagging mango fruit (*Mangifera indica*) in order to improve the quality of the fruit of late-maturing cultivars. The authors studied the fruit of the 'Keitt' variety and all bagging treatments increased the percentage of the skin area with yellow color. The percentage of skin with red color and its intensity decreased with increasing bagging duration. Fruit weight, pulp color, total soluble solids, acidity and quality of consumption were generally not affected by bagging. In the end, these authors concluded that bagging can improve fruit quality by reducing disease and pathophysiology, and this benefit outweighed the negative effects of bagging on skin color in cultivar 'Keitt'. In general, we agree with these considerations made by these authors in their work on the mango cultivar 'Keitt'. In this sense, Bentley and Viveros (1992) in a Granny Smith apple bagging study.

Table 4
Statistical analysis showing the influence of the pomegranate variety (V), the bagging technique (B) and the V × B interaction on the chemical parameters of the pomegranate fruits.

Parameter	V: Pomegranate variety	B: Bagging	V × B
pH	ns	ns	ns
Total soluble solids (TSS)	*	*	ns
Titulable acidity (TA)	**	ns	ns
Maturity index (MI)	*	**	ns
ABTS	ns	ns	ns
DPPH	*	ns	*
FRAP	ns	ns	ns
Total phenols (TP)	*	ns	ns
Glucose	**	*	ns
Fructose	**	ns	ns
Citric acid	*	ns	ns
Malic acid	ns	ns	ns
Quinic acid	ns	ns	ns
α punicalagin	ns	*	**
β punicalagin	ns	ns	ns
α + β punicalagin	ns	*	**
Ellagic acid	ns	*	**
Anthocyanins	**	ns	ns

Where ns: not significative; *: $p < 0.05$; **: $p < 0.01$; $p < 0.001$ according to the multifactorial ANOVA.

Table 5
External and internal color results of the pomegranate fruits, for each variety of pomegranate with and without bagging. The results indicate the mean (n = 12) ± standard error.

	'MR-Mix'		'Purple Queen®'	
	Without bagging	With bagging	Without bagging	With bagging
External color				
L*	46.68 ± 0.71[a]	47.60 ± 0.48[a]	45.45 ± 0.46[a]	47.12 ± 0.55[b]
a*	42.63 ± 0.58[a]	42.63 ± 0.35[a]	42.12 ± 0.41[a]	41.92 ± 0.39[a]
b*	18.34 ± 0.34[a]	20.84 ± 0.39[b]	18.02 ± 0.28[a]	19.10 ± 0.29[b]
C*	46.47 ± 0.59[a]	47.55 ± 0.32[a]	45.84 ± 0.45[a]	46.11 ± 0.39[a]
H*	23.33 ± 0.41[a]	26.06 ± 0.50[b]	23.14 ± 0.25[a]	24.51 ± 0.36[b]
Internal Color				
L*	39.12 ± 1.86[a]	38.89 ± 2.27[a]	39.61 ± 2.26[a]	45.69 ± 3.27[a]
a*	19.63 ± 1.25[a]	19.10 ± 1.73[a]	20.01 ± 2.63[a]	18.76 ± 2.63[a]
b*	10.91 ± 0.66[a]	11.38 ± 0.72[a]	11.87 ± 0.99[a]	13.43 ± 0.82[a]
C*	22.54 ± 1.29[a]	22.42 ± 1.67[a]	23.54 ± 2.57[a]	23.89 ± 2.09[a]
H*	29.42 ± 1.41[a]	31.98 ± 2.58[a]	32.37 ± 3.02[a]	39.55 ± 4.99[a]

The different letters within the rows for each pomegranate variety indicate significant differences.

3.4. Statistical analysis

The Principal Components Analysis (PCA) results showed that the first two components explained 99.99% of the total variance, represented as PC1 and PC2. In this sense, PC1 represents 59.72% of the variance and basically explains acidity, TSS, acids and sugars, punicalagins, anthocyanins, color and fruit size. The PC2 component represents 40.27% of the variance and mainly explains total phenols, ellagic acid, juice color, number of fruits affected by Cryptoblabes and number of cracked fruits. The PCA results graphically presented (Fig. 1) clearly discriminates between the two varieties studied, as well as the influence of the bagging technique.

4. Conclusions

- The fruit bagging influences the size and shape of the fruit, represented by the W, ED, A and ED/A ratio parameters. The size of the fruit is greater in the non-bagged fruits compared to the bagged ones, however, it did not show influence about Pt.
- Bagging affects the chemical parameters TSS, MI, glucose, α punicalagin, α + β punicalagin, and ellagic acid, while did not show

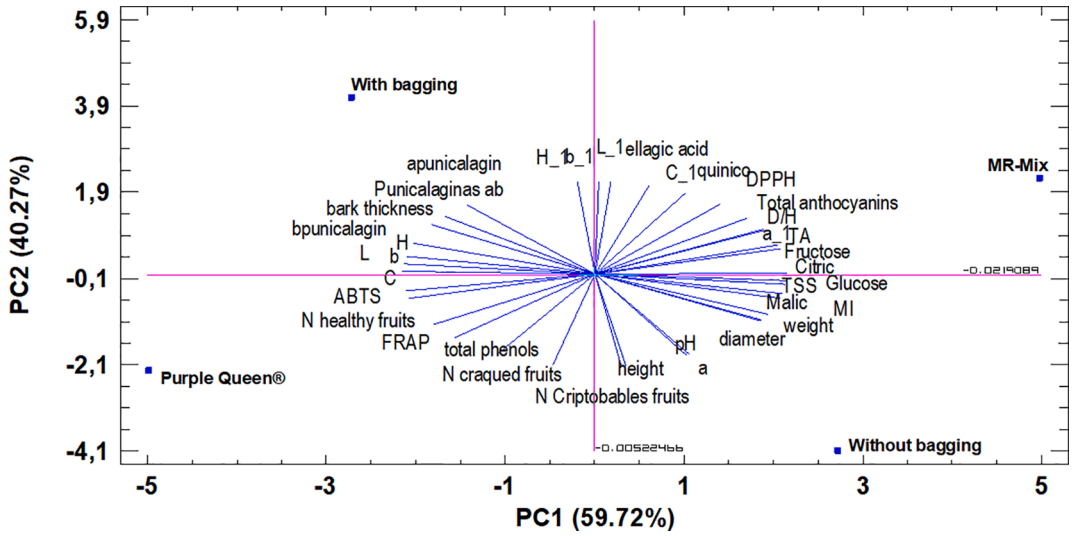

Fig. 1. Principal component analysis (PC1 and PC2) of pomegranate fruits (var. Purple Queen ® and MR – Mix) cultivated with and without bagging technique. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

influence on pH, TA, ABTS, DPPH, FRAP, total phenols, fructose, citric, malic and quinic acids, β punicalagin and anthocyanins.

- With bagging both cultivars presented a similar value of DPPH, neverthless, without bag, 'MR-Mix' variety presents a significantly higher DPPH value than the cultivar 'Purple Queen®'. For the cultivar 'Purple Queen®' the content of α punicalagin is the same with and without bagging, while for the cultivar 'MR-Mix' the content of α punicalagin with bagging is significantly higher than that without bagging. A very similar trend occurs for ellagic acid content.
- Bagging does not affect the internal coloration of the fruit in the two cultivars tested. However, the external color of the fruit is affected and unevenly depending on the cultivar. The bagged fruits showed greater luminosity and a greater predominance of yellow hue than the non-bagged ones.
- Finally, based on the results, it can say that bagging can improve fruit quality by reducing damage from pests and pathophysiological conditions, and this benefit compensates or even exceeds the negative effects of bagging on peel color.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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