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Prestorage Heat Treatment To Maintain Nutritive and Functional Properties during Postharvest Cold Storage of Pomegranate

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Heat treatments have been used to extend storability of several fruits, although no information is available about their effects on nutritive and functional properties in pomegranates, which was the objective of this research. Thus, pomegranate fruits were heat treated (dips at 45 °C for 4 min) and stored at 2 °C for 90 days. Every 15 days, samples were taken and further stored 2 days at 20 °C for shelf life study. Arils from heat-treated pomegranates exhibited higher total antioxidant activity than controls, which was correlated primarily to the high levels of total phenolics and to lesser extent to ascorbic acid and anthocyanin contents. Additionally, the levels of sugars (glucose and fructose) and organic acids (malic, citric, and oxalic acids) remained also at higher concentrations in arils from treated fruits. With this simple and non-contaminant technology, the functional and nutritive properties, after long periods of storage, could then be even greater than in recently harvested fruits, thus providing a high content in health-beneficial compounds to consumers after the intake of these fruits.

KEYWORDS: Organic acids; phenolics; sugars; total antioxidant activity

INTRODUCTION

Pomegranate (*Punica granatum* L.) is considered one of the oldest known edible fruit and probably originated in northern Turkey (*1*). The arils are the edible part of the fruit, which contain around 80% juice and 20% seed. The juice is rich in sugars, organic acids, vitamins, polysaccharides, and essential minerals (*2*). In addition, pomegranates have been used broadly in the folk medicine of many cultures (*3*), but recently other properties have been claimed, such as antioxidant activity, anticancer, and against atherosclerosis, among others (*4*, *5*).

During postharvest, pomegranate exhibits important quality loss due to several physiological and enzymatic disorders, the major storage problem being water loss leading to browning symptoms in both peel and arils. These symptoms increase with storage temperature below 5 °C (*6*). However, to avoid excessive desiccation and decay occurrence, storage at low temperatures is therefore necessary. Moreover, loss of firmness, aril color, vitamin C, and acidity was reported, which were accompanied by reduction of acceptability in terms of freshness, juiciness, and taste (*7*, *8*). To extend storability and marketing of several fruits, good results were obtained with heat treatments, such as vapor, water immersion, and hot water rinsing and bruising. These treatments reduced the postharvest ripening and external skin damage during storage, and induced resistance to chilling injury and fungal infections (*9*−*12*). In pomegranate, intermittent warming at 20 °C every 6 days at 2 or 5 °C, as well as film wrapping have been tested with satisfactory results in maintaining pomegranate quality during storage, in terms of retention of anthocyanin and titratable acidity, reduction of decay, and alleviation of chilling injury (*7*, *8*, *13*).

However, no information is available about the use of heat treatments (temperature over 35 °C during short periods) in pomegranate, neither on the organoleptic nor nutritive and functional properties of the arils. In this sense, the aim of this work was to study the effect of prestorage heat treatment (hot water dip at 45 °C during 4 min) on the nutritive (sugars and organic acids) and functional properties (ascorbic acid, total phenolic compounds, total anthocyanins, and total antioxidant activity) during postharvest storage of pomegranate.

MATERIALS AND METHODS

Plant Material and Experimental Design. Pomegranates (*Punica granatum* L. cv. Mollar de Elche) were picked on October 10, 2005 in a commercial orchard in Orihuela (Alicante). This cultivar is late-ripening with delicious sweet arils containing soft tegmen. Fruits were harvested when fully mature according to commercial practice and immediately transported to the laboratory. Pomegranates with defects (sunburn, crack, bruise, and cut in the husk) were discarded. The remaining fruits were randomized and divided into two lots of 175 fruits for the following treatments in quintuplicate (each replicate

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Table 1. ANOVA for Dependent Variables for Treatment Applied, Storage Time, and Their Interactions for Pomegranate Arils[a]

	time	treatment	time treatment
total antioxidant activity	***	***	***
phenolic compounds	*	***	NS
total anthocyanins	*	***	NS
ascorbic acid	**	***	NS
oxalic acid	**	***	NS
citric acid	***	***	**
succinic acid	***	**	NS
malic acid	***	***	*
glucose	NS	**	*
fructose	***	***	*
sucrose	***	NS	NS

[a] ***, **, and * represent significance at the 0.01, 0.01, and 0.05 levels, respectively, and NS represents nonsignificance at $P < 0.05$.

contained 35 individual fruits): control (distilled water at 25 °C for 4 min) and heat treatment (hot water dip at 45 °C for 4 min). The heat treatment was based on previous experiments (*14*, *15*). Following treatments, fruits were placed on Kraft paper and allowed to dry before storage the next day at 2 °C (considered as day 0) in a temperature-controlled chamber, in permanent darkness and with relative humidity of 90%. After 15, 30, 45, 60, 75, and 90 days, 25 fruits for each treatment (5 from each replicate) were sampled and further stored at 20 °C for 3 days (shelf life, SL). Next, each husk was carefully cut at the equatorial zone with a sharpened knife, and then arils were manually extracted. The arils of each replicate were combined and frozen in liquid N_2, milled, and stored at −20 °C until analytical determinations.

Total Antioxidant Activity and Total Phenolic Compounds. For each sample, 5 g of arils was homogenized in 10 mL of 50 mM phosphate buffer pH = 7.8 and then centrifuged at 10 000*g* for 15 min at 4 °C. The supernatant was used for total antioxidant activity (TAA) and total phenolic compounds quantification in duplicate, as previously described (*16*). For TAA, L-ascorbic acid was used for calibration curve, and the results were expressed as mg ascorbic acid equivalent 100 g^{-1} fw (fresh weight). The total phenolic compounds were expressed as mg gallic acid equivalent 100 g^{-1} fw. For both cases, results were the mean of determinations made in duplicate in each one of the five samples.

Organic Acid and Sugars Content. For organic acid and sugar determinations, the same extract as above was used and previously described (*16*). One milliliter of the extract was filtered through a 0.45 μm Millipore filter and then injected into a Hewlett-Packard HPLC series 1100. The elution system consisted of 0.1% phosphoric acid running isocratically with a flow rate of 0.5 mL min^{-1}. The organic acids were eluted through a Supelco column (Supelcogel C-610H, 30 cm × 7.8 mm, Supelco Park, Bellefonte, USA) and detected by absorbance at 210 nm. A standard curve of pure organic acids (L-ascorbic, malic, citric, oxalic, and succinic acids) purchased from Sigma (Poole, Dorset, UK) was used for quantification. Results were expressed as mg ascorbic acid 100 g^{-1} and g 100 g^{-1} (%) for the remaining acids. For sugar concentrations, the same HPLC, elution system, flow rate, and column were used. The detection of sugars was obtained by refractive index detector. A standard curve of pure sugars (glucose, fructose, and sucrose) purchased from Sigma was used for quantification. Results were expressed as g 100^{-1} (%).

Total Anthocyanins. The method described by García-Viguera et al. (*17*) was adapted to pomegranate. Five grams of arils was homogenized in 4 mL of methanol and left 1 h at −18 °C. Extracts were centrifuged at 15 000 rpm for 15 min at 4 °C. The supernatant was loaded onto a C18 Sep-Pak cartridge, previously conditioned with 5 mL of methanol, 5 mL of pure water, and then 5 mL of 0.01 N HCl. Cartridge was washed with 5 mL of pure water and then eluted with acidified MeOH (0.01% HCl). Absorbance of the collected fraction was measured at 530 nm. Total anthocyanin was calculated using cyanidin-3-glucoside (molar absorption coefficient of 23 900 L cm^{-1} mol^{-1} and molecular weight of 449.2 g mol^{-1}), and results were expressed as mg 100 g^{-1} fw and were the mean of determinations made in duplicate in each one of the five samples.

Statistical Analysis. Data for the analytical determinations were subjected to analysis of variance (ANOVA). Sources of variation were storage and treatment. Mean comparisons were performed using HSD and Tukey's test to examine if differences were significant at $P < 0.05$. To know the compounds that contribute to TAA, linear regressions were performed among the functional parameters for all sampling data (from both control and treated). All analyses were performed with SPSS software package v. 12.0 for Windows (*18*).

RESULTS

A summary of the statistical results is shown in **Table 1**.

Total Antioxidant Activity (TAA) and Total Phenolics. The application of the heat treatment led to a significant ($P < 0.001$) increase of the TAA, which was detected 1 day after treatment, with values of 57.21 ± 0.77 and 35.19 ± 2.55 mg equiv ascorbic acid 100 g^{-1} for treated and control fruits, respectively (**Figure 1a**). During storage and subsequent shelf life (SL) periods, TAA was significantly higher ($P < 0.001$) in heat-treated than in control fruits, with final levels of 82.47 ± 1.77 and 73.54 ± 2.46 mg equiv ascorbic acid 100 g^{-1}, respectively, after 90 days

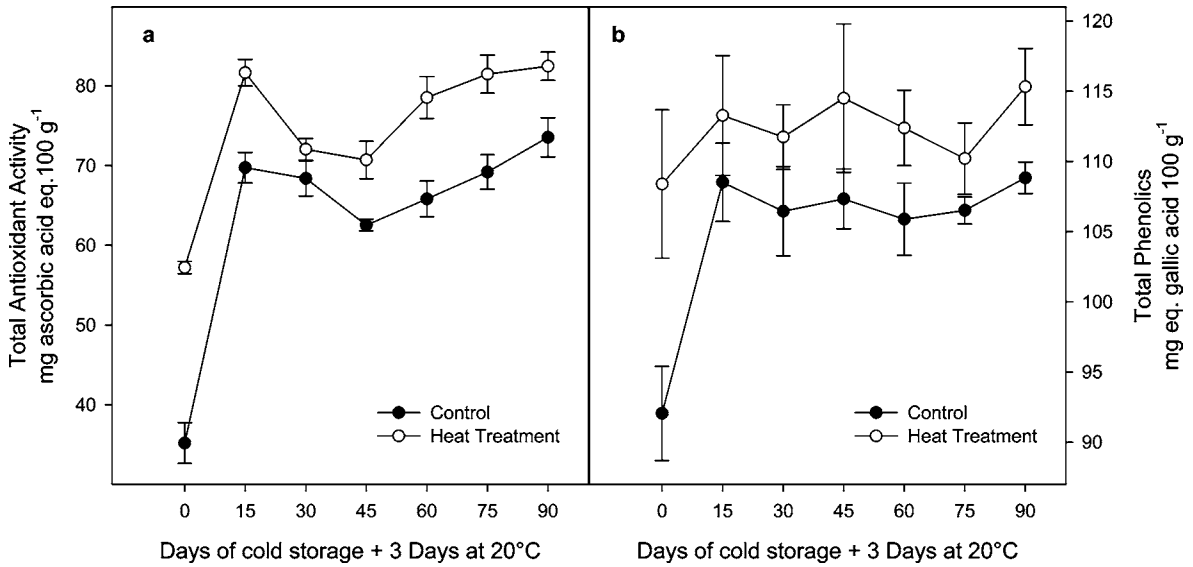

Figure 1. Total antioxidant activity (**a**) and total phenolics content (**b**) in arils during cold storage + 3 days at 20 °C (SL) of control and heat-treated pomegranates.

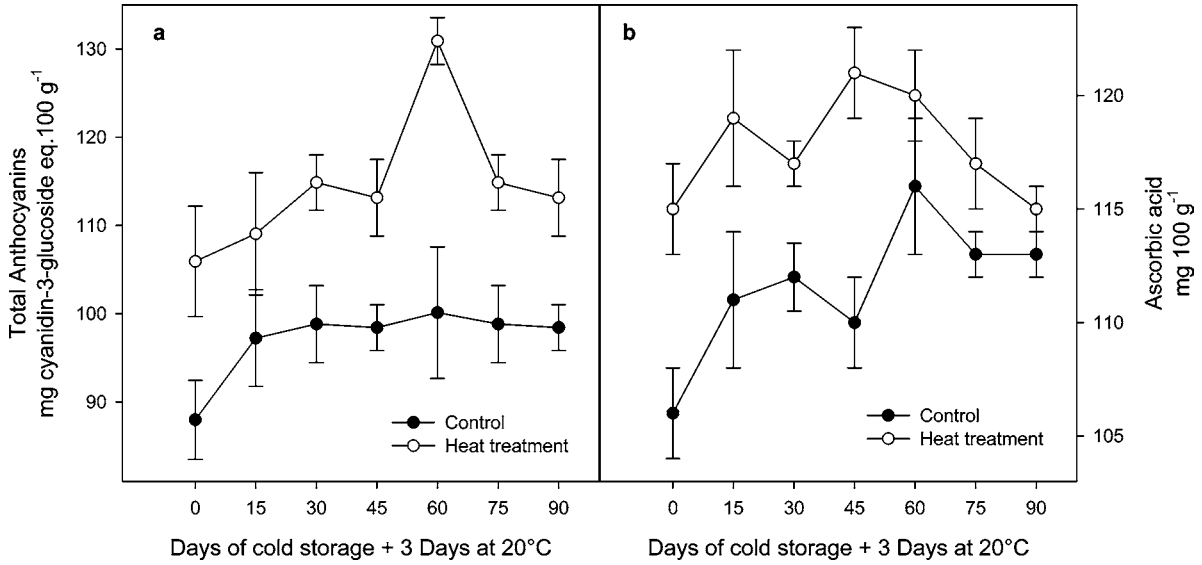

Figure 2. Total anthocyanins (**a**) and ascorbic acid content (**b**) in arils during cold storage + 3 days at 20 °C (SL) of control and heat-treated pomegranates.

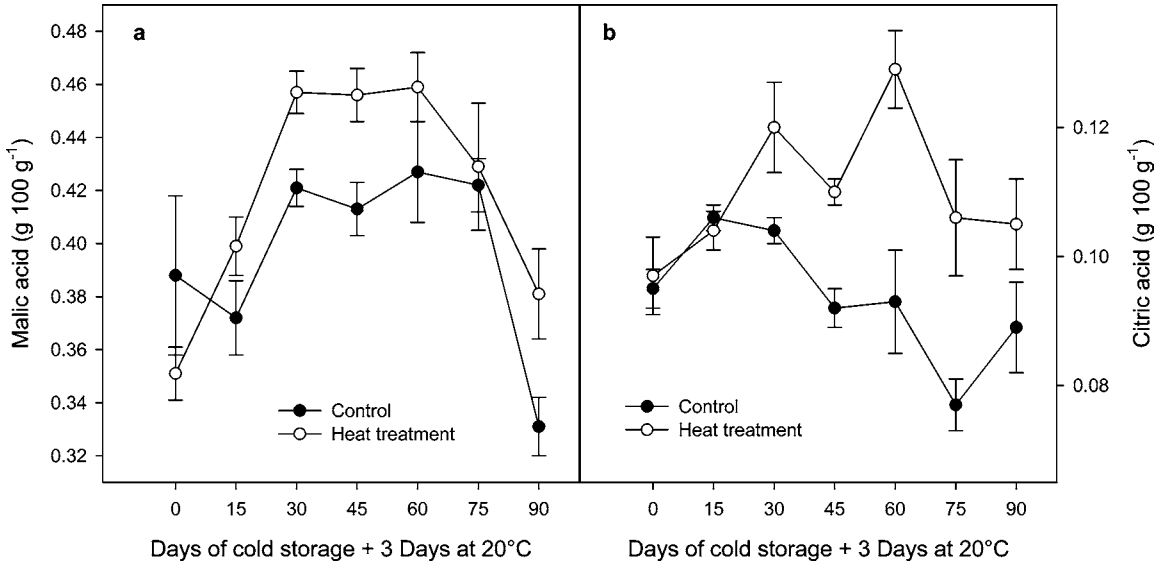

Figure 3. Malic (**a**) and citric acid (**b**) content in arils during cold storage + 3 days at 20 °C (SL) of control and heat-treated pomegranates.

of cold storage + 3 days SL. The same behavior was found for total phenolic compounds (**Figure 1b**), for which heat-treated fruits exhibited significantly ($P < 0.05$) higher phenolic content (108.39 ± 5.28 mg equiv gallic acid 100 g^{-1}) immediately after treatment as compared to control arils (92.05 ± 3.36 mg equiv gallic acid 100 g^{-1}). The increase in total phenolics content throughout complementary 3 days at 20 °C due to thermal treatment showed a trend not always significantly different ($P < 0.05$) from the control.

Total Anthocyanins and Ascorbic Acid. The level of total anthocyanins remained significantly higher ($P < 0.001$) in treated than in control arils (**Figure 2a**). In addition, a significant increase ($P < 0.01$) in total anthocyanins occurred in heat-treated with the maximum peak being after 60 days of storage + SL (130.89 ± 2.66 mg equiv cyanidin-3-glucoside 100 g^{-1}). On the contrary, in control arils, the content of total anthocyanin remained unchanged during storage. With respect to ascorbic acid (**Figure 2b**), the effect of heat treatment was clear in maintaining a higher concentration of ascorbic acid, which was detectable immediately after treatment, with concentrations of 115.0 ± 1.98 and 106.2 ± 2.01 mg 100 g^{-1} for treated and control arils, respectively. During storage, the levels of ascorbic acid remained also significantly higher ($P < 0.001$) in treated than in control fruits until 45 days of cold storage + SL. No heat treatment effect was observed after 45 days of cold storage + SL.

Organic Acid and Sugar Contents. The results of organic acids by HPLC revealed that the main organic acid was malic acid (**Figure 3a**), with concentration ranging 0.35−0.46 g 100 g^{-1}, followed by citric acid (**Figure 3b**), which ranged 0.09−0.13 g 100 g^{-1}. The levels of both organic acids were generally higher in heat-treated than in control arils, which were significantly different ($P < 0.05$) between 30 and 60 days of cold storage + SL. The other two organic acids detected occurred at much lower concentrations, 0.02−0.06 and 0.01−0.03 g 100 g^{-1} for succinic and oxalic acids, respectively, while fumaric acid occurred at traces or was nondetectable (data not shown). An initial increase (0.056 ± 0.005 g 100 g^{-1}) followed by a decrease was observed for succinic acid, without significant differences ($P > 0.05$) between heat-treated and control arils (**Figure 4a**). On the contrary, oxalic acid was affected by heat-treatment (**Figure 4b**), because significantly ($P < 0.001$) higher concentrations were observed in heat-treated than in control arils during storage.

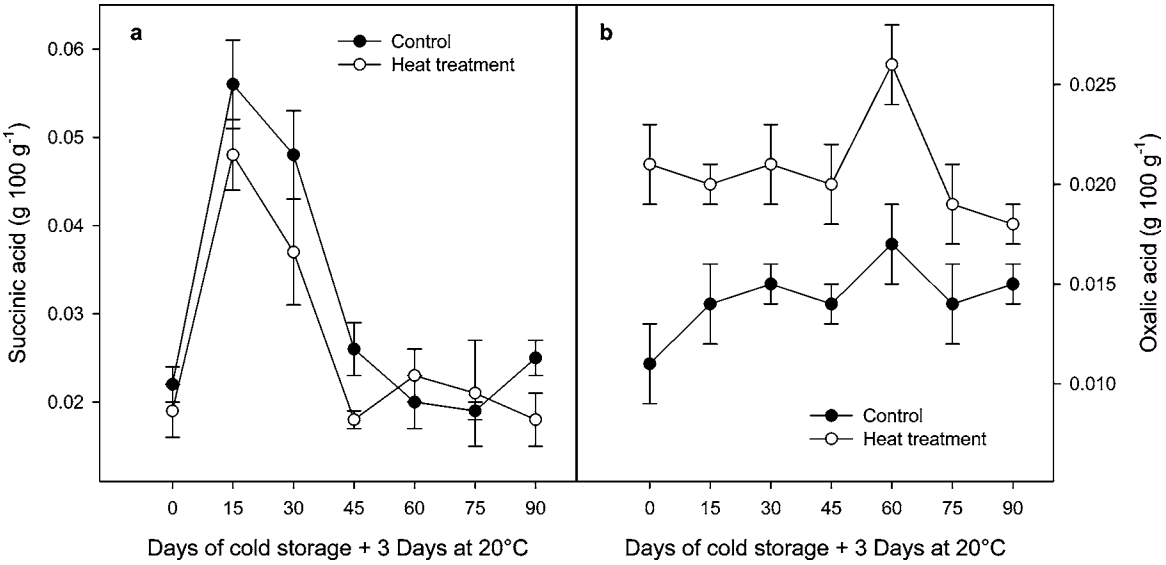

Figure 4. Succinic (**a**) and oxalic acid (**b**) content in arils during cold storage + 3 days at 20 °C (SL) of control and heat-treated pomegranates.

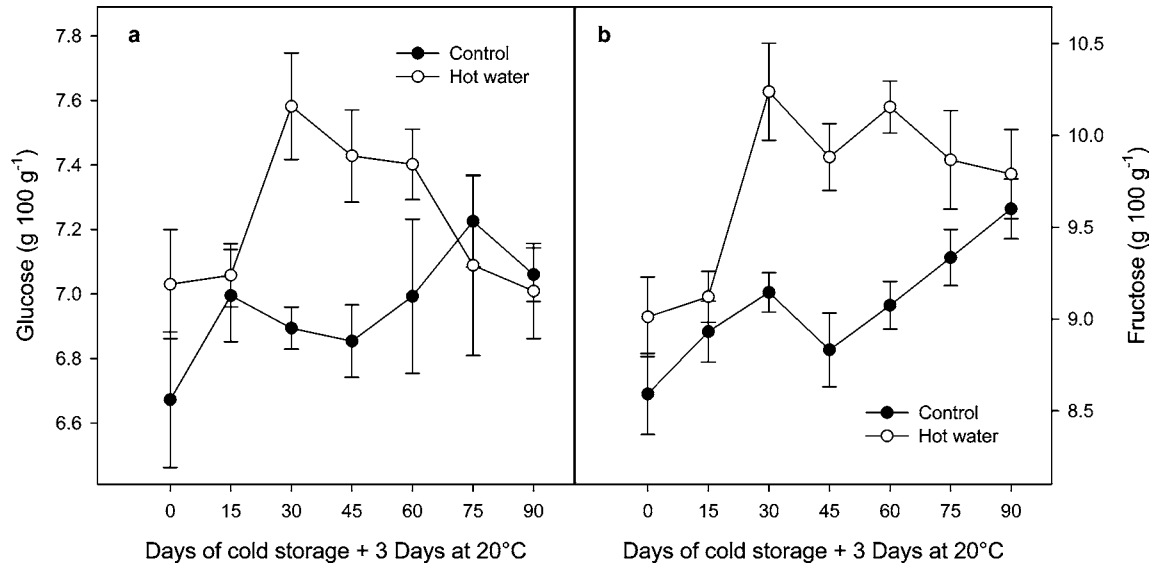

Figure 5. Glucose (**a**) and fructose (**b**) content in arils during cold storage + 3 days at 20 °C (SL) of control and heat-treated pomegranates.

With respect to sugars, the predominant were glucose (6.6−7.6 g 100 g^{-1}) and fructose (8.6−10.3 g 100 g^{-1}), while sucrose occurred at low concentrations (0.25−0.44 g 100 g^{-1}). For this sugar, a significant ($P < 0.05$) increase was observed during storage, although no differences were detected due to treatment (data not shown). Contrarily, the levels of glucose and fructose remained significantly higher ($P < 0.05$) in heat-treated than in control arils during storage (**Figure 5a**,**b**), although final concentrations were similar for glucose (∼7 g 100 g^{-1}) and fructose (∼9.6 g 100 g^{-1}) in both treated and control fruits.

DISCUSSION

The consumption of fruit imparts beneficial health effects based on the content of several compounds with antioxidant activity, including ascorbic acid, flavonoids, and phenolic compounds such as anthocyanins (*19*). We report herein that "Mollar de Elche" pomegranate is rich in these compounds, because higher concentrations of ascorbic acid and total phenolics were found as compared to "Taifi", "Wonderful", and "Ganesh" cultivars (*2*, *8*, *20*). However, during postharvest storage of fruit and vegetables, loss of health-beneficial compounds has been reported, such as in table grape (*21*) and broccoli (*22*). In pomegranate, loss of ascorbic acid (vitamin C) also occurred either during cold storage or at ambient temperatures (*8*).

During pomegranate growth and maturation, increases of anthocyanins and decreases of total phenolics and ascorbic acid are reported in arils (*23*). Because pomegranate has been described as a non-climacteric fruit (*24*), the harvest period marks the levels of these functional compounds. Several methods have been used to maintain or increase the functional properties of fruits and vegetables during postharvest storage, such as the use of edible coating in table grape (*21*), or modified atmosphere packaging (MAP) in broccoli (*22*) and pomegranate (*8*). Moreover, when MAP was combined with essential oils, greater retention and lower losses in functional compounds in table grapes were reported (*25*). All of these treatments also have shown effectiveness on extending shelf life by reducing rates of fruit deterioration, as has been reported for heat treatments (*9*−*12*). However, how heat treatment affects functional and nutritive compounds is still unknown.

The application of heat treatment to pomegranate husks induced higher levels of total phenolic compounds, ascorbic acid, and total anthocyanins, as well as higher TAA during

storage than in control arils. Accordingly, heat-treated strawberries showed increases in both ascorbic acid and TAA due to a stimulation of protective enzymes against oxidative molecules (*26*). Because bioavailability of these compounds from pomegranate juice after in vitro gastrointestinal digestion has been demonstrated (*27*), heat treatment could be a simple and noncontaminant technology that might contribute to protecting humans by increasing TAA during storage of pomegranate. In pomegranate arils, anthocyanin, ascorbic acid, and phenolics are responsible for the TAA, alone or in combination (*23*), as has been observed in several fruits (*28*). In the "Mollar de Elche" cultivar, TAA was highly correlated with total phenolics ($r^2 = 0.70$), while the contribution of ascorbic acid and total anthocyanins to TAA was less significant ($r^2 = 0.50$). During pomegranate fruit development, TAA decreased in the arils, which was correlated to the reduction in the content of total phenolics (*23*). Among the phenolic compounds, punicalagin has been described as the major compound in pomegranate arils contributing to TAA (*29*) as well as derivatives of ellagic acid (*20*), while delphinidin, cyanidin, and pelargonidin were suggested as anthocyanidins participating in the TAA of arils (*30*).

With respect to nutritive compounds, in "Mollar de Elche" cultivar the main sugars were glucose and fructose, while sucrose was found as a minor, according to previous reports of other pomegranate cultivars (*2*, *31*). For organic acids, in this cultivar the predominant acid was malic acid, with citric acid being the next most abundant, while oxalic, succininc, and fumaric were found at much lower concentrations. Different patterns for the organic acid profile have been reported depending on cultivar, with the main organic acids being citric and malic acid (*31*, *32*), although oxalic and tartaric acid were found as main acids in "Assaria" cultivar (*33*). The effect of heat on increasing the sugar concentrations could be attributed to the increase in glucosidase, galactosidase, and arabinase activities, which would release sugars from the cell wall polymers, as has been proposed in kiwifruit after heat treatments (*34*). In addition, higher levels of malic, citric, and oxalic acids were also found in heat-treated pomegranates than in control ones. The higher sugars and organic acids content after heat treatment would maintain the organoleptic quality of the arils, because these compounds are related to the taste and flavor (*2*, *32*). No information has been found about the behavior of organic acids during storage of pomegranates, but these higher concentrations could be related to lower respiration rate, because these compounds are substrates for the respiration and heat treatments were shown to inhibit respiration rate in plums (*15*). Thus, further research is necessary to determine the causes of these changes in pomegranates.

In summary, the application of mild heat treatments to pomegranate could be considered as a non-contaminant postharvest tool with good results in terms of maintenance and/or increase in TAA, total phenolics, total anthocyanins, and ascorbic acid, as well as in sugar and organic acid concentrations, which led to higher functional and nutritive properties after long periods of postharvest storage than in recently harvested pomegranates.

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