

Effect of Hot Air Treatment on Physio-Chemical Properties of Pomegranate Arils

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ABSTRACT

Hot air treatment has been applied on a wide range of horticultural crops to control postharvest decay and to maintain quality characteristics. To evaluate the effect of hot air treatment on physico-chemical properties of pomegranate arils, an experiment was carried out in a factorial experiment using a completely randomized design with four replications in 2015. About 200 g of "Rabab-e-Neyriz" pomegranate arils were placed in 350 mg polypropylene boxes with three holes on top for ventilation. Boxes were heated at 35 °C, 40 °C and 45 °C and 80% relative humidity for 30, 60, and 120 min in oven. After being cooled at an ambient temperature, boxes were stored at 5 °C (RH = 70-80%) for 15 days. The scored results of decay assay showed that heated "Rabab" arils at 35 °C till 45 °C decayed lesser than those untreated during storage period. Hot air temperature at 45 °C for 120 min resulted in the highest weight loss at the end of storage period. The highest total soluble solids content was belonged to the arils heated at 45 °C for 30 min. Hot air treatments increased pH and TSS/TA ratio of aril juice. Hot air temperature treatment decreased antioxidant activity, total phenolic compounds and total anthocyanin of arils during storage period, whilst hot air treatment had no significant effect on color values. Generally, the application of mild heat treatments could be considered as a non-contaminant postharvest tool to maintain functional and nutritive properties of arils during postharvest storage.

Introduction

Pomegranate (*Punica granatum* L.) is a popular fruit of tropical and subtropical regions, belonging to family Punicaceae. It is extensively cultivated throughout the Middle East and Caucasus region, north and tropical Africa, the Indian subcontinent, Central Asia, the drier parts of southeast Asia, and parts of the Mediterranean Basin (Patil and Karade, 1996). The edible part of the fruit (arils) contains a considerable amount of sugars,

vitamins, polysaccharides, minerals and polyphenols (Bchir et al., 2012). The arils serve as an antioxidant fruit due to their polyphenol compounds (i.e. anthocyanins), condensed tannins (i.e. proanthocyanidins) and hydrolysable tannins (i.e. ellagitannins and gallotannins) (Jaiswal et al., 2010). Although pomegranate fruit plays a key role in human health, the preservation of its aril is difficult that caused a reduction of all these advantages (Defilippi et al., 2006). Development of decay is one of the most limiting factors, which is often caused by the

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presence of fungal inoculum in the blossom end of the fruit. During long-term storage, arils face to some postharvest disorders such as rind scald, weight loss, pathogen infection and physiological discoloration (Defilippi et al., 2006).

Heat treatment, a physical method, attracted so much attention as a novel means to extend the storage life of arils (Lurie, 1998; Palou et al., 2008; Schirra et al., 2000). There are three methods of using heat commodities; hot water, vapor heat and hot air treatments. In general, cultivar, fruit condition prior to treatment, temperature, treatment duration, and the mode of heat application were depended on fruit responses to heat. The fruit physiological responses are varied by season and growing location, and can be as a result of differences in climate, soil type, season, cultivation practices, maturity at harvest, and fruit size (Fallik, 2004). Moreover, hot air treatment has been shown not only to reduce pathogens, but also induce resistance of host against fungal attack, thereby inhibiting decay incidence in postharvest fruits (Sabehat et al., 1998). Artés et al. (2000) found that hot air treatment could maintain high levels of organic acids and antioxidant activities in pomegranate fruit and broccoli heads. The combination of hot air treatment and antagonistic yeast had notable inhibitory effects on the infections in peach fruit's wound (Zhao et al., 2019). Hot air treatments at 39 °C for 3 days in peach fruit was found to delay internal breakdown development, although it enhanced the red coloration in both peel and flesh (Bustamante et al., 2012; Lauxmann et al., 2012). Pal et al. (1999) found that mangoes treated with hot air produced a more attractive skin color than the control. If the temperature and duration are inaccurately handled, the internal quality of the fruit would be affected and skin burns may result. Thus, hot air treatment has a great potential as a postharvest treatment for reducing decay and maintaining fruit quality.

Previous studies in heat treatments of pomegranate were related to the hot water treatment; hence, the objective of this study was to evaluate the effects of different hot air treatments on natural decay and fruit quality of pomegranate arils.

Materials and Methods

Plant material

Pomegranate (*Punica granatum* L.) fruits of "Rabab-e-Neyriz" cultivar were bought from NeyRiz, Fars, Iran and stored at 5 °C in cold storage for 7 days until the beginning of the experiment. Pomegranate with defect (sunburn, crack, bruise, and cut in the husk) were discarded and the remaining fruits were thoroughly washed with water and disinfected with hypochlorite sodium (5%) to remove the dirt before applying heat treatments. The husk was carefully cut at the equatorial zone with stainless-steel sharpened knives and arils were manually separated.

Treatments and storage conditions

Approximately 200 g of arils were placed in 350 mg polypropylene boxes, ventilated with three holes of 5 mm diameter on top and then heated at 35 °C, 40 °C and 45 °C and RH = 80 % in hot air oven. Arils were taken out from oven following meeting different heating durations (30, 60, 120 min) and after being cooled at an ambient temperature, the boxes were stored 15 days in incubator at 5 °C with RH = 70-80%. Corresponding controls were not heated and directly stored at the same conditions. Samples of treated arils were evaluated after 15 days to determine the effect of heat treatments on aril quality. The following attributes were determined after 15 days.

Fungal mycelium development assessment (Aril decay)

The development of fungal mycelium on the arils was assessed in control group and hot air

treatments after 15 days. Samples were scored for aril decay according to the following quantitative scale: 0 = no lesion (visible infected area) or no fungal mycelium present, 1 = mycelium present on the aril surface, 2 = lesion $\leq 25\%$ of aril surface, 3 = lesion on 26–50% of aril surface, 4 = lesion $> 50\%$

$$\text{Weight loss (\%)} = \frac{\text{aril weight before storage (g)} - \text{aril weight after storage (g)}}{\text{aril weight before storage (g)}} \times 100$$

Aril color values

Pomegranate aril color was measured using a colorimeter (Chroma Meter, Minolta, Japan). Juice color was assessed according to the Commission International del Eclairage (CIE) and expressed as L*, a*, b*, C, and H° color values. In this coordinate system, the L* value is a measure of lightness, ranging from 0 (black) to +100 (white); the a* value ranges from -100 (greenness) to +100 (redness), and the b* value ranges from -100 (blueness) to +100 (yellowness). The chroma or intensity (C*) were calculated according to the following equation (Romano et al., 2008):

$$C = (a^{*2} + b^{*2})^{1/2}$$

Antioxidant activity

Antioxidant activity of the aril extracts was assessed using 2,2-diphenyl1picrylhydrazylhydrate (DPPH). The DPPH radical scavenging activity of aril extracts was quantified according to the method reported by Mirdehghan et al. (2006) with minor modifications. Briefly, 1 ml of extract was mixed with 1 ml of Tris and 1 ml of DPPH. After leaving for 30 minutes in the dark at room temperature, the absorbance was recorded at 517 nm using a UV visible spectrophotometer (UV 1601, Shimadzu, Japan). Blank is considered with Tris. The antioxidant activity was calculated based on the following equation.

$$\text{A.A (\%)} = [1 - (A_{\text{sample}} / A_{\text{control}})] \times 100$$

Aril weight loss

Weight loss of stored samples was recorded by weighting the samples before and after storage with digital balance and percentage of aril weight loss was calculated by the following equation:

Where, A_{sample} is read by spectrophotometer, A_{control} is mixture of 1 ml of Tris and 1 ml of DPPH.

Total phenolic compounds

Total phenolic content in aril juice was determined using the Folin-Ciocalteu method (Ghasemnezhad et al., 2013) with some modifications. Briefly, 900 μL of diluted juice was mixed with 180 μL of 50% Folin-Ciocalteu reagent and 900 μL of 2% sodium carbonate. The mixture was allowed to stand for 90 min at room temperature in the dark before the absorbance was measured using a UV-visible spectrophotometer (Shimadzu UV 240, Cambridge, USA) at 760 nm. The results were expressed as mg gallic acid equivalent in 100 mL of juice (mg GAE/100 mL of juice).

Anthocyanin content

Anthocyanin content was determined using the pH differential method described by Kirca et al. (2007). Two samples of 150 μl were taken from the upper phase of pomegranate juice, and each one was placed in 25 ml flask. The first flask was diluted with buffer solution pH 1 (1.49 g KCl/100 ml and 0.2 N HCl) and the second one with buffer solution pH 4.5 (1.64 g sodium acetate/100 ml). After standing for 30 min at room temperature, the absorbance of samples was measured at 520 and 700 nm, using a UV-visible spectrophotometer (Shimadzu UV 240, Cambridge, USA). Pigment content was calculated, based on cyanidin-3-

glucoside using the following equation:

$$A = (A_{520} - A_{700}) \text{ PH } 1.0 - (A_{520} - A_{700}) \text{ PH } 4.5$$

The monomeric anthocyanin pigment content in the original sample was calculated according to the following formula:

$$AC = A \times MW \times DF \times 1000 / \epsilon L$$

Where, A- difference of sample absorbance between pH 1.0 and 4.5, ϵ - molar extinction coefficient for cyanidin-3-glucoside (26,900); L- path length of the spectrophotometer cell (1.0 cm), DL- dilution factor and molecular weight (MW) of cyanidin-3- glucoside (449.2 g/mol), 1000- factor for conversion from g to mg. The result was expressed as mg cyanidin-3-glucoside equivalent/100 g extract.

Titratable acidity (TA), Total soluble solids (TSS) and pH

To measure the titratable acidity, 5 ml of extracted aril juice was diluted to 40 ml with distilled water and titrated with 0.1 N sodium hydroxide. Titratable acidity was calculated as percentage of citric acid by the following formula (Nielsen, 2010):

$$\% \text{ acid (W/V)} = N \times V_1 \times \text{Eq wt} / V_2 \times 10$$

Where N = normality of titrant (usually NaOH (mEq/mL)), V1 = volume of titrant (mL), Eq. wt. = Equivalent weight of predominant acid (mg/mEq), V2 = volume of sample (mL).

Total soluble solids in the extracted juice of aril was measured by a hand-held refractometer (Extech Co., Model RF 10, Brix, 0–32 %, USA), and the results were expressed as percentage.

pH of the juice was also evaluated using a digital pH meter (Extech Co., USA).

Vitamin C

Vitamin C content of arils was determined by the 2, 6-dichlorophenolindophenol method (Tefera et al., 2007). An aliquot of 100 μ L of aril juice extract was diluted to 10 mL with 2% metaphosphoric acid and vortexed for 30 s. Then 1 mL of that mixture was taken and diluted with 9 mL of indophenol. After vortexing, the ascorbic acid content was measured at 515 nm using a UV-visible spectrophotometer (Shimadzu UV 240, Cambridge, USA).

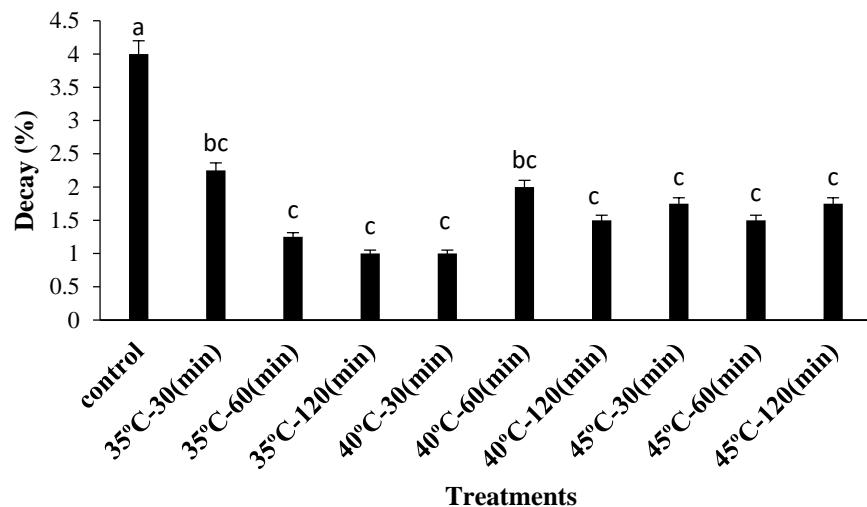
Statistical analysis

This experiment was conducted in a factorial experiment using a completely randomized design with four replicates. Data analysis was conducted using analysis of variance (ANOVA) procedure. The comparison of mean values was carried out according to the Least Significant Difference ($P \leq 0.05$). The SAS software version 9.00 was used on all analyses.

Results

Aril decay and weight loss

After 15 days of storage, the highest decay (internal decay) was appeared in control treatment compared to different heat treatments (Fig. 1). Arils treated with hot air treatments exhibited lower decay than those untreated arils. Arils treated with hot air at 35 °C for 120 min had the lowest decay level; whilst, the difference was not significant with other heat treatments.

**Fig. 1.** Effects of different hot air temperature and duration treatments on "Rabab" pomegranate aril decay.

Means with the same letters are not significantly different at 5% level, LSD

As shown in Table 1, hot air treatment at 35 °C for 60 min had the lowest weight loss (5.07 g); whilst, the difference was not significant with arils heated at 35 °C for 30 min (5.66 g). The highest weight loss was recorded in arils heated at 45 °C for 120 min, while having no significant difference with some treatments.

Titratable acidity (TA), total soluble solids (TSS), TSS/TA ratio and pH

15-day aril storage caused a significant decrease in TA of arils. The highest TA of arils was recorded in control treatment and arils

heated at 45 °C for 120 min (0.94 % and 0.92%, respectively) (Table 1). Arils treated at 40 °C for 30 min had the lowest TA (0.78%). Nonetheless, there was no significant difference with some treatments. Arils heated at 45 °C for 30 min had the highest TSS content (22.90%), compared with the other treatments (Table 1). The ratio of TSS/TA was markedly the highest both in heated arils with 40 °C for 30 min and 45 °C for 30 min. The lowest TSS/TA ratio was recorded in untreated arils (20.69%) (Table 1).

Table 1. Effects of different hot air temperature and duration treatments on aril weight loss, TA, TSS and TSS/TA Ratio of "Rabab" pomegranate aril.

| Temperature °C | Duration of hot air treatment (min) | Aril weight loss (g) | TA (%) | TSS (%) | TSS/TA |
|----------------|-------------------------------------|----------------------|---------------------|---------------------|----------------------|
| Control | 0* | 12.59 ^{ab} | 0.94 ^a | 19.50 ^{bc} | 20.69 ^f |
| | 30 | 5.66 ^c | 0.81 ^{c-e} | 18.37 ^c | 22.51 ^{c-e} |
| 30 | 60 | 5.07 ^c | 0.83 ^c | 19.00 ^{bc} | 22.67 ^{cd} |
| | 120 | 6.47 ^{bc} | 0.81 ^{c-e} | 18.75 ^{bc} | 23.18 ^c |
| 40 | 30 | 9.88 ^{bc} | 0.78 ^e | 19.77 ^b | 25.12 ^{ab} |
| | 60 | 7.17 ^{bc} | 0.91 ^{ab} | 19.27 ^{bc} | 21.14 ^{ef} |
| | 120 | 13.1 ^{ab} | 0.80 ^{de} | 19.25 ^{bc} | 23.84 ^{bc} |
| 45 | 30 | 9.73 ^{bc} | 0.90 ^b | 22.90 ^a | 25.31 ^a |
| | 60 | 8.81 ^{bc} | 0.81 ^{cd} | 19.12 ^{bc} | 23.39 ^c |
| | 120 | 18.48 ^a | 0.92 ^{ab} | 19.65 ^{bc} | 21.28 ^{d-f} |

*Means in each column with the same letters are not significantly different at 5% level, LSD.

Significantly higher pH was obtained in hot air treated arils in comparison with the control. The highest value was recorded in arils heated at 40 °C for 30 min (3.46); whilst, the control treatment showed the lowest value (3.38) (Table 2).

Total phenolic compounds, anthocyanin content and antioxidant activity

After 15-day storage, significant decrease in

total phenolic compounds, total anthocyanin content and antioxidant activity were recorded in arils. Higher total phenolic compounds, total anthocyanin and antioxidant activity were obtained in control compared with the other treatments (Table 2). As shown in Table 2, the lowest total phenolic compounds, total anthocyanin and antioxidant activity were recorded in arils heated at 45 °C for 120 min.

Table 2. Effects of different hot air temperature and duration treatments on pH, total phenol, anthocyanin content and antioxidant capacity of “Rabab” pomegranate aril.

| Temperature °C | Duration of hot air treatment (min) | pH | Total phenol content (mg GAE per 100 mL) | Anthocyanin content (mg per 100 g) | Antioxidant activity (%) DPPHsc) |
|----------------|-------------------------------------|--------------------|--|------------------------------------|----------------------------------|
| Control | 0* | 3.38 ^c | 80.95 ^a | 16.60 ^a | 70.77 ^a |
| | 30 | 3.44 ^{ab} | 81.01 ^a | 14.56 ^b | 67.10 ^{ab} |
| 35 | 60 | 3.42 ^b | 80.61 ^a | 13.68 ^b | 64.59 ^{bc} |
| | 120 | 3.42 ^b | 77.11 ^b | 11.26 ^c | 62.87 ^{cd} |
| 40 | 30 | 3.46 ^a | 76.65 ^b | 11.16 ^c | 59.82 ^{de} |
| | 60 | 3.44 ^{ab} | 75.54 ^{bc} | 10.20 ^{cd} | 58.86 ^{d-f} |
| | 120 | 3.42 ^b | 73.27 ^d | 10.41 ^b | 57.12 ^{ef} |
| 45 | 30 | 3.43 ^{ab} | 75.01 ^{b-d} | 9.24 ^d | 57.48 ^{ef} |
| | 60 | 3.44 ^{ab} | 73.98 ^{cd} | 9.16 ^d | 56.10 ^{ef} |
| | 120 | 3.42 ^b | 68.51 ^e | 7.69 ^e | 54.93 ^f |

*Means in each column with the same letters are not significantly different at 5% level, LSD.

Vitamin C

The highest and the lowest values of vitamin C were recorded in control and hot air treatment at 45 °C for 120 min, respectively (Fig. 2).

Extended treatment duration (120 min) significantly reduced the vitamin C content of arils compared with the control treatment (Fig. 2).

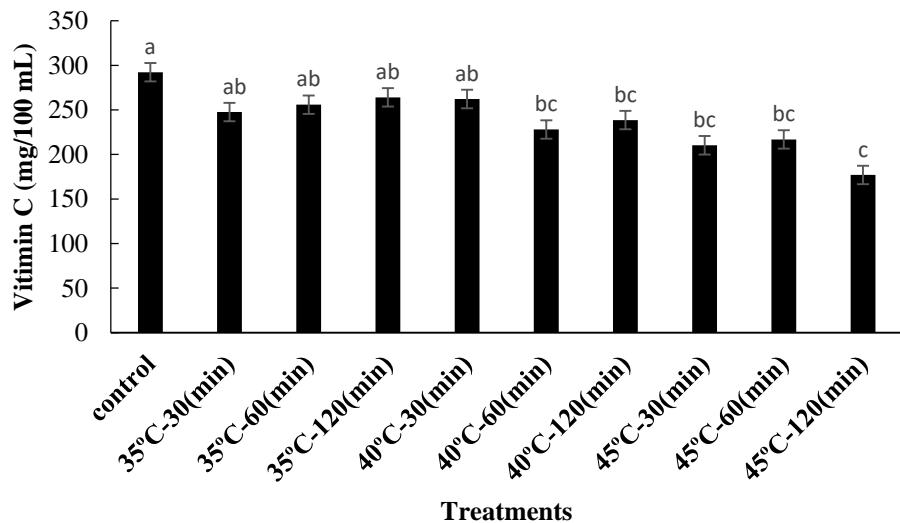


Fig. 2. Effects of different hot air temperature and duration treatments on Ascorbic Acid of “Rabab” pomegranate arils. Means with the same letters are not significantly different at 5% level, LSD

Aril color values

No significant differences were observed in aril color (L^* , a^* , b^*) between heated arils and control treatment (data not shown).

Discussion

The results obtained in this study revealed that the use of different heat treatments caused a significant effect on qualitative and quantitative traits of arils.

In the current study, heated arils had the lowest decay symptoms. Heat treatments have been used to prevent fungal decay, control insect pests, increase tolerance to chilling injury, delay ripening, and extend postharvest life of various fruits and vegetables (Lurie, 1998). In line with our results, Smith and Worthington (1965) and Pan et al. (2004) found that hot air treatment was more effective than hot water dip in reducing decay in strawberries. It seems that the mechanism of the heat treatment application in variety of different commodities may be via either direct interaction with the fungus itself or via physiological response of the fruit tissue through the up-regulation of defense mechanisms or delaying ripening and senescence (Maghoumi et al., 2013). In

accordance with the present study, the detrimental effect of heat treatment on microorganism growth could be direct and/or indirect. Directly, it could retard fungal spore germination; while indirectly, it could activate defense responses in fruit, mainly as an effect of heat shock. Thus, our result revealed that high temperature in short time is more capable to rapid inactivation of microorganisms. So, it was suggested to be used high temperature /short time (HTST) term (Civello et al., 1997).

In this study, it was found that arils heated at 45 °C for 120 min led to significantly higher weight loss than the control. It seems that the longer the heat treatment duration, the greater the weight loss was occurred (Table 1). Additionally, in other studies reported that some fruits are even more perishable and their quality declines more rapidly if subjected to inappropriate heat treatment, which was in accordance with the current study. According to finding of Civello et al. (1997), hot air treatment at 50 °C for 2, 4 or 5 h resulted in damage in strawberry fruit, with greater fungal growth and enhanced quality deterioration compared with the control. In another case, hot air treatment at 58 °C caused tissue damage and increased weight loss and berry color darkening in table grape fruit

(Lydakis and Aked, 2003). Previous studies have found that hot air treatment (45 °C for 4 h) were confirmed to decrease fruit respiration rate and ethylene production, and keep the integrity of membrane structure (Zhao et al., 2019).

According to the results of the present study, heat treatments decreased titratable acidity in arils. The reduction of TA by high temperature treatment has been reported in apples and tomatoes (Lurie, 1998), which was in line with the current study. Furthermore, a decrease in total acidity after application of heat treatment on 'Selva' (Vicente et al., 2002) and 'Tudla' (Garci'a et al., 1995) strawberries, apple, nectarine, grapefruit and tomato (Vicente et al., 2002) were found. It seems that heat treatments increased the rate of respiration, resulting in use of organic acid as a substrate for the process (Vicente et al., 2002).

In the present study, arils treated at 45 °C for 30 min had the highest TSS content. It seems that heat treatments postponed the decrease of soluble solids content, led to change in the metabolism of mono and disaccharides (Garci'a et al., 1995). In the current study the highest TSS /TA ratio might be due to the heat stress of aril which improved the permeability of vacuolar membrane and enabled acid to enter cytoplasm, and caused the decrease of TA content (Zhao et al., 2019).

The current study showed that treated arils recorded the highest pH content. In line with our results, an increase in pH content in heat-treated fruit has been reported in 'Anna' apples (Lurie and Klein, 1990) and 'Tudla' strawberries (Garci'a et al., 1995) that might be due to the increased membrane permeability in heated fruit leading to enzymatic degradation of organic acids which released from the vacuole.

In the present study the reduction of total phenolic compounds, total anthocyanin content and antioxidant activity can be as a

result of thermal degradation of anthocyanins and phenolic acid in heated arils (Madrigal-carballo et al., 2009; Devic et al., 2010) and also the astringency reduction of the fruits, which is considered a desirable sensory attribute in pomegranate (Meighani et al., 2017). Additionally, the lowest total phenolic compounds can be related to the activity of polyphenol oxidase due to use as the substrate, which was in accordance with the Madrigal-carballo et al. (2009) findings. In the current study, hot air treatments reached the highest antioxidant activity, which was in agreement with the findings of Madrigal-carballo et al. (2009) and Devic et al. (2010), who reported that phenolics are the most important compounds contributing to the maintenance of antioxidant statue. Moreover, it was suggested that antioxidant compounds are considered as an indicator of the quality of food processing due to its low stability during thermal process (Saxena et al., 2010). This reduction of antioxidant activity could be due to the loss of different components (i.e. phenolics acids, flavonoid and ascorbic acid), which are responsible for the antioxidant activity of pomegranate arils, during heating treatments (Saxena et al., 2010). Our results revealed that although natural antioxidants are lost during heating, the overall antioxidant properties of aril pomegranate could be maintained or enhanced by the formation of new antioxidant compounds with enhanced antioxidant properties, which was in line with the findings of Vega-Galvez et al. (2009). Increase in antioxidant activity following thermal treatment has been also reported in many vegetables (Kang et al., 2006; Meighani et al., 2014). Moreover, it seems that high activity of antioxidant compounds can reduce the accumulation of reactive oxygen species, leading to enhancing storage quality of arils (Ranjbari et al., 2016).

The application of hot air treatment led to the significant difference of the ascorbic acid concentration. Slight negative effect of hot air

treatment on vitamin C concentration compared with control was detected. The higher the temperature, the more vitamin C degradation (Oms-Oliu et al., 2008). Hot air treatments have been reported to affect vitamin C either negatively or positively in the other crops. The highest content of total ascorbic acid was observed in non-heated tomato (Soto-Zamora et al., 2005), but in strawberry higher level of ascorbic acid was found in heat treated fruit than in the control (Vicente et al., 2006).

In the current study, aril color values were not affected by different heat treatments that was not in accordance with the finding of Pal et al. (1999), who reported that mangoes treated with hot air produced more attractive husk color than the control.

Conclusion

In this study, we found that hot air treatment not only reduced decay, but also caused higher levels of TSS/TA ratio and pH during the subsequent storage. Thus, hot air treatment might be a useful technique to control fruit decay and to maintain quality in pomegranate aril during postharvest storage and transportation. As a whole, the application of mild heat treatments to pomegranate could be considered as a non-contaminant postharvest tool to maintain functional and nutritive properties during postharvest storage.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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