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Original Article

Effect of Modified Atmosphere Packaging on Aril Physico-chemical and Microbial Properties of Two Pomegranate Cultivars (*Punica granatum* L.) Grown in Iran

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ABSTRACT

Background and Objectives: Edible parts of pomegranate fruits are a rich source of bioactive compounds. The present research examines the effect of modified atmosphere packaging on the fruit physico-chemical and microbial properties of two commercial pomegranate cultivars grown in Iran.

Materials and Methods: The arils were packaged and stored under four different atmospheres. All of the packaged samples were stored at 4 °C for 15 days.

Results: The results revealed an increase in total acidity of all treatments. The highest total soluble solid (TSS) was observed in 'Yousef-Khani' stored in 10% O_2 + 15% CO_2, while 'Malas-e-Saveh' treated with 20% O_2 + 5% CO_2 showed the highest degree of TSS. The mean value of a* in 'Malas-e-Saveh' arils packed with normal and (15% O_2 + 10% CO_2 + 75% N_2) atmosphere increased significantly. The L* showed a decrease in 'Yousef-Khani'. Total phenolic compounds gradually increased during storage. After storage, decreases in total anthocyanin contents ranged from 12 to 30% for 'Yousef-Khani'. The overall antioxidant activity of arils in 'Yousef-Khani' showed a 6-15% increase during storage. However, a reverse effect was observed for 'Malas-e-Saveh'. The lowest microbial counts were recorded under the atmosphere containing 10 and 15% CO_2.

Conclusions: Packaging of 'Malas-e-Saveh' arils in 15% O_2 + 10% CO_2 and 'Yousef-Khani' in 15% O_2 + 10% CO_2 or 10% O_2 + 15% CO_2 is recommended to extend the shelf-life of ready-to-eat arils.

Keywords: Pomegranate, Modified atmosphere, Phenolic compounds, Anthocyanin, Antioxidant activity

Introduction

The pomegranate (*Punica granatum* L.), cultivated extensively in tropical and subtropical countries, is widely recognized as one of the oldest fruit crops known to humans. There is a broad consensus that the pomegranate fruit is native to Iran, and from there, it has diversified into many other countries, including China, India, Greece, and Spain (1, 2). Based on statistical data, the total pomegranate production of Iran in 2013 was ~910,000 tons (3). During the past decade, there has been a considerable increase in the commercial production of pomegranates worldwide, which could be mainly attributed to its unique sensory properties and potential health benefits. The edible parts of pomegranate fruit are a rich source of acids, sugars, vitamins, polysaccharides, polyphenols, essential minerals, and various kinds of antioxidants, including anthocyanins and ellagitannins, ellagic acid, punicalagin and punicalin. These compounds have the potential to inhibit oxidative stress, have a positive effect on controlling the risk factors for cardiovascular diseases (CVDs), and can stop the progress of such diseases (4, 5).

Despite the above-mentioned beneficial health effects, there are some reasons, which have deterred the widespread consumption of pomegranates. These include the difficulty of extracting the arils (6), and high concentrations of phenolic compounds in the husk (7), as well as oxidative enzymes that could stain and sometimes irritate sensitive skin, when removing the outer covering of the fruit. It seems that industrial processing of pomegranates could be regarded as a highly productive method to obtain ready-to-eat arils, with intact sensory properties and nutritive values. This, in turn, can increase the production and consumption of pomegranates. Moreover, packaging of pomegranate arils makes it possible to exclude

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those fruits with external defects (bruised, cracked, and sunburnt) that are considered commercially unacceptable and unsuitable for fresh marketing. Accordingly, to meet the increasing demand from consumers for fresh-cut and ready-to-eat fruits (8), minimal processing of pomegranates has gained popularity among both producers and those who buy and use these products. Modified atmosphere packaging (MAP), defined as a packaging technology that modifies or alters the gas composition around the products in food packages, has occupied an important place (9). The principal objective of MAP is to produce a product with minimal processing and fresh characteristics similar to those at harvest, especially flavor, color and texture. It also aims to extend the post-harvest shelf-life of the fruit and reduce the microbial hazards yet maintaining the sensory and nutritional properties.

Many studies have been conducted to examine the potential effect of MAP on both quality preservation and shelf-life extension in fruits (10- 13). Gil *et al*. (1996) reported a 12.92% decrease in the total anthocyanin content of pomegranate arils after 7 days of storage at 4^{o}C and under an atmospheric composition of 13.5% O_2 plus 7.5% CO_2 using polypropylene (PP) baskets (7). López-Rubira *et al*. (2005) found a 2.46% increase in total anthocyanin content of pomegranate arils harvested in October, UV-C treated and stored under MAP conditions (26.9-29.2kPa CO_2 plus <1kPa O_2) after 15 days of storage (6). The authors estimated a minimum of 10 days shelf-life for pomegranate arils stored under MAP at 1^{o}C. Garcia *et al*. (2000) suggested that MAP of pomegranate arils at 4^{o}C and under the 7% O_2 plus 15% CO_2 with semi-permeable polymer cannot significantly prolong the shelf-life of minimally processed arils (14). Moreover, after 10 days of storage at 5 ^{o}C with modified atmosphere (6.5% O_2 + 11.4% CO_2) in polypropylene, no significant change, except for an increase in acidity, was found in the chemical activities of pomegranate arils. Despite the studies mentioned above, there is still particular concern about the use and influence of atmosphere modification on the functional and bioactive properties (e.g. anthocyanins) of fresh-cut products, in general, and pomegranate arils, in particular.

In spite of Iran's enormous success as an exporter and producer of pomegranates in the world, there is not enough information about the potential effect of MAP on the physico-chemical properties of Iranian cultivars.

Therefore, the present study aims to investigate the effect of MAP and storage on the physico-chemical and microbial properties of pomegranate arils of two Iranian commercial cultivars (Malas-e-Saveh and Yousef-Khani).

Materials and Methods

Chemicals: The anthocyanins standards were purchased from Apin Chemicals Co. Ltd., (Oxfordshire, England), and HPLC grade Methanol (MeOH) was purchased from Caledon Laboratories (Ontario, Canada). Formic acid, acetonitrile, sodium hydroxide, Folin–Ciocalteu reagent, and plate count agar (PCA) were obtained from Merck Chemical Co. (Darmstadt, Germany). Radical 2, 2-diphenyl-1-picrylhydrazyl (DPPH) was purchased from Fluka (Germany).

Sample preparation: Two commercial pomegranate cultivars ('Malas-e-Saveh' and 'Yousef-Khani') were purchased from the Agricultural Research Centre of Saveh, Iran. The fruits were transported, on the same day, by a ventilated car to a laboratory at Tarbiat Modares University (Tehran, Iran). After discarding defective ones visually, each fruit was thoroughly rinsed with cold water and drained; then the arils were manually extracted. The separated and homogenized arils were vacuum-packaged, using a packaging machine (Henkelman model 200, USA), under four different atmospheres: (A) (20% O_2 + 5% CO_2 + 75% N_2), (B) (15% O_2 + 10% CO_2 + 75% N_2), (C) (10% O_2 + 15% CO_2 + 75% N_2), and normal atmosphere. Moreover, we used a 3-layer coating comprising polyethylene (LDPE), polyamide, and polyethylene (thickness 60 µm) (Tahavol Kala Novin Co. (Nadipack, Iran).

The vacuum-sealing machine utilized (MAP) in the present study relies on a technology that automatically carries out the vacuum process by extracting the air from the product once the lid is closed. Then the cycle completes by adding a gas immediately followed by sealing of the package. A mixture of nitrogen (N_2), carbon dioxide (CO_2) and oxygen (O_2) is then added using a pump. All the packed samples were stored at 4 °C for 15 days. Microbial and chemical properties of the samples were determined before and after packaging on days 5, 10 and 15 of storage. In each stage, the samples' juice was centrifuged (10000 rpm) for 2 min at -4 ^{o}C (4).

Determination of physico-chemical properties: The amount of titratable acidity was determined potentiometrically using 0.1 M NaOH to the titration

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end point of pH 8.1 and was expressed as %citric acid (4). A refractometer was employed to measure total soluble solids (TSS), expressed as °Brix, at 20 °C.

Color: Based on the method described by Alighourchi and Barzegar (2009), aril color was evaluated using a colorimeter (Colourflex, VA, USA). Furthermore, CIELAB parameters, L* (lightness and darkness), a* (redness and greenness) and b* (yellowness and blueness) were reported (15).

Total phenolic content: Total polyphenols were estimated colorimetrically according to Folin–Ciocalteu method, as described in Mousavinejad *et al*. (2009), using gallic acid as a standard. The results were expressed as mg gallic acid L^{-1} juice. Moreover, absorption was measured at 760 nm (1).

Individual and total anthocyanins: The samples` anthocyanins were separated and determined by HPLC equipped with Empower Software (Waters Corp., Milford, USA), UV–Vis detector and a Waters 600E high-pressure pump. The separation was done by a Nucleodur C18 Gravity column (250 mm × 4.6 mm, dp 5 µm) from Macherey-Nagel (Düren, Germany). Prior to HPLC injection, the juice samples were centrifuged (10000 rpm) for 2 min at 4°C and passed through a Sep-pak C18 cartridge (Millipore, Milford, USA). A Millipore Swinnex type filter (pore size = 0.45 µm) was used to remove the particles. After that, 20 µL of clarified juice was injected onto the HPLC with three replicates. The separations were performed using gradient HPLC method with the following mobile phase program: formic acid 10% (A) and acetonitrile (B). The linear gradient started from 95% A and 5% B for 1.67 min; 90% A and 10% B for 3.34 min; 80% A and 20% B for 20 min; and finally, 95% A and 5% B for 25 min. The flow rate was 0.8 mL min^{-1}, and the chromatograms were recorded at 520 nm. It is to be pointed out that the methodology reported by Del Carpio Jimenez *et al*. (2011), which was further improved by Alighourchi *et al*. (2013), was applied to determine the anthocyanins (16, 17). Anthocyanins were identified by comparing their retention times with those of pure standards, and the their concentration was calculated using an external standard method. Finally, total anthocyanins were estimated by adding the amounts of the six individual anthocyanins previously identified in each chromatogram.

Total antioxidant activity: The antioxidant activity was assessed by DPPH radical scavenging method (18). The absorbance values of the final solutions were determined at 517 nm by a UV–Visible spectrophotometer (SCINCO, Seoul, South Korea). Moreover, radical scavenging activity was presented as inhibition percentage. The antioxidant activity was calculated using the following equation:

$$\textit{Antioxidant activity}\ (\%) = [1 - (A_{sample}/A_{control})] \times 100$$

Microbial analysis: Microbiological quality was assessed by total plate count. To enumerate the microbial load present in the arils, the samples were serially diluted and plated by pour-plating on plate count agar (PCA, Merck). Agar plates were incubated at 30°C for 48–72 h. The results are presented as log CFU mL^{-1} (6).

Statistical analysis: A minimum of three replicates were used for each treatment. The experimental data obtained were analyzed by the Statistical Analysis System (SAS) software using analysis of variance (ANOVA) and the differences among means were determined for significance at $P < 0.01$. The LSD Multiple Range Test was also applied when appropriate.

Results

Total soluble solids (TSS) and total acidity (TA): At harvest, TA values for fresh-cut Malas-e-Saveh and Yousef-Khani cultivars were 1.05±0.00 and 0.71±0.01 (% citric acid), respectively. As indicated in Figure 1, there was a significant increase ($P < 0.01$) in the TA of both Malas-e-Saveh and Yousef-Khani cultivars at the end of the storage time. These results agree with those of Gil *et al*. (1996) and Ding *et al*. (2013) in which Dabai fruit (*Canarium odontophyllum* Miq.) samples were stored under modified atmosphere conditions (7, 11).

The initial TSS (°Brix) values of fresh-cut 'Malas-e-Saveh' and 'Yousef-Khani' arils were 17.00±0.10 and 17.15±0.05, respectively. After 15 days of storage, the highest level of TSS was observed in cv. Yousef-Khani stored in 10% O_2 plus 15% CO_2. However, the differences among the three treatments (A and C; A and B; and control) as compared to fresh-cut fruit were not statistically significant. At the end of storage time, no significant difference was observed in the TSS values of Malas-e-Saveh in comparison with the fresh-cut fruit (treated with 20% O_2 plus 5% CO_2). However, decreases in TSS values were observed for the other treatments.

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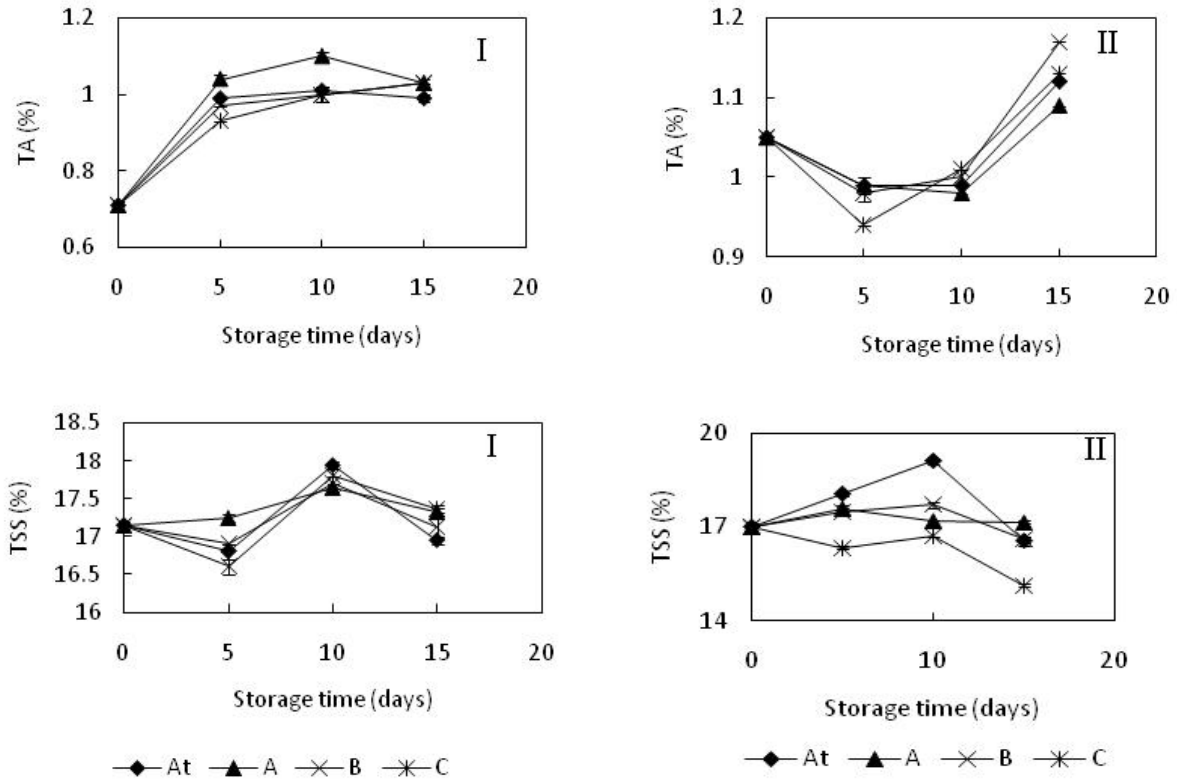

Figure 1. Evaluation of titrable acidity (TA) and total soluble solids (TSS) of pomegranate arils packed under modified atmosphere and control during storage. I: cv. Yousef-Khani, II: cv. Malas-e-Saveh; At: normal air; A: 20:5:75 O_2:CO_2:N_2; B: 15:10:75 O_2:CO_2:N_2; and C: 10:15:75 O_2:CO_2:N_2.

Changes in pomegranate color: The values for CIE a* (redness), b* (yellowness) and L* (lightness) parameters for pomegranate arils both before and after packaging are presented in Figure 2. According to the color evaluations, a* of 'Yousef-Khani' arils decreased in all atmospheres while air-stored 'Malas-e-Saveh' arils and those treated with 15% O_2 plus 10% CO_2 showed higher red color at the end of the storage time. There were no significant differences in b* value of 'Yousef-Khani' arils among different treatments. However, 'Malas-e-Saveh' arils showed significantly lower yellowness. The L* value of 'Yousef-Khani' arils was reduced while higher levels of L* were observed for 'Malas-e-Saveh' arils (Figure 2).

Evaluation of total phenolic content: The initial total phenolic content values varied ranging from 1040.00±30.00 to 953.30±42.90 (mg gallic acid L^{-1} juice) for the arils of 'Yousef-Khani' and 'Malas-e-Saveh', respectively. As seen from Figure 3, at the 5th day of storage, the TPC of pomegranate arils decreased significantly as compared to the initial levels however; it then started to increase significantly for all the treatments. The patterns of the observed variations were in accordance with those reported by Giacalone and Chiabrando (2013) for strawberries and by D'Aquino *et al*. (2010) for film-wrapped pomegranates (10, 5).

At the end of the storage time, air-stored 'Yousef-Khani' arils showed the lowest loss of phenolic content (19.22%). It is worth noting that there was no significant difference between the C and B treatments. On the other hand, the TPC of cv. Malas-e-Saveh arils packed with normal atmosphere (with a 0.69% increase) and those packed under the B treatment (with a 2.12% increase) was not statistically different in comparison to their initial level. Moreover, no significant difference was observed between the A (with a 9.82% increase in TPC) and B treatments.

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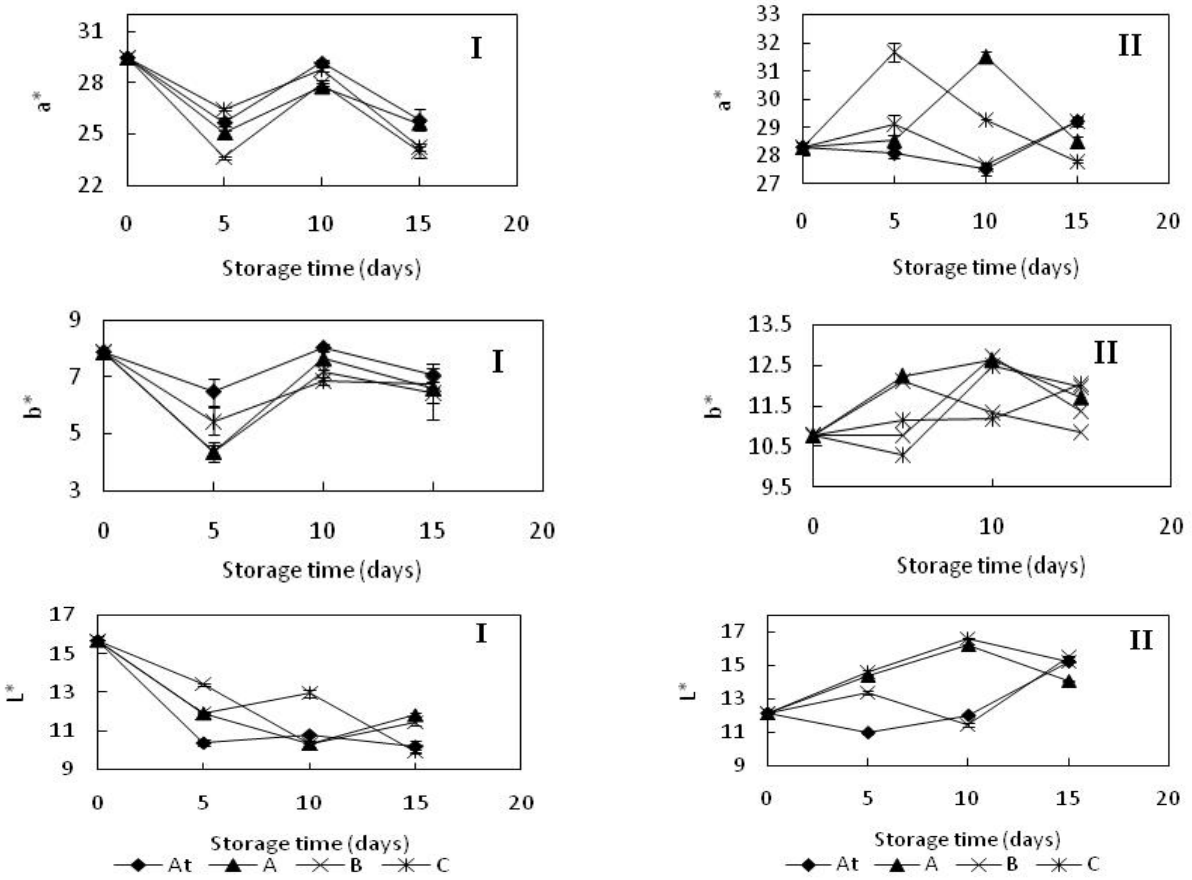

Figure 2. Evaluation of a*, b* and L* of pomegranate arils packed under modified atmosphere and control during storage. I: cv. Yousef-Khani, II: cv. Malas-e-Saveh; At: normal air; A: 20:5:75 O_2:CO_2:N_2; B: 15:10:75 O_2:CO_2:N_2; and C: 10:15:75 O_2:CO_2:N_2.

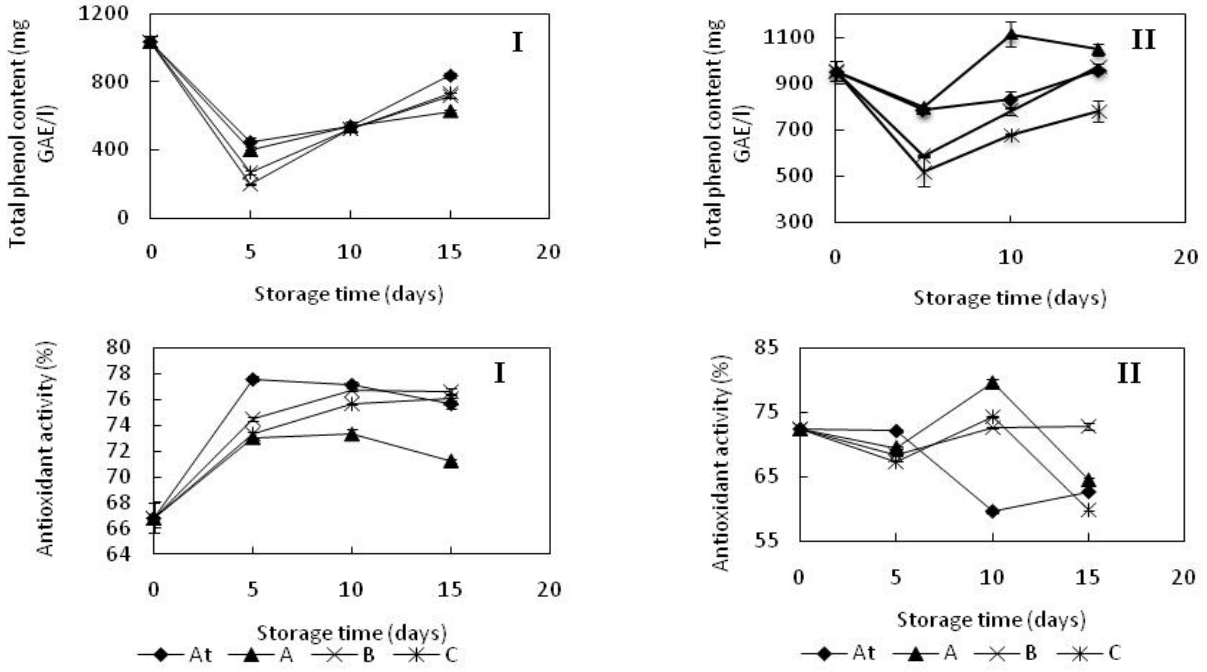

Figure 3. Evaluation of total phenol content and antioxidant activity of pomegranate arils packed under modified atmosphere and control during storage. I: cv. Yousef-Khani, II: cv. Malas-e-Saveh; At: normal air; A: 20:5:75 O_2:CO_2:N_2; B: 15:10:75 O_2:CO_2:N_2; and C: 10:15:75 O_2:CO_2:N_2

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Measurement of anthocyanin content: The predominant anthocyanins found in the pomegranate aril juices were as follows: delphinidin 3, 5-diglucoside (Dp35) and 3-glucoside (Dp3), cyanidin 3, 5-diglucoside (Cy35), 3-glucoside (Cy3), pelargonidin 3, 5-diglucoside (Pg35), and 3-glucoside (Pg3). The profile of the identified anthocyanins was in accordance with the previous studies (1, 4). The initial total anthocyanin content for Yousef-Khani and Malas-e-Saveh cultivars was 686.64 and 563.74 (mg L^{-1}), respectively. The results further indicated a decrease in the anthocyanin content of both cultivars in all the treatments except for air-stored 'Malas-e-Saveh' arils and those stored in 15% O_2 plus 10% CO_2 (Figures 4 and 5). At the end of the storage time, there was 13.62% increase in the total anthocyanin content of 'Malas-e-Saveh' under normal atmosphere; this increase was about 9.25% under the B treatment (15% O_2 + 10% CO_2). On the other hand, in the case of 'Yousef-Khani' arils, the least decrease in total anthocyanin content was 12.83% for control; this decrease ranged from 18.68 to 29.71% for the other treatments.

Table 1 also shows the individual anthocyanin contents of two studied cultivars. According to the data, cyanidin 3, 5-diglucoside was found to be the most representative anthocyanin in both cultivars. Anthocyanins responsible for pigmentation of pomegranate arils were also quantified during the storage time (Figures 4 and 5). The results indicated that neither the different atmospheres nor even the storage time affected the anthocyanins profile. Moreover, during the storage time, cyanidin 3, 5-diglucoside had the highest and pelargonidin 3-glucoside had the lowest concentration.

After 15 days of storage, the delphinidin, pelargonidin, cyanidin glucoside contents of Malas-e-Saveh *cultivars* stored under normal atmosphere increased by 13-24%, and under the B treatment, this increase amounted to 6-55%. However, there was 16.43% decrease in delphinidin-diglucoside value under normal atmosphere and about 28% under the B treatment. The arils stored under the A and C atmospheres showed significant decreases in all anthocyanins, except for delphinidin 3-glucoside under the A atmosphere. Previous studies have indicated that delphinidin glycoside derivatives are among the best substrates for enzymatic oxidation (7), and that the decrease in these anthocyanins was more significant as compared to other anthocyanins. In addition, it has been reported that delphinidin diglucoside derivatives are more stable than monoglucoside ones (4).

The HPLC analysis of Yousef-Khani cultivar showed different results. Mono/di glucoside derivatives of delphinidin and cyanidin decreased under all atmospheres in comparison to the initial values. The lowest loss (12.59-14.31%) for delphinidin derivatives was recorded under atmospheres B and C. On the other hand, the lowest decrease (6-23.09%) in cyanidin derivatives was recorded for the arils packaged under normal atmosphere. Nevertheless, pelargonidin 3, 5-diglucoside content increased considerably.

Evaluation of changes in antioxidant activity: The antioxidant activity of fresh-cut 'Yousef-Khani' and 'Malas-e-Saveh' arils was 66.83±1.16% and 72.40±0.10%, respectively. As the data indicate (Figure 3), the antioxidant activity of 'Yousef-Khani' arils increased during the storage time, with the highest recorded value (12.82%) was for the arils stored in 15% O_2 plus 10% CO_2. However, the arils stored under the B and C treatments did not show a significantly higher antioxidant activity than the control arils. By contrast, the antioxidant activity of 'Malas-e-Saveh' arils decreased. At the end of the storage time, the only treatment that did not result in a significant change in the antioxidant activity of 'Malas-e-Saveh' arils was treatment B.

Table 1. Anthocyanin composition for fresh-cut Malas-e-Saveh and Yousef-Khani cultivars (mg L^{-1}) *

Cultivar	Dp3G	Dp35dG	Pg3G	Pg35dG	Cy3G	Cy35dG	Total
Yousef-Khani	99.65	106.33	5.66	11.99	125.72	337.27	686.64
Malas-e-Saveh	49.19	83.96	4.69	13.75	75.40	336.72	563.74

* Dp3G: delphinidin-3-glucoside; Dp35dG: delphinidin-3, 5-diglucoside; Pg3G: pelargonidin-3-glucoside; Pg35dG: pelargonidin-3, 5-diglucoside; Cy3G: cyanidin-3-glucoside; and Cy35dG: cyanidin-3, 5-diglucoside.

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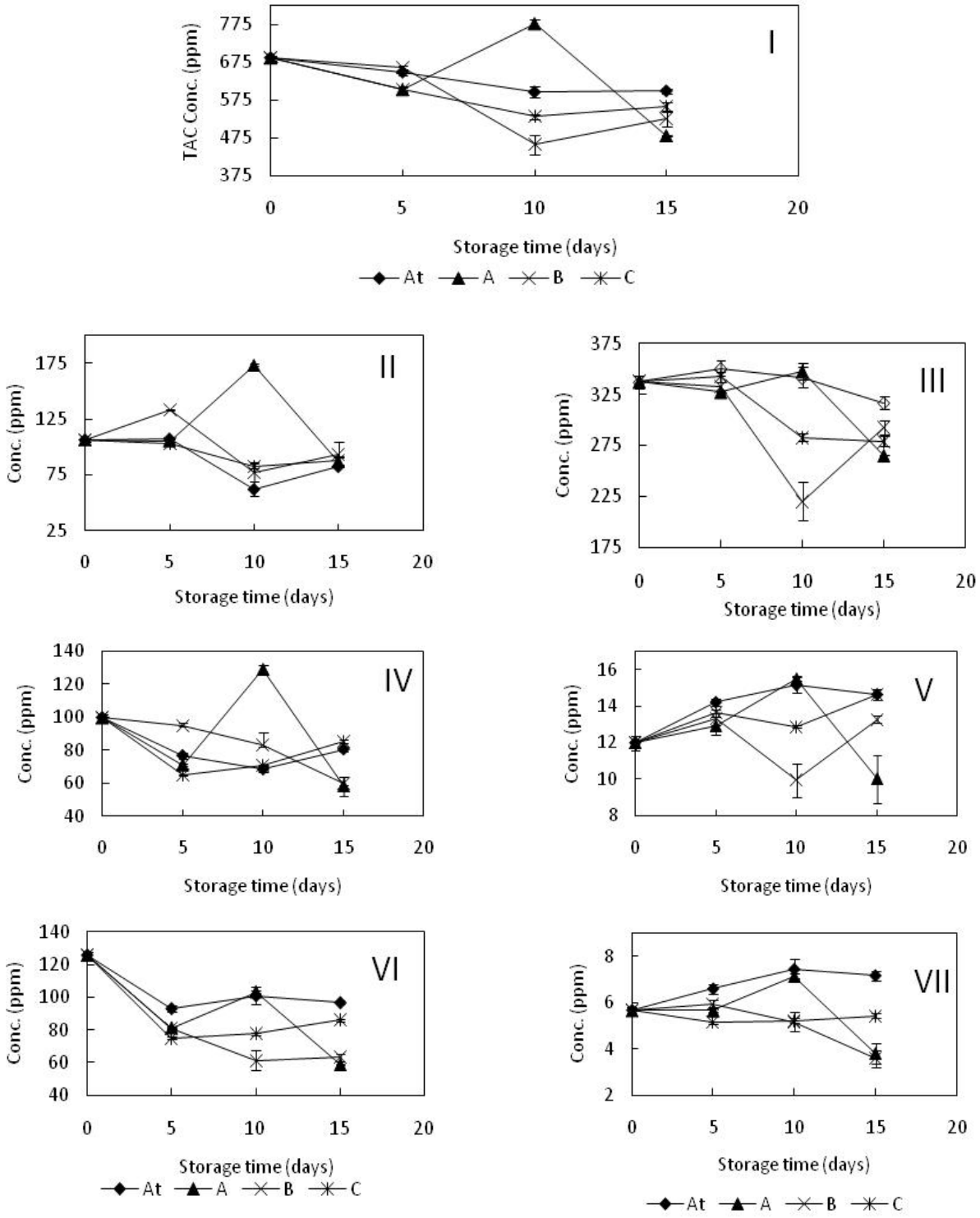

Figure 4. Evaluation of total anthocyanin content of pomegranate arils of Yousef-Khani cultivar packed under modified atmosphere and control (I) and individual anthocyanins content during storage. (II: delphinidin-3, 5-diglucoside, III: cyanidin-3, 5-diglucoside, IV: delphinidin-3-glucoside, V: pelargonidin-3, 5-diglucoside, VI: cyanidin-3-glucoside and VII: pelargonidin-3-glucoside); At: normal air; A: 20:5:75 O_2:CO_2:N_2; B: 15:10:75 O_2:CO_2:N_2; and C: 10:15:75 O_2:CO_2:N_2.

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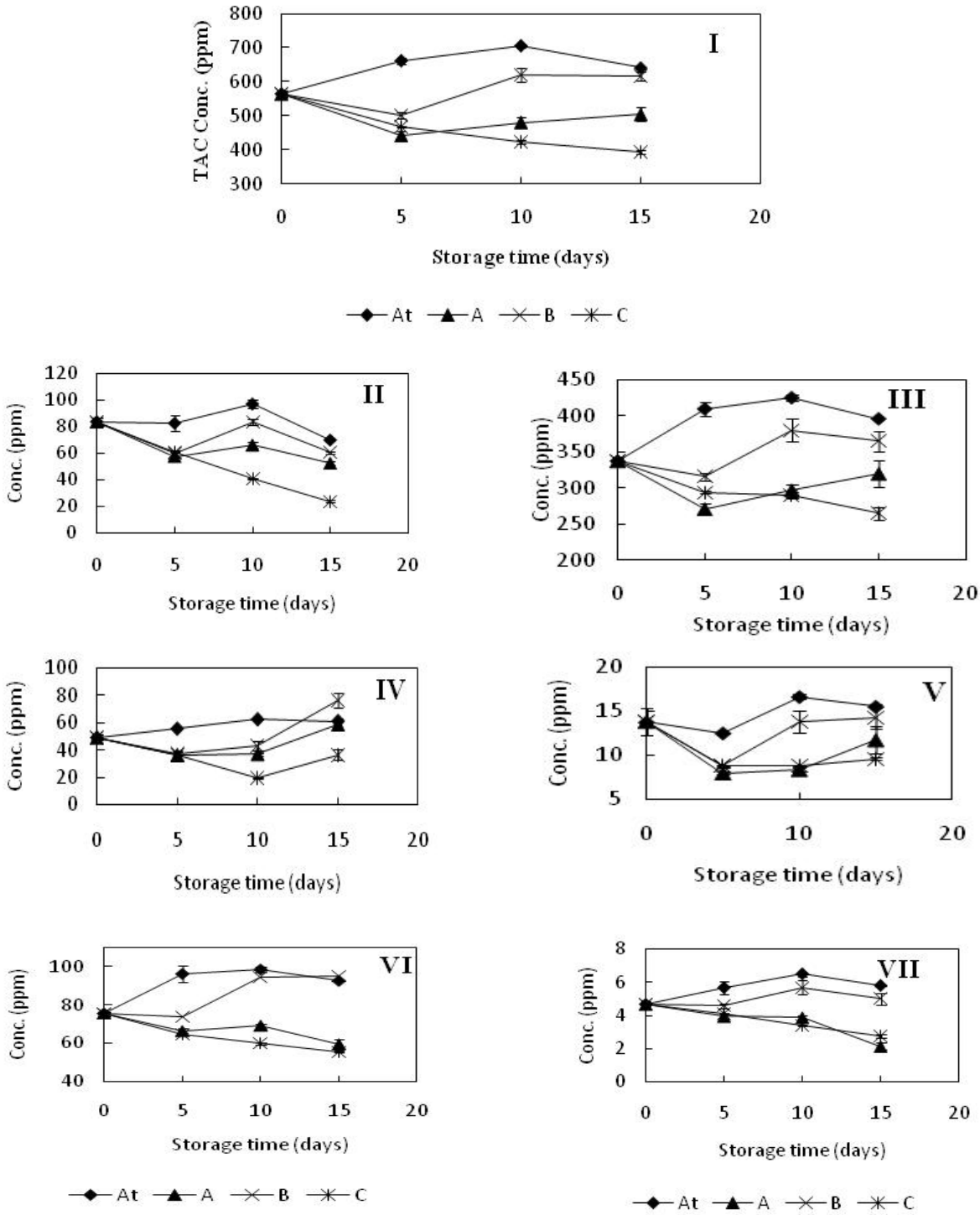

Figure 5. Evaluation of total anthocyanin content of pomegranate arils of Malas-e-Saveh cultivar packed under modified atmosphere and control (I) and individual anthocyanins content during storage (II: delphinidin-3, 5-diglucoside, III: cyanidin-3, 5-diglucoside, IV: delphinidin-3-glucoside, V: pelargonidin-3, 5-diglucoside, VI: cyanidin-3-glucoside and VII: pelargonidin-3-glucoside); At: normal air; A: 20:5:75 O_2:CO_2:N_2; B: 15:10:75 O_2:CO_2:N_2; and C: 10:15:75 O_2:CO_2:N_2.

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Changes in microbial property: As mentioned before, the standard total plate count method was used to enumerate total microbial populations. The initial number of microorganisms in the pomegranate juice for 'Malas-e-Saveh' and 'Yousef-Khani' arils was 2.26 and 2.67 log colony forming units (cfu) mL^{-1}, respectively. The arils treated with 15% O_2 + 10% CO_2 and also those stored in 10% O_2 plus 15% CO_2 showed the lowest count both during the storage period and at the end of the storage time as compared to those treated with higher O_2 concentrations (Figure 6). It is worth noting that no significant microbial growth (<10 cfu mL^{-1}) was observed for the arils treated with the above gas compositions from the 5th day to the end of the storage period. Moreover, the differences in the microbial load for these two conditions were not statistically significant. However, the air-stored arils and those treated with 20% O_2 plus 5% CO_2 showed higher counts.

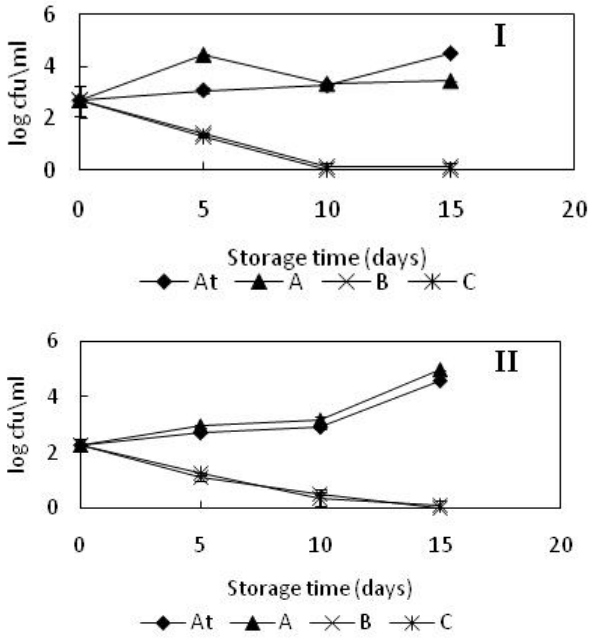

Figure 6. Evaluation of microbial load of pomegranate arils packed under modified atmosphere and control during storage. I: cv. Yousef-Khani, II: cv. Malas-e-Saveh; At: normal air; A: 20:5:75 O_2:CO_2:N_2; B: 15:10:75 O_2:CO_2:N_2; and C: 10:15:75 O_2:CO_2:N_2.

Discussion

Some previous studies (7, 11) have demonstrated that different levels of atmosphere (e.g. high levels of CO_2 and low levels of O_2) in the packages may cause an increase in TA concentration. Moreover, this increase could be explained by other factors such as the amount of water loss of fruit samples as well as microbial growth and activity during the storage time.

The small but insignificant increases in TSS could be attributed to moisture loss or increase in sugar concentrations together with the polysaccharides' breakdown during storage (10, 11).

Based on our analysis of the bioactive compounds (see below), the changes in the a* (redness) value could be related to the changes in total anthocyanin content during the storage time. Similar to this study, Holcroft *et al.* (1998) reported that changes in color characteristics of pomegranate arils were correlated with the increase in anthocyanin concentration. They also observed that the arils stored in normal atmosphere had higher red color intensity than those stored in CO_2-enriched environments (19).

The initial decrease in the TPC of pomegranate arils could be attributed to the specific activity of polyphenol oxidase and its role in TPC degradation (4). On the other hand, the Folin-Ciocalteu assay, besides estimating phenolic compounds, measures the levels of other compounds such as ascorbic acid and anthocyanins, which are highly unstable and may decompose both during the storage time and after sampling. Therefore, the decrease in TPC could also be due to the presence of these compounds. It is worth mentioning that total anthocyanins for both cultivars in the first five days decreased in most of the treatments as compared to their initial level at harvest.

The synthesis of phenolic compounds and the responsible enzymes in this process can be modulated by application of atmosphere modification. Moreover, the increase in phenolic content during the storage time could be explained by changes in the enzyme activities of phenylalanine ammonia-lyase (PAL) as expressed in Kader (1985)(20). Kader reported that PAL acts as the key enzyme contributing to the biosynthesis of phenolic compounds. Furthermore, some previous studies (19, 21) have demonstrated that, in the presence of higher levels of O_2, the activity of PAL in pomegranate arils increases significantly. Based on the obtained results for cv. Malas-e-Saveh arils in the present study, it could be argued that certain levels of CO_2 may positively affect the TPC of pomegranate arils. Gil *et al.* (1998) also reported similar results (22).

The pattern of the observed changes in anthocyanin accumulation for cv. Malas-e-Saveh was in line with that of Holcroft *et al.* (1998) (19). They reported that the anthocyanin concentration of arils from the fruits stored in air with 10 kPa CO_2 increased after 4-6 weeks, and the anthocyanin concentration of

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pomegranates stored in 20 kPa CO_2 for 6 weeks was lower than their initial concentration. Gil *et al*. (1996), however, found a 12.92% decrease in total anthocyanin content in pomegranate arils after 7 days of storage at 4 ^oC and under an atmospheric composition of 13.5% O_2 plus 7.5% CO_2 (7). Moreover, López-Rubira *et al*. (2005), with regard to pomegranate arils harvested in October, UV-C treated and stored under different MAP conditions (26.9 - 29.2 kPa CO_2 and <1 kPa O_2), reported a 2.46% increase in total anthocyanin content after 15 days of storage (6). The authors further observed 28.73% decrease in the total anthocyanin content of arils from the same cultivar harvested in December and after 13 days of storage at 3^oC. Gil *et al*. (1997) and Holcroft and Kader (1999) reported that air and controlled atmosphere with a certain level of CO_2 affected and accumulated anthocyanin levels in strawberries, but these changes were progressively reduced in fruit with elevated CO_2 levels (12, 21). Gil *et al*. (1998) suggested that fresh processed 'Lollo Rosso' lettuce decreased by 37.47% in anthocyanin pigment concentration (cyanidin 3-malonylglucoside) when stored in air and by 69.60% when MAP-stored in the presence of 8% O_2 and 3% CO_2.

The possible effect of 'atmosphere modification' on the anthocyanins' content of arils can arise from two concurrent factors: the enzymatic oxidation of anthocyanins and their biosynthesis in controlled-atmosphere conditions with certain levels of oxygen and carbon dioxide. Based on the findings, it could be concluded that different CO_2 and O_2 concentrations may have an impact on the degradation of anthocyanins as well as on their synthesis (19). The decrease in anthocyanins' content may occur due to the possible effects of enzymatic oxidation on some phenolic compounds such as Dp 3, 5dG, Dp 3G, Cy 3G, etc. (23). Moreover, Brownmiller *et al*. (2008) reported that polymerization of anthocyanins may reduce anthocyanins during storage (24). The authors believe that polymeric compounds formed during storage may occur due to the condensation reactions of anthocyanins with other phenolic compounds. On the other hand, in line with the previous findings (19, 20, 21), our findings revealed that one possible reason for increase in anthocyanins such as Pg35dG could be the post-harvest biosynthesis of phenolic compounds, which is, in turn, dependent on the enzyme activity of the biosynthetic pathway, such as PAL in the arils. Furthermore, it appears that the possible effect of atmosphere modification (10-15% CO_2) on the overall concentration of anthocyanins could be dependent on fruit type. The findings of the present study are in accordance with the previously reported results in this regard (5, 19).

The increase in total antioxidant activity of cv. Yousef-Khani arils, despite the reduced levels of total phenolics, could be explained by the increased amount of organic acids. Hansawasdi *et al*. (2006) found similar results in the case of strawberries, indicating the key role organic acids could play in the antioxidant activity (25). However, a reverse trend was observed for cv. Malas-e-Saveh arils, and a decrease in the total antioxidant activity was found. It could be concluded that the correlation between total antioxidant activity, on the one hand, and total phenolic contents and anthocyanins, on the other, depends on the type of the particular cultivar under study. The same result has been found in Usenik *et al*. (2008) for strawberries (26). Similarly, Ahn *et al*. (2005) have shown that MAP packaging has a positive effect on maintaining the antioxidative activity of the Chinese cabbage (*Brassica Rapa* L.) (27).

The microbial analysis may suggest that the use of coating alone, without altering the normal atmosphere, may not prevent subsequent fungal growth. Similar results have been reported for the 'Malas-e-Saveh' arils stored in polyethylene bags and exposed to hot water treatment (28). In other words, it appears that coating causes a highly moisturized atmosphere and provides conditions that make fungal growth easy. On the other hand, higher metabolic activities of the arils treated with normal air or 20% O_2 plus 5% CO_2 may produce more moisture and warmth, and a subsequent microbial growth should be expected. However, higher levels of CO_2 (10-15%) may be effective in inhibiting microbial growth. Martinez-Ferrer *et al*. (2006), who examined the effect of MAP on minimally processed mango and pineapple fruits, have reported similar findings (13). Bieganska-Marecik (2004) found more or less the same results in the case of apples (29). Both O_2 and CO_2 have important preventive functions. Based on the findings, it could be argued that oxygen inhibits the growth of anaerobic bacteria; also in the present study, lower concentrations of O_2 were found to prevent mold growth as well as the growth of aerobic bacteria. On the other hand, CO_2, by reducing enzymatic activities and reactions and also by altering cell membrane permeability (which, in turn, leads to changes in cell pH and physico-chemical parameters) can inhibit the growth of microorganisms; this

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preventive function increases significantly in temperatures less than 10 ºC (30).

Based on the observed changes in pomegranate juice antioxidant activity, bioactive compounds and microbial growth, packaging of 'Malas-e-Saveh' arils in 15% O_2 + 10% CO_2 (i.e. treatment B) is recommended as the desirable gas composition to extend the shelf-life of this cultivar. It is worth noting that, in the case of cv. 'Yousef-Khani', increases in anthocyanin contents and higher antioxidants in the fruits treated with the modified atmospheres B (15% O_2 + 10% CO_2) and C (10% O_2 + 15% CO_2) were observed at the end of the storage time. Based on the microbial analysis results for 'Yousef-Khani' arils, moderate CO_2 concentrations (10% CO_2) can have some additional advantages such as controlling the post-harvest decay. Therefore, to extend the commercial shelf-life, packaging the arils of this cultivar with an atmospheric composition of 15% O_2 + 10% CO_2 + 75% N_2 or 10% O_2 + 15% CO_2 + 75% N_2 can yield more desirable results compared to air.

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