

Effects of modified atmosphere packaging on the storage and shelf life of Hicaznar pomegranate fruits

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Abstract: This study compared the postharvest quality of Hicaznar pomegranate fruit unpackaged and packaged with 5-kg or 12-kg bags of two different modified atmosphere packaging (MAP) materials (X5, X12, L5, and L12) during 6 months of storage at 6 °C. The fruits were also evaluated during a simulated shelf life period at 20 °C for 7 days after each cold storage period. The MAP bags significantly reduced the loss of titratable acidity and ascorbic acid content and retarded husk discoloration of the fruit during cold storage and the shelf life period. The incidence of fungal decay was lower in fruit packaged with MAP bags than unpackaged fruit. X5 and X12 MAP bags were more effective in reducing weight loss and husk scald than L5 and L12 MAP bags. X5 and X12 MAP bags maintained initial red aril color intensity and antioxidant properties of Hicaznar pomegranates throughout cold storage and the shelf life period. Unpackaged control fruit remained marketable for only 4 months at 6 °C plus shelf life period, while packaged fruit maintained visual quality for up to 6 months at 6 °C plus shelf life period.

Key words: Bioactive compounds, modified atmosphere packaging, pomegranate, quality, shelf life, storage

1. Introduction

The production and marketing of pomegranates (*Punica granatum* L.) have increased during the recent years due to consumer demand for fresh pomegranate fruit and juices, which are a very rich source of antioxidant phenolics and anthocyanins (Gil et al., 2000). The antioxidant properties of pomegranates possess therapeutic benefits in the prevention of cancer, cardiovascular disorders, diabetes, male infertility, and Alzheimer disease (Ramezani and Erkan, 2017).

Turkey is one of the major producers and exporters of pomegranates in the world. According to the data of the Turkish Statistical Institute (<http://www.tuik.gov.tr/>), Turkey produces 465,000 t of pomegranates in mainly the Mediterranean and Aegean regions and exports 184,000 t to major destinations, such as the European Union, the Russian Federation, Ukraine, Iraq, and other Middle Eastern countries. Hicaznar, with its sour-sweet flavor, dark red husk, and aril color, is the major export pomegranate cultivar of Turkey (Özgüven and Yilmaz, 2000). Pomegranates are chilling-sensitive fruits below 5 °C (Kader et al., 1984). In order to avoid chilling injury, pomegranates could be stored at 5 °C for up to 2 months and a safe storage temperature range of 6 to 8 °C is recommended for longer durations with 90%–95%

relative humidity (Erkan and Kader, 2011). The average monthly prices of pomegranate fruit in European markets reach their highest levels during the months of March, April, and May (Rymon, 2012); therefore, the marketing of pomegranates aims to extend the commercial storage period until at least late March (Selcuk and Erkan, 2015). However, long-term storage of pomegranate fruit is often limited by weight loss, decay development, husk scald, and loss of aril quality and taste (Porat et al., 2016).

Modified atmosphere packaging (MAP) has been found to be successful in reducing water loss, visible shriveling symptoms, husk scald, and decay of pomegranate fruit during cold storage (Artés et al., 2000; Nanda et al., 2001; D'Aquino et al., 2010; Selcuk and Erkan, 2014, 2015; Porat et al., 2016). MAP bags have become widely used for pomegranate storage and shipping in pomegranate-exporting countries, including Turkey. However, the use of improper MAP bags leads to excessive decay, husk scald, and weight loss, which impairs visual quality and reduces marketability. In this study, we compared 5-kg and 12-kg bags of two different MAP materials for maintaining postharvest quality and antioxidant properties of the Hicaznar pomegranate cultivar during storage at 6 °C for 6 months and subsequent shelf life of 7 days at 20 °C.

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2. Materials and methods

2.1. Plant material and postharvest treatments

Pomegranate fruits (cultivar Hicaznar) were harvested from a commercial orchard located in Antakya, Hatay, Turkey, at commercial maturity (<1.85% of titratable acidity and >17% of total soluble solids) during the 2015 and 2016 seasons. After harvest, pomegranates were transported to the cold storage facilities of the Department of Horticulture at Mustafa Kemal University (Antakya, Hatay). Fruit at uniform size and maturity without defects (growth cracks, bruises, decay) were then packaged in 5-kg or 12-kg bags of two different MAP materials: Life Pack® [5-kg (L5) and 12-kg (L12) bags, patent no. 2007 45625, Aypek Ambalaj Co., Bursa, Turkey] and Xtend® [5-kg (X5) bag code: 815-PG28/m, 12-kg (X12) bag code: 815-PG18F/R, patent no. 6190710, Serpak Co., Antalya, Turkey]. Unpackaged fruit stored in plastic boxes served as the control. L5 and X5 MAP bags contained 5 kg of fruit while L12 and X12 MAP bags contained 12 kg of fruit. Packaged fruits were cooled to 6 °C for 24 h before sealing the MAP bags and then stored together with control fruits at 6 ± 0.5 °C and 90 ± 5% RH for 6 months. Packaged and control fruits were removed from cold storage at 2-month intervals. After unsealing the packages, 5 fruits per replication were placed in a plastic box for each treatment and kept at 20 ± 1 °C and 70 ± 5% relative humidity for 7 days to simulate a shelf life period.

2.2. Postharvest quality evaluation

Percentage of weight loss was determined in reference to the initial weight (Selcuk and Erkan, 2015) by using the following formula: %WL = [(Wi–Wf) / Wi] × (100), where %WL = percentage weight loss, Wi = initial fruit weight (g), and Wf = final fruit weight (g) at the indicated storage or shelf life period.

Headspace O₂ and CO₂ concentrations inside the MAP bags were monitored using a Check Point model O₂/CO₂ analyzer (PBI-Dansensor America Inc., Glen Rock, NJ, USA). Husk color values were obtained from 3 different measurements taken from the equatorial region of each fruit and aril color values were from 3 different measurements of 20 g of arils in a petri dish (Artés et al., 1998) for each replication of treatments. Husk and aril color were measured based on the CIE L*a*b* color space using a Minolta Chroma Meter CR-300 (Osaka, Japan). Chroma (C*) and hue angle (h°) values were computed from these values. The arils of 5 fruit per replicate were squeezed through cheesecloth by a hand press. The juice obtained was analyzed for total soluble solids (TSS) content using a refractometer (Atago Model ATC-1E, Tokyo, Japan) and titratable acidity (TA) by titration of 5 mL of juice with 0.1 N NaOH to a pH of 8.1, and expressed as percent of citric acid equivalents. Ascorbic acid (AsA) was extracted based on the method described by Lee and Coates (1999).

Briefly, 5 mL of pomegranate juice was mixed with 5 mL of 2.5% metaphosphoric acid and homogenized using an Ultra-Turrax homogenizer for 2 min. After centrifugation at 9418 × g for 5 min at 5 °C, the supernatant was recovered and kept at –20 °C until analysis. HPLC analysis of L-ascorbic acid was conducted as described previously (Candir et al., 2017). The results were expressed as mg 100 mL⁻¹. A 1:5 ratio of diluted pomegranate juices was used to analyze total monomeric anthocyanin (TMA) content, total phenolic (TP) content, and antioxidant capacity (Gil et al., 2000). The TMA and TP contents were determined by the pH-differential method (Lee et al., 2005) and Folin–Ciocalteu method (Singleton et al., 1999), respectively, using a UV-1208 model UV-VIS spectrophotometer (Shimadzu, Kyoto, Japan). TMA content was expressed as mg cyanidin-3-glucoside equivalents (molecular weight = 493.5 g mol⁻¹; molar extinction coefficient = 26,900 in L mol⁻¹ cm⁻¹) per L of juice and TP as gallic acid equivalents (mg GAE L⁻¹). For estimation of antioxidant capacity, both the ferric reducing antioxidant power (FRAP) and the Trolox equivalent antioxidant capacity (TEAC) assays (Ozgen et al., 2006) were used. Antioxidant capacity was expressed as Trolox equivalents (TE) (mmol TE L⁻¹). The overall visual quality was assessed by 10 trained panelists on a 5-point scale, where 1 = very poor, 2 = poor (limit of marketability), 3 = good, 4 = very good, and 5 = excellent (Selcuk and Erkan, 2015). The taste of the fruit was also evaluated by 10 trained panelists using a hedonic scale of 1 = disliked to 9 = liked and represented as eating quality. The fruit was examined visually for fungal decay and husk scald as described previously (Defilippi et al., 2006). The incidence of fungal decay and husk scald were expressed as a percentage of total number of fruits in each replicate. Severity of scald was evaluated on the following scale: 1 = no scald, 2 = <10%, 3 = 11%–25%, 4 = 25%–50%, 5 = 50%–75%, and 6 = 75%–100% of the husk surface affected.

2.3. Statistical analysis

The experimental design was completely randomized. Data were obtained from 3 replicates of each treatment using 5 kg or 12 kg of fruit per replication, depending on MAP bag type, and analyzed by analysis of variance (ANOVA) using SAS 9.4 software (SAS, 2017). Means were compared by Fisher's least significant difference (LSD) test at a P < 0.05 level using the SAS Proc GLM procedure.

3. Results and discussion

3.1. Headspace O₂ and CO₂ concentrations

Changes in O₂ and CO₂ concentrations inside the MAP bags are presented in Figures 1A and 1B, respectively. In all MAP bags, O₂ concentrations decreased significantly during storage with a significant or a slight increase after 4 months. The CO₂ concentration inside the MAP bags increased throughout the storage period, except

for a slight decrease after 4 months. The increase in O₂ level and decrease in CO₂ level after 4 months indicated equilibration of gases across the package film because a typical equilibration of internal and external gases inside MAP bags involves a rapid decline in O₂ and increase in CO₂, followed by elevation of O₂ and decrease in CO₂ (Smith et. al., 1987). The decrease in CO₂ level was slight due to the fact that CO₂ diffuses more slowly in air than O₂ (Wade and Graham, 1987). After 6 months of storage, final O₂ and CO₂ levels in decreasing order of permeability of MAP bags were 15.30 kPa and 7.45 kPa in L5, 14.38 kPa and 8.37 kPa in L12, 13.47 kPa and 9.40 kPa in X12, and 12.73 kPa and 10.20 kPa in X5 MAP bags, respectively (Figures 1A and 1B). Significantly higher O₂ and lower CO₂ concentrations were achieved inside the L5 MAP bags than other MAP bags while the X5 MAP bags resulted in significantly lower O₂ and higher CO₂ concentrations than other MAP bags. The O₂ and CO₂ levels were lower inside the X12 and L12 MAP bags during storage compared to the X5 MAP bags. Based on the O₂ and CO₂ levels, the least permeable MAP bag was X5, followed by X12, L12, and L5

MAP bags. Previous studies demonstrated that MAP bags with steady-state modified atmosphere of 13.5–17.60 kPa of O₂ and 4.40–8.1 kPa of CO₂ minimized weight loss and decay and maintained the overall visual quality of Hicaznar and Hicrannar pomegranate cultivars for 120–210 days at 6 °C (Selcuk and Erkan, 2014, 2015). MAP bags with 8 kPa of O₂ and 10 kPa of CO₂ reduced weight loss and husk scald development for pomegranate cultivar Mollar de Elche after 12 weeks at 2 °C (Artés et al., 2000). The MAP bags tested in this study provided the desired modified atmosphere for the Hicaznar pomegranate cultivar.

3.2. Weight loss

Weight loss increased as storage time was extended. Weight loss percentage reached 20.36% in unpackaged control fruit, 7.85% in L12, 9.64% in L5, 4.63% in X12, and 3.94% in X5 MAP bags after 6 months of storage (Figure 2A). An additional weight loss of 22.50% occurred in the control fruit during the shelf life period following 6 months of cold storage (Figure 2B). Weight loss of packaged fruit was 10.95% in L12, 12.52% in L5, 4.32% in

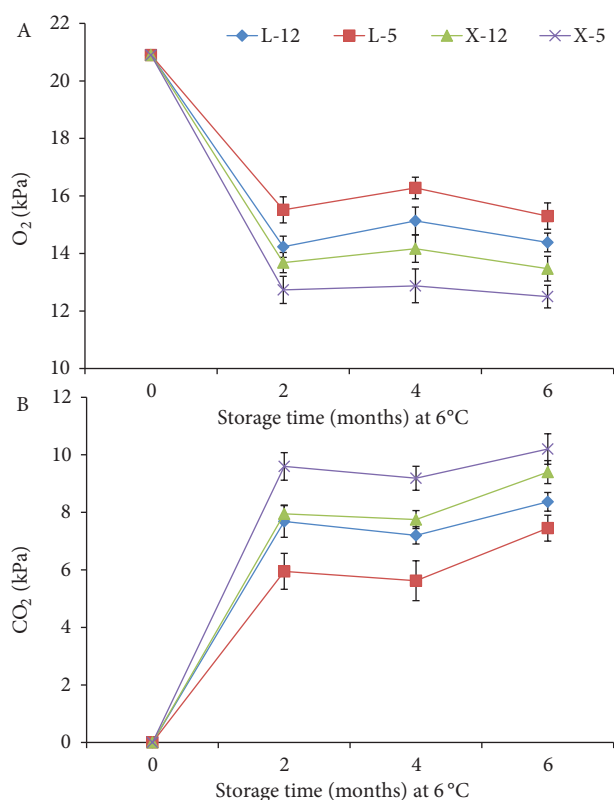


Figure 1. Changes in headspace O₂ (A) and CO₂ (B) concentrations (%) of MAP bags containing Hicaznar pomegranate fruit during storage for 6 months at 6 °C. Error bars represent the standard deviation of the mean of 3 replicates and when absent fall within the area of the symbol.

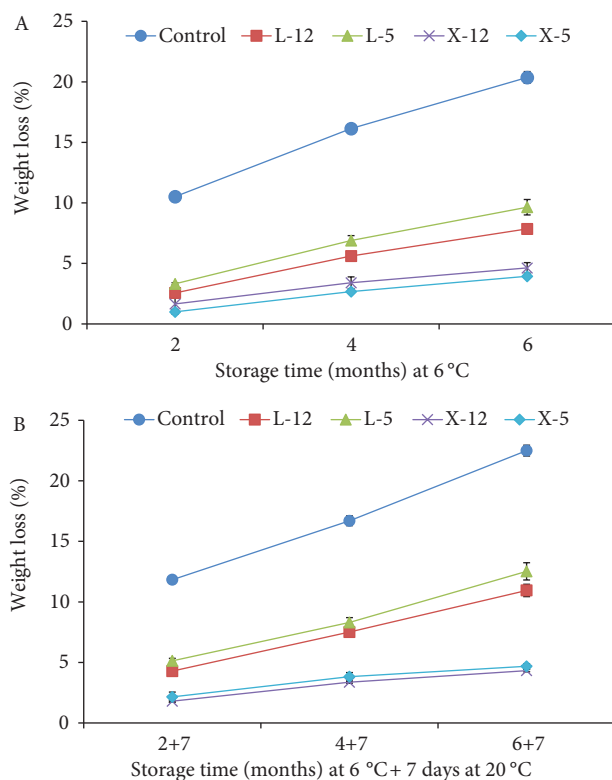


Figure 2. Changes in the weight loss of Hicaznar pomegranate fruit during storage for 6 months at 6 °C (A) and subsequent shelf life period of 7 days at 20 °C (B). Treatments are the unpackaged control fruit and packaged fruit with L12, L5, X12, and X5 MAP bags. Error bars represent the standard deviation of the mean of 3 replicates and when absent fall within the area of the symbol.

X12, and 4.68% in X5 MAP bags during the subsequent shelf life period after 6 months of storage. Weight loss was lower in packaged fruit than in unpackaged control fruit. X5 and X12 MAP bags reduced weight loss by 5- to 6-fold while L5 and L12 MAP bags reduced it by only 2- to 3-fold during storage and shelf life compared to the control. MAP was previously reported to minimize weight loss of pomegranate fruit during storage, depending on the type of MAP film (Artés et al., 2000; Nanda et al., 2001; D'Aquino et al., 2010; Şen et al., 2013; Selcuk and Erkan, 2014, 2015; Porat et al., 2016; Selçuk and Erkan, 2016). Unpackaged control fruits exhibited severe visible shriveling symptoms, such as hard and darkened husk, after 6 months of storage and subsequent shelf life periods (Figure 3) since weight loss of these fruits exceeded the threshold (5% of the initial weight) suggested by Kader et al. (1984). No shriveling was observed in the fruit from X5 and X12 MAP bags (Figure 3). Shriveling symptoms were slight on fruit from L5 and L12 MAP bags (Figure 3). Şen et al. (2013) found significant differences in weight loss among MAP bag types, depending on water vapor permeability of the tested MAP bags, and observed visible shrinkage symptoms on the husk of pomegranate fruit from some MAP bags. Our data indicated that X5 and X12 MAP bags had lower permeability to water vapor than L5 and L12 MAP bags.

3.3. TSS content and TA

TSS content and TA decreased significantly during storage and the shelf life period compared to the initial values at harvest (Tables 1 and 2). Consistent with our findings, previous studies reported a decrease in TSS content and TA in pomegranate fruit with increasing storage duration (Kader et al., 1984; D'Aquino et al., 2010; Selcuk and Erkan, 2015; Selçuk and Erkan, 2016). This indicates utilization of acids and sugars by pomegranate fruit for the respiration process (Selçuk and Erkan, 2016). There was no significant difference in TSS content between the control and MAP treatments during cold storage period (Table 1), but TSS content was lower in X5 and X12 MAP treatments than in control and other MAP treatments after 7 days of shelf life period at 20 °C following 6 months of cold storage (Table 2). The effect of reduced O₂ and/or elevated CO₂ in the CA/MAP on reducing respiration rate has been suggested as maintaining TSS and TA content, depending on fruit species (Kader et al., 1989). Many reports have shown that, in pomegranate fruit, TSS content and TA are not significantly affected by gas composition of CA/MAP (Artés et al., 1996, 2000; Palou et al., 2007; D'Aquino et al., 2010; Selcuk and Erkan, 2015; Selçuk and Erkan, 2016). Higher weight loss in the control, L5 MAP, and L12 MAP treatments might lead to higher TSS content than in X5 MAP and X12 MAP treatments after shelf life at the end of the cold storage period. Similarly, a higher TSS content

was observed in pomegranate fruit when higher weight loss occurred due to a concentration effect of water loss on sugars (Arendse et al., 2014a; Selcuk and Erkan, 2015). The X5 MAP bag, least permeable to gases, maintained higher TA compared to the control after the cold storage period, while other MAP bags resulted in TA values similar to the control treatment. There was no significant difference in TA between treatments during the shelf life period following cold storage. Previous studies on different pomegranate cultivars showed that MAP treatment resulted in a better retention of TA than the control treatment (Nanda et al., 2001; Selcuk and Erkan, 2014). However, slight or no significant differences in TA between control and MAP-treated pomegranate fruit were detected at the end of cold storage and the shelf life period (Artés et al., 2000; Selcuk and Erkan, 2015). In our study, the least permeable X5 MAP bag was more effective in retaining TA than other MAP bags during cold storage and its effect disappeared when fruits were kept at 20 °C for 7 days. In contrast to our result, Nanda et al. (2001) reported no significant differences in retention of TA between low and highly permeable MAP films during storage at 8 °C and the effect of low permeability MAP film on TA of pomegranate fruit continued during shelf life at 25 °C.

3.4. Husk scald, fungal decay, and visual quality

Chilling injury symptoms were not observed in either control or MAP treatments during storage and the shelf life period. A brown discoloration of husk without affecting the arils or the white segments, described previously as a typical symptom of scald (Ben-Arie and Or, 1986; Defilippi et al., 2006), occurred in both unpackaged and packaged fruit. Typical scald symptoms developed on the husk of packaged fruit later and at a lower rate than unpackaged control fruit. After 4 months of storage, plus 7 days at 20 °C, a scald incidence of 17.78% was observed only on unpackaged control fruit (Table 2). Scald incidence was highest in unpackaged control fruit and reached 41.48% and 40.00% by the end of storage and subsequent shelf life period, respectively (Tables 1 and 2). The lowest scald incidence was observed in X5 and X12 MAP bags, followed by L5 and L12 MAP bags after 6 months of storage and shelf life. In all treatments, the severity of scald was low since typical scald symptoms covered only <10% of the husk's surface area. Husk scald was suggested to occur as a result of oxidation of phenolic compounds on the husk of pomegranates stored at >5 °C (Ben-Arie and Or, 1986). Lower scald incidence was reported in Wonderful and Primosole pomegranates stored in MAP bags after 12 to 16 weeks of cold storage and shelf life period in comparison to unpackaged control fruit (D'Aquino et al., 2010; Porat et al., 2016) due to the fact that lower O₂ levels in MAP bags reduced or delayed oxidation of phenolic compounds on the husk (D'Aquino et al., 2010).



Figure 3. Severe (hard and darkened husk), slight, and no visible shriveling symptoms of Hicaznar pomegranate fruit from control (A and B), L5 and L12 MAP (C and D), and X5 and X12 MAP (E and F) treatments, respectively, after 6 months of storage at 6 °C. MAP: Modified atmosphere packaging; L5 and L12: 5-kg and 12-kg Life Pack® MAP bags; X5 and X12: 5-kg and 12-kg Xtend® MAP bags.

The incidence of fungal decay was not observed in any treatments for 4 months of cold storage and subsequent shelf life period (Tables 1 and 2). After 6 months of cold storage and subsequent shelf life period, decay incidence reached about 37% in the control treatment. There was

no observable decay in fruit packaged in L5 MAP bags. Other MAP treatments resulted in lower decay percentage than the control. We observed fungal decay mostly caused by *Botrytis cinerea*, *Penicillium* sp., *Alternaria* sp., and *Aspergillus* sp., as previously reported (D'Aquino et al., 2010;

Table 1. Effects of treatments on the total soluble solids (TSS) content, titratable acidity (TA), husk scald, decay incidence, and visual and eating quality of Hicaznar pomegranate fruit during storage at 6 °C.

Storage time and treatments	TSS (%)	TA (%)	Huskscald (%)	Severity of scald ²	Fungal decay (%)	Visual quality ³	Eating quality ⁴
At harvest	18.30 a ¹	1.43 a	0.00 e	1.00 b	0.00 c	5.00 a	9.00 a
2 months at 6 °C							
Control	17.47 bc	1.27 b	0.00 e	1.00 b	0.00 c	4.87 a	7.50 bc
L12	17.93 ab	1.34 ab	0.00 e	1.00 b	0.00 c	5.00 a	8.17 ab
L5	17.40 bc	1.27 b	0.00 e	1.00 b	0.00 c	5.00 a	8.50 a
X12	17.97 ab	1.29 b	0.00 e	1.00 b	0.00 c	5.00 a	8.50 a
X5	17.37 bcd	1.35 ab	0.00 e	1.00 b	0.00 c	5.00 a	8.17 ab
4 months at 6 °C							
Control	17.23 b-e	1.09 de	0.00 e	1.00 b	0.00 c	4.27 b	6.33 d
L12	17.27 b-e	1.08 de	0.00 e	1.00 b	0.00 c	4.93 a	7.17 d
L5	17.07 c-f	1.11 de	0.00 e	1.00 b	0.00 c	4.77 a	7.33 bc
X12	16.87 c-f	1.25 bc	0.00 e	1.00 b	0.00 c	5.00 a	7.50 bc
X5	16.83 c-f	1.27 b	0.00 e	1.00 b	0.00 c	5.00 a	7.33 bc
6 months at 6 °C							
Control	16.57 def	1.02 e	41.48 a	2.12 a	37.78 a	1.00 d	6.42 d
L12	16.97 c-f	1.07 de	25.00 b	2.08 a	13.33 b	3.62 c	6.83 cd
L5	16.50 ef	1.07 de	16.67 c	2.14 a	0.00 c	3.59 c	6.89 cd
X12	16.30 f	1.13 de	12.22 cd	2.08 a	12.22 b	4.27 b	6.83 cd
X5	16.40 f	1.14 cd	7.78 d	2.09 a	12.22 b	4.28 b	6.75 cd

¹ Means (n = 3) followed by different letters within a column are significantly different at P < 0.05 according to Fisher's LSD test.
² Severity of scald was assessed on a 1–6 scale (1 = no scald; 2 = <10%; 3 = 11%–25%; 4 = 25%–50%; 5 = 50%–75%; 6 = 75%–100% of the fruit surface affected).
³ Evaluated based on a 5-point scale where: 1 = very poor, 2 = poor (limit of marketability), 3 = good, 4 = very good, and 5 = excellent.
⁴ Evaluated based on a hedonic scale of 1 = disliked extremely to 9 = liked extremely.

Selcuk and Erkan, 2014, 2015). The fungal decay appeared mostly on fruit showing husk scald. This indicates that husk scald increases the susceptibility of pomegranate fruit to decay, as reported by Defilippi et al. (2006). D'Aquino et al. (2010) observed similar decay incidence in wrapped and unwrapped control pomegranate fruit after 6 and 12 weeks of cold storage, but wrapped fruit had significantly higher decay percentage than unwrapped control fruit after 12 weeks of storage and shelf life period. For pomegranate cultivars Mollar de Elche (Artés et al., 2000) and Wonderful (Porat et al., 2016), the MAP did not affect decay incidence during cold storage for 12 or 16 weeks followed by 1 week at 20 °C. In Hicrannar and Hicaznar pomegranates, MAP reduced or increased fungal decay depending on the type of MAP bag in comparison to control fruit after 120 to 210 cold storage days and an additional 3 days at 20 °C (Selcuk and Erkan, 2014, 2015). In this study, L5 MAP bags led to higher weight loss, probably due to lower relative humidity

microenvironment than other MAP bags, and did not show fungal decay.

The visual appearance of fruit was affected by the incidence of husk scald, fungal decay, and weight loss at the end of storage shelf life period. After 4 months of storage plus 7 days at 20 °C, control fruit reached the limit of marketability, while the visual quality of fruit from other treatments was rated as acceptable (Table 2). The loss of visual quality was most evident in unpackaged control fruits, which showed shriveling, browning, and hardening of the husk, and they finally became unmarketable after 6 months of storage and the subsequent shelf life period (Tables 1 and 2). Packaged fruits were rated as good and still marketable after 6 months of storage and subsequent shelf life period. Similar observations on visual quality were made in previous studies that compared packaged and unpackaged pomegranate fruit (D'Aquino et al., 2010; Selçuk and Erkan, 2016). X5 and X12 MAP bags

Table 2. Effects of treatments on the total soluble solid (TSS) content, titratable acidity (TA), husk scald, decay incidence, and visual and eating quality of Hicaznar pomegranate fruit after 7 days of shelf life period at 20 °C following storage at 6 °C.

Storage time and treatments	TSS (%)	TA (%)	Husk scald (%)	Severity of scald ²	Fungal decay (%)	Visual quality ³	Eating quality ⁴
At harvest	18.30 a ¹	1.43 a	0.00 e	1.00 d	0.00 c	5.00 a	9.00 a
2 months at 6 °C plus 7 days at 20 °C							
Control	17.27 bc	1.20 c	0.00 e	1.00 d	0.00 c	4.87 a	7.83 b
L12	17.47 b	1.28 b	0.00 e	1.00 d	0.00 c	5.00 a	7.50 b
L5	17.17 bc	1.31 b	0.00 e	1.00 d	0.00 c	5.00 a	7.44 bc
X12	17.30 bc	1.40 a	0.00 e	1.00 d	0.00 c	4.93 a	7.33 bc
X5	16.90 bcd	1.44 a	0.00 e	1.00 d	0.00 c	5.00 a	7.33 bc
4 months at 6 °C plus 7 days at 20 °C							
Control	16.58 cd	1.01 d	17.78 c	2.27 b	0.00 c	2.20 d	6.38 d
L12	16.37 d	1.20 c	0.00 e	1.00 d	0.00 c	4.75 a	6.44 d
L5	16.87 bcd	1.25 bc	0.00 e	1.00 d	0.00 c	4.57 a	6.73 cd
X12	16.65 cd	1.26 bc	0.00 e	1.00 d	0.00 c	4.55 a	6.28 d
X5	16.83 bcd	1.26 bc	0.00 e	1.00 d	0.00 c	4.43 ab	6.22 d
6 months at 6 °C plus 7 days at 20 °C							
Control	16.87 bcd	1.02 d	40.00 a	2.35 a	36.67 a	1.00 e	5.28 e
L12	16.93 bcd	1.02 d	26.67 b	2.24 b	5.19 b	3.20 c	5.33 e
L5	16.65 cd	1.03 d	28.89 b	2.42 a	2.22 bc	3.20 c	5.44 e
X12	15.33 e	1.02 d	4.45 e	2.07 c	2.22 bc	3.98 b	5.33 e
X5	15.27 e	1.01 d	11.11 d	2.13 c	5.19 b	3.93 b	5.17 e

¹ Means (n = 3) followed by different letters within a column are significantly different at P < 0.05 according to Fisher's LSD test.
² Severity of scald was assessed on a 1–6 scale (1 = no scald; 2 = <10%; 3 = 11%–25%; 4 = 25%–50%; 5 = 50%–75%; 6 = 75%–100% of the fruit surface affected).
³ Evaluated based on a 5-point scale where: 1 = very poor, 2 = poor (limit of marketability), 3 = good, 4 = very good, and 5 = excellent.
⁴ Evaluated based on a hedonic scale of 1 = disliked extremely to 9 = liked extremely.

maintained visual quality better than L5 and L12 MAP bags due to lower weight loss and scald incidence during storage and shelf life period, but fruit from L5 and L12 MAP bags were still rated as acceptable. The eating quality of fruit received lower scores as storage time was extended in all treatments. The taste of the fruit was rated as acceptable (>5) after 6 months of cold storage plus shelf life period. Panelists did not detect a significant difference in fruit taste among treatments.

3.5. Husk and aril color

The L* (lightness) and C* (intensity) values of the husk decreased while the h° value of the husk increased in both the unpackaged control and packaged fruit during the storage and shelf life periods (Tables 3 and 4), indicating loss of husk color lightness and intensity and yellowing of husk. A decrease in intensity and lightness, husk color redness, and an increase in h° value during cold storage and shelf life periods have been previously reported

with different pomegranate cultivars (Holcroft et al., 1998; D'Aquino et al., 2010; Selcuk and Erkan, 2014). In comparison to control treatment, MAP treatments delayed changes in L*, C*, and h° values of husk color and resulted in significantly higher values of L* and C* and lower values of h° during storage and the shelf life period (Tables 3 and 4). A significant decline in L* (darker fruit) was observed in the control treatment because of excessive weight loss, as reported by Selcuk and Erkan (2015). Similarly, delay in husk color loss of pomegranate fruit by MAP has been observed in previous studies (Artés et al., 2000; D'Aquino et al., 2010; Selcuk and Erkan, 2014, 2015). Holcroft et al. (1998) reported that a loss of husk color intensity in the air was noticeable after 4 weeks of storage and husk C* value was maintained better when fruit was stored in air enriched with CO₂ than in air alone.

When compared to values at harvest, aril color lightness (L*) decreased in all treatments throughout storage and

the shelf life period, but was better maintained by MAP treatments compared to the control (Tables 3 and 4). Aril color intensity (C^*) showed a significant increase in the control treatment to a lesser extent in MAP treatments throughout cold storage (Table 3). The h° values decreased significantly in all treatments with an extended cold storage period. After shelf life periods following 4 or 6 months of cold storage, all treatments suffered a pale color of the aril (loss of red color) due to decreasing C^* values and increasing h° values. Differences in C^* values between packaged and control fruit became insignificant when the control fruit showed a significant red color loss after shelf life at the end of the cold storage period (Table 4). Changes in C^* and h° values were a result of changes in a^* values, while b^* values remained quite stable (data not shown). There was no observable browning of arils in any of the treatments. Consistent with our results, Arendse et al. (2014a) found that aril red coloration (a^*) and intensity (C^*) of Wonderful pomegranates after storage at 5 °C for 2 to 3 months were considerably higher than the initial

values at harvest. The authors also noted a decline in red coloration and lightness of aril as h° values increased and C^* and L^* values decreased with extended storage period. In contrast to our findings, Fawole and Opara (2013) reported no significant change in aril color redness and intensity for pomegranate fruit cultivars Bhagwa and Ruby during 16 weeks of storage at 5 °C. The initial increase in aril color intensity (C^*) indicates more intense red aril color due to biosynthesis and accumulation of anthocyanin pigments, as reported previously in pomegranate fruit by Holcroft et al. (1998). The loss of red color with prolonged storage period is probably a result of enzymatic oxidation, inducing discoloration of anthocyanin pigments (Artés et al., 2000; Meighani et al., 2015). X5 and X12 MAP bags resulted in slightly paler aril red coloration than the control and other treatments after 4 months of storage and subsequent shelf life, but still maintained initial color intensity throughout the storage period (Tables 3 and 4). In Mollar de Elche pomegranate, the C^* of arils decreased only in unperforated polypropylene bags, while

Table 3. Effects of treatments on the husk and aril color L^* , chroma (C^*), and hue angle (h°) values of Hicaznar pomegranate fruit during storage at 6 °C.

Storage time and treatments	Husk color			Aril color		
	L^*	C^*	h°	L^*	C^*	h°
At harvest	43.90 a ¹	49.68 abc	25.11 d	32.70 ab	25.64 d	29.91 a
2 months at 6 °C						
Control	42.32 cd	44.92 fg	28.69 ab	32.03 abc	27.84 bc	26.19 b
L12	43.65 ab	48.32 bcd	25.23 d	32.77 a	26.83 cd	24.29 b
L5	43.79 ab	51.04 a	25.65 d	32.62 ab	26.70 cd	24.52 b
X12	43.31 abc	51.02 a	25.64 d	32.80 a	25.85 d	25.33 b
X5	43.01 abc	50.23 ab	25.20 d	32.47 ab	25.38 d	23.74 b
4 months at 6 °C						
Control	40.61 e	43.03 g	28.87 ab	28.28 e	30.35 a	24.22 b
L12	43.05 abc	46.12 def	26.26 cd	32.88 a	29.28 ab	24.49 b
L5	43.07 abc	46.74 def	26.22 cd	32.75 a	29.16 ab	26.27 b
X12	43.68 ab	45.68 ef	26.24 cd	32.96 a	26.34 cd	25.13 b
X5	43.18 abc	47.75 cde	26.48 cd	32.43 ab	26.54 cd	24.36 b
6 months at 6 °C						
Control	38.89 f	40.71 h	29.70 a	25.57 f	26.42 cd	23.90 b
L12	41.69 de	46.60 def	27.74 bc	28.40 e	26.76 cd	25.39 b
L5	42.67 bcd	47.40 de	26.18 cd	30.44 cd	26.40 cd	25.08 b
X12	42.38 cd	45.09 fg	26.46 cd	30.94 bcd	25.57 d	24.96 b
X5	42.69 bcd	47.12 def	26.23 cd	29.56 de	25.65 d	25.52 b

¹ Means (n = 3) followed by different letters within a column are significantly different at $P < 0.05$ according to Fisher's LSD test.

Table 4. Effects of treatments on the husk and aril color L*, chroma (C*), and hue angle (h°) values of Hicaznar pomegranate fruit after 7 days of shelf life period at 20 °C following storage at 6 °C.

Storage time and treatments	Husk color			Aril color		
	L*	C*	h°	L*	C*	h°
At harvest	43.90 ab ¹	49.68 a	25.11 cd	32.70 a	25.64 c	29.91 a
2 months at 6 °C plus 7 days at 20 °C						
Control	43.43 ab	44.83 cde	24.75 cd	25.65 de	32.13 a	25.13 b
L12	44.53 a	45.97 bc	24.95 cd	29.12 bc	32.09 a	23.49 b
L5	44.24 ab	46.87 b	24.38 d	29.01 bc	31.59 a	24.90 b
X12	44.16 ab	47.14 b	24.14 d	31.49 ab	28.84 b	24.07 b
X5	44.16 ab	47.02 b	24.59 cd	31.16 ab	28.79 b	24.52 b
4 months at 6 °C plus 7 days at 20 °C						
Control	42.58 bc	41.52 h	29.06 b	25.96 de	31.44 a	30.42 a
L12	44.78 a	43.62 efg	25.95 c	29.14 bc	28.49 b	30.08 a
L5	44.20 ab	45.14 cd	25.41 cd	29.87 abc	28.68 b	29.14 a
X12	44.04 ab	44.03 def	24.54 cd	31.30 ab	26.81 c	30.23 a
X5	44.03 ab	43.61 efg	25.67 cd	31.56 ab	26.65 c	31.25 a
6 months at 6 °C plus 7 days at 20 °C						
Control	41.41 c	42.22 gh	30.77 a	23.18 e	26.42 c	30.31 a
L12	43.67 ab	43.72 def	28.28 b	29.30 bc	26.42 c	28.03 a
L5	43.74 ab	43.67 ef	27.78 b	28.07 cd	26.70 c	31.68 a
X12	44.10 ab	43.34 fg	25.30 cd	29.76 bc	25.72 c	29.62 a
X5	44.15 ab	43.84 def	25.45 cd	29.90 abc	26.47 c	31.16 a

¹ Means (n = 3) followed by different letters within a column are significantly different at P < 0.05 according to Fisher's LSD test.

unpackaged control and perforated polypropylene bag treatments maintained the initial red color of the arils during storage at 5 °C (Artés et al., 2000).

3.6. AsA, TMA, and TP contents and antioxidant capacity

The initial AsA content of 10.71 mg 100 mL⁻¹ decreased during storage and the shelf life period (Tables 5 and 6). Control fruit had lower AsA content than packaged fruit during storage and the shelf life period. No significant difference in AsA content was detected among MAP treatments. The loss of AsA content during storage and shelf life period was previously reported (Artés et al., 1996; Nanda et al., 2001; Arendse et al., 2014b; Selcuk and Erkan, 2015). Consistent with our results, Nanda et al. (2001) reported higher AsA content in shrink-wrapped Ganesh pomegranates compared to those unwrapped. However, Selcuk and Erkan (2015) found no significant differences in AsA content between control and MAP-treated Hicaznar pomegranate fruit.

TMA and TP contents significantly increased during 4 months of storage and then decreased at the end of cold storage in all treatments (Table 5). The increases in TMA

and TP content were also observed for all treatments during the shelf life period following 2 months of storage; thereafter, all treatments showed a decrease in TMA and TP contents (Table 6). Similar changes in total phenolic and anthocyanin concentrations were observed in Wonderful (Arendse et al., 2014b, 2015) and Hicaznar (Selcuk and Erkan, 2015) pomegranate fruit during cold storage and the shelf life period. In the Mollar de Elche pomegranate cultivar, fruits from perforated polypropylene bags and unpackaged control treatments showed an increase in total anthocyanins content after a cold storage period at 5 °C, but all treatments exhibited a decrease in total anthocyanins content at the end of shelf life (Artés et al., 2000). In contrast to our results, Artés et al. (1998) reported no increase in the total anthocyanin content of arils of Mollar de Elche pomegranates after both storage and shelf life compared to values at harvest. The observed increases in anthocyanin and phenolic concentrations during cold storage have been attributed to biosynthesis and accumulation of anthocyanin continuing after harvest in several fruit, including pomegranate, particularly those

Table 5. Effects of treatments on ascorbic acid (AsA), total monomeric anthocyanin (TMA) and total phenolic (TP) contents, and antioxidant capacity by the ferric reducing antioxidant power (FRAP) and Trolox equivalent antioxidant capacity (TEAC) assays of Hicaznar pomegranate fruit during storage at 6 °C.

Storage time and treatments	AsA (mg 100 mL ⁻¹)	TMA (mg L ⁻¹)	TP (mg GAE L ⁻¹)	FRAP (mmol TE L ⁻¹)	TEAC (mmol TE L ⁻¹)
At harvest	10.71 a ¹	180.65 c	1729.71 f	5.31 f	6.55 e
2 months at 6 °C					
Control	9.03 cd	320.40 a	2196.00 bc	7.05 de	6.90 ab
L12	10.67 a	202.46 bc	2143.40 bc	7.11 de	6.91 ab
L5	10.57 a	200.46 bc	2164.48 bc	6.87 de	6.93 a
X12	10.53 a	178.00 c	2132.14 c	6.60 e	6.84 c
X5	10.43 a	180.55 c	2100.41 cd	6.55 e	6.86 bc
4 months at 6 °C					
Control	9.01 cd	307.54 a	2358.86 a	10.04 a	6.91 ab
L12	9.50 bc	257.53 ab	2276.00 ab	9.63 a	6.92 a
L5	9.42 bc	295.50 a	2120.80 c	7.62 cd	6.93 a
X12	9.55 bc	172.75 c	2080.00 cd	7.44 de	6.88 abc
X5	9.76 b	163.34 c	2078.86 cd	7.45 de	6.90 ab
6 months at 6 °C					
Control	8.53 d	196.68 bc	1932.00 e	8.60 b	6.60 de
L12	9.40 bc	200.57 bc	1910.57 e	8.54 bc	6.61 d
L5	9.27 bc	190.76 bc	1977.29 de	7.65 bcd	6.61 d
X12	9.59 bc	178.46 c	1893.43 e	7.83 bcd	6.58 de
X5	9.69 bc	184.15 c	1980.56 de	7.72 bcd	6.58 de

¹ Means (n = 3) followed by different letters within a column are significantly different at P < 0.05 according to Fisher's LSD test.

stored in air at low storage temperatures (Holcroft et al., 1998; Holcroft and Kader, 1999). The decrease in total phenolic content of pomegranate fruit was suggested to be related to enzymatic oxidation of phenolic compounds during storage (Fawole and Opara, 2013).

X5 and X12 MAP treatments resulted in lower TMA and TP contents than the control during 4 months of cold storage and the subsequent shelf life period (Tables 5 and 6). However, TMA and TP contents were similar in packaged and unpackaged control fruits at the end of cold storage plus shelf life when higher reductions occurred in control fruit compared with the previous period. Our results are consistent with the earlier report by Selcuk and Erkan (2015), where the total anthocyanin and phenolic contents of Hicaznar pomegranates were higher in control fruit than those stored in MAP bags during storage at 6 °C or after 3 days at 20 °C. D'Aquino et al. (2010) reported that total polyphenol and anthocyanin content did not change in unpackaged control fruit

over the storage period, but decreased in wrapped fruit, especially during shelf life periods. Holcroft et al. (1998) suggested a lower anthocyanin concentration in the arils of Wonderful pomegranates stored in CO₂-enriched atmospheres (10–20 kPa) than in air-stored fruit due to anthocyanin biosynthesis being suppressed by high CO₂. In our study, the higher CO₂ levels in X5 and X12 MAP bags probably delayed anthocyanin synthesis, resulting in lower anthocyanin and phenolic concentrations of arils and less intense aril color than other treatments during the storage and shelf life period.

A significant increase followed by a decrease occurred in FRAP and TEAC antioxidant capacity, similar to TP and TMA contents, during storage and shelf life (Tables 5 and 6), as reported previously in cold-stored pomegranate fruit (Fawole and Opara, 2013; Selcuk and Erkan, 2015). We found no significant effects of treatments on TEAC antioxidant capacity, but FRAP antioxidant capacity was lower in X5 and X12 MAP treatments compared to the

Table 6. Effects of treatments on ascorbic acid (AsA), total monomeric anthocyanin (TMA) and total phenolic (TP) contents, and antioxidant capacity by the ferric reducing antioxidant power (FRAP) and Trolox equivalent antioxidant capacity (TEAC) assays of Hicaznar pomegranate fruit after 7 days of shelf life period at 20 °C following storage at 6 °C.

Storage time and treatments	AsA (mg 100 mL ⁻¹)	TMA (mg L ⁻¹)	TP (mg GAE L ⁻¹)	FRAP (mmol TE L ⁻¹)	TEAC (mmol TE L ⁻¹)
At harvest	10.71 a ¹	180.65 e	1729.71 e	5.31 c	6.55 e
2 months at 6 °C plus 7 days at 20 °C					
Control	9.05 de	297.28 a	2293.71 a	8.90 a	6.92 a
L12	9.89 bc	269.50 ab	2208.10 ab	8.85 a	6.92 a
L5	9.85 bc	251.04 bc	2212.00 ab	8.99 a	6.93 a
X12	10.49 a	249.73 bc	2217.90 ab	8.82 a	6.90 ab
X5	10.35 ab	248.81 bc	1981.81 cd	7.23 b	6.92 a
4 months at 6 °C plus 7 days at 20 °C					
Control	8.98 e	263.73 ab	2171.36 b	8.61 a	6.90 ab
L12	9.73 c	264.74 ab	2170.57 b	8.72 a	6.92 a
L5	9.43 cde	249.85 bc	2164.54 b	8.73 a	6.92 a
X12	9.85 bc	211.82 de	1996.21 c	7.21 b	6.87 bc
X5	9.91 bc	210.08 de	1881.86 d	7.15 b	6.84 c
6 months at 6 °C plus 7 days at 20 °C					
Control	8.32 f	250.43 bc	1974.73 cd	7.28 b	6.64 d
L12	9.52 cd	246.05 bcd	1974.97 cd	7.36 b	6.63 d
L5	9.40 cde	233.80 bcd	1935.63 cd	7.33 b	6.62 d
X12	9.59 c	224.92 cd	1875.33 d	6.71 b	6.62 d
X5	9.60 cd	221.37 cd	1895.33 d	6.77 b	6.61 d

¹ Means (n = 3) followed by different letters within a column are significantly different at P < 0.05 according to Fisher's LSD test.

control after 4 months of cold storage and subsequent shelf life period. At the end of the cold storage plus shelf life period, all treatments showed similar FRAP antioxidant capacity. In agreement with our findings, Selcuk and Erkan (2015) reported that control fruit exhibited a significant increase in antioxidant activity during storage and this increase was retarded by MAP treatments. On the contrary, D'Aquino et al. (2010) found that total polyphenol and anthocyanin content and antioxidant capacity did not change in unpackaged control fruit over the storage period, but gradually decreased in wrapped fruit, especially during shelf life periods.

3.7. Conclusions

All MAP bag types were successful in reducing fungal decay and loss of AsA content and delaying husk discoloration of the fruit during cold storage and the shelf life period. Unpackaged control fruit remained marketable for only 4 months at 6 °C plus 7 days at 20 °C while packaged fruit

maintained visual quality for up to 6 months at 6 °C plus 7 days at 20 °C. L5 and L12 MAP bags were less effective in reducing weight loss and husk scald and maintaining overall visual quality compared to X5 and X12 MAP bags. By utilizing the less permeable MAP bags of X5 and X12, it was possible to maintain acceptable visual quality, initial red aril color intensity, and antioxidant properties of Hicaznar pomegranates for 6 months of cold storage plus shelf life.

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