

Overall Quality and Shelf Life of Minimally Processed and Modified Atmosphere Packaged "Ready-to-Eat" Pomegranate Arils

ZEHRA AYHAN AND OKAN EŞTÜRK

ABSTRACT: Minimally processed ready-to-eat pomegranate arils have become popular due to their convenience, high value, unique sensory characteristics, and health benefits. The objective of this study was to monitor quality parameters and to extend the shelf life of ready-to-eat pomegranate arils packaged with modified atmospheres. Minimally processed pomegranate arils were packed in PP trays sealed with BOPP film under 4 atmospheres including low and super atmospheric oxygen. Packaged arils were stored at 5 °C for 18 d and monitored for internal atmosphere and quality attributes. Atmosphere equilibrium was reached for all MAP applications except for high oxygen. As a general trend, slight or no significant change was detected in chemical and physical attributes of pomegranate arils during cold storage. The aerobic mesophilic bacteria were in the range of 2.30 to 4.51 log CFU/g at the end of the storage, which did not affect the sensory quality. Overall, the pomegranate arils packed with air, nitrogen, and enriched oxygen kept quality attributes and were acceptable to sensory panelists on day 18; however, marketability period was limited to 15 d for the low oxygen atmosphere. PP trays sealed with BOPP film combined with either passive or active modified atmospheres and storage at 5 °C provided commercially acceptable arils for 18 d with high quality and convenience.

Keywords: anthocyanin content, minimal processing, modified atmosphere packaging, polyphenols, pomegranate, ready-to-eat, shelf life, total antioxidant activity

Introduction

Pomegranate is generally consumed fresh or processed into juice, syrup, jams, or wine. In recent years, minimally processed "ready-to-eat" pomegranate arils have become popular due to their convenience, high value, unique sensory characteristics, and health benefits.

Studies showed that pomegranate has chemopreventive properties such as antimutagenicity, antihypertension, antioxidative potential, and reduction of liver injury due to its high anthocyanin content (Hertog and others 1997; Lansky and others 1998).

Most of the studies on pomegranates dealt with chemical composition, the chemical and physical changes during ripening of whole fruit and the determination of shelf life at different storage conditions (Hernandez and others 1999; Artes and others 2000a, 2000b; Melgarejo and others 2000; Al-Maiman and Ahmad 2002; Poyrazoğlu and others 2002; Kulkarni and Aradhya 2005). However, there are few studies on the preservation of pomegranate arils (seeds) using disinfection methods, modified atmosphere packaging, different plastic materials, and cold storage (Gil and others 1996; Nanda and others 2001; Lopez-Rubira and others 2005).

Minimal processing of pomegranate mainly consists of washing with sanitizing agents to reduce the initial microbial load, pH modifications, use of antioxidants, modified atmosphere packaging, and temperature control (Sepulveda and others 2000).

MS 20081049 Submitted 12/22/2008, Accepted 3/26/2009. Authors are with Mustafa Kemal Univ., Dept. of Food Engineering, Hatay, Turkey. Direct inquiries to author Ayhan (E-mail: zehra.ayhan@gmail.com).

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Modified atmosphere packaging (MAP) has been suggested to extend the shelf life of minimally processed arils (Sepulveda and others 2000; Lopez-Rubira and others 2005). Sepulveda and others (2000) reported that minimally processed pomegranate var. Wonderful were able to be stored for 14 d at 4 °C ± 0.5 with the use of semipermeable film. This study focused on the effect of different types of semipermeable films and antioxidant solutions on the quality of pomegranate arils; however, only passive modification was used in this study.

Lopez-Rubira and others (2005) studied the shelf life and overall quality of minimally processed pomegranate (var. Mollar Elche) arils treated with UV-C and packaged under passive atmosphere using polypropylene (PP) baskets sealed with BOPP film. They reported that unclear results were obtained on the effect of UV-C radiation on the microbial growth of pomegranate arils. They also reported that the harvest date had an effect on the quality parameters and the shelf life of the arils. The shelf lives of 10 and 14 d were obtained for late and earlier harvested fruits, respectively.

Since extracting the pomegranate arils is very difficult and time consuming, its consumption is not widespread (Lopez-Rubira and others 2005). Commercial production of pomegranate arils is now possible with today's technologies. The shelf life of the pomegranate arils commercially produced in Turkey is suggested as 10 d using 100% nitrogen in PET packages. However, drip loss was a big problem with the product.

The objective of this study was to monitor overall quality parameters (physiological, physical, chemical, sensory, and microbiological) and to extend the shelf life of ready-to-eat pomegranate arils packaged with modified atmospheres including low and super atmospheric oxygen. For this purpose, pomegranate arils were packaged in PP trays sealed with BOPP film under 4 different

atmospheres: low oxygen (5% O₂ + 10% CO₂ + 85% N₂), enriched oxygen (70% O₂ + 10% CO₂ + 20% N₂), 100% nitrogen, and air.

Materials and Methods

Materials

Pomegranate fruits (*Punica granatum* L.) cv. Hicaznar grown under Mediterranean climatical conditions in Antalya, Turkey, were obtained at the commercial harvest date (mid-October) from a company, IC Tahal A.S. (Antalya, Turkey). Average weight of the fruit was 300 g with 50% yield. Chemicals were supplied from Merck (Darmstadt, Germany). PP trays (mono PP with the dimensions of 144 × 190 × 50 mm) and biaxially-oriented polypropylene film (BOPP, 20 μ with the OTR of 2300 cm³ m⁻² day⁻¹ at 23 °C and 0% RH, and WVTR of 6.5 g m⁻² day⁻¹ at 38 °C and 75% RH) were provided by Huhtamaki (Istanbul, Turkey) and Polinas (Manisa, Turkey), respectively.

Fruit processing and packaging procedures

The damaged fruits were removed and the outer skins of healthy fruits uniform in size and appearance were washed in 200 μLL⁻¹ chlorine (NaOCl) solution using a brush. Husks were carefully cut at the equatorial zone with sharpened knives and the arils were manually extracted. The extracted arils were collected in a tray and mixed to assure uniformity. The samples were dipped in a solution containing 1% citric acid (w/v) and 100 μLL⁻¹ chlorinated water which is suggested by Gil and others (1996), and washed arils were dried for 15 min. Dried arils were weighed as 350 g in PP trays previously sanitized with 3% (v/v) hydrogen peroxide (H₂O₂). PP trays were sealed with BOPP film using a modified atmosphere packaging machine (MECA 501, Champigny sur Marne, France) combined with triple gas mixer (KM60-3, Witt, Germany). Four different gas compositions were selected as packaging atmospheres: air (MAP1), 100% N₂ (MAP2), 5% O₂ 10% CO₂ 85% N₂ (MAP3), and 70% O₂ 10% CO₂ 20% N₂ (MAP4). Two replicates of each atmosphere were made. Packaged samples were stored at 5 °C and 75% relative humidity for 18 d, and sampling was carried out on 0, 3, 6, 9, 12, 15, and 18 d of storage. Two packs were analyzed for each application on sampling days.

Analysis

Internal headspace composition and gas production analysis. Before opening the packages, the gas composition (oxygen and carbon dioxide) inside the packages was determined using a gas analyzer (PBI Dansensor, Ringsted, Denmark). Gas analysis was performed by inserting a needle attached to the gas analyzer through an adhesive seal fixed on the lidding material. A total of 15 mL of gaseous sample was extracted from the headspace using an airtight syringe attached to the analyzer. The measurements were taken at 2 different sides of each package. Total of 4 measurements were made at 2 packs for each treatment. The results were represented as O₂ and CO₂%. Immediately after completing the gas analysis, packages were opened and used for microbial, sensory, physical, and chemical analyses.

Physical Attributes

Color. Color measurements were performed using a Minolta chromameter model CR-400 (Osaka, Japan). Twenty grams of arils were weighed into a petri dish and the color of the arils was measured on 5 different points of the dish (Artes and others 2000a). Measurements were done at 2 packages and the mean of 10 measurements was calculated for each application. In the CIE L*, a*, b* uniform color space, L* indicates lightness, a* chromacity on a

green (-) to red (+) axis, and b* chromacity on a blue (-) to yellow (+) axis. Before each measurement, the apparatus was standardized against a white plate (Illuminants C: Y = 93.6, x = 0.3133, y = 0.3195).

Texture. Textural analysis was carried out using a texture analyzer (TA-XT Plus, Stable Micro Systems, Surrey, England). Twelve grams of arils were weighed into a 28 cm² metal plate and were crushed using a 5-cm diameter cylindrical probe. Maximum force (N) was measured and expressed as firmness. A speed of 5 mm/s and penetration distance of 7 mm were used. A total of 10 measurements were made for each application.

Chemical Attributes

Titrateable acidity, pH, and total soluble solids. A total of 60 g of arils were hand pressed for extracting the juice and filtered using cheesecloth. The juice of the sample was directly used for pH and soluble solid content (AOAC 2003). pH measurements were performed using a pH meter (Model pH-315i; WTW, Weilheim, Germany). Total soluble solids (TSS) were measured by hand refractometer (Model N-50E; Atago, Tokyo, Japan) and expressed as °Brix at 20 °C. Total titrateable acidity was determined potentiometrically using 0.1 N NaOH to the titration end point of pH 8.1 using 5 mL of juice diluted with 50 mL of distilled water and expressed as citric acid%. All analyses were done as 4 replicates.

Total phenolic content. Total polyphenols were determined by the Folin-Ciocalteu method (Singleton and Rossi 1965) using gallic acid as the standard. The results are expressed in milligrams of gallic acid equivalents per 1 L of juice.

Total anthocyanin content. Total juice anthocyanin content was determined by the pH-differential method described by Lee and others (2005) using 2 buffer systems: potassium chloride buffer, pH 1 (0.025M), and sodium acetate buffer, pH 4.5 (0.4M). The sample diluted with corresponding buffer and the absorbance was measured at 520 and 700 nm. Total anthocyanins were calculated as cyanidin-3-glucoside according to the following equation:

$$\text{Total anthocyanins (mg/L)} = \frac{A \times MW \times DF \times 1000}{\epsilon \times l}$$

where A = (A₅₂₀ - A₇₀₀) pH 1 - (A₅₂₀ - A₇₀₀) pH 4.5; MW (molecular weight) = 449.2 g/mol for cyanidin-3-glucoside; DF = dilution factor; l = pathlength in cm; ε = 26900 molar extinction coefficient in liter per mol per centimeter for cyanidin-3-glucoside; 1000 = conversion from grams to milligrams. All analyses were done as 4 replicates (n = 4).

Total antioxidant activity. Total antioxidant activity of pomegranate juice was determined by the DPPH method described by Moon and Terao (1998). Fresh pomegranate juice (0.1 mL) was mixed with 0.9 mL of 100 mM Tris-HCl buffer (pH 7.4) to which 1 mL of DPPH (500 μM in ethanol) was added. The mixture was shaken vigorously and left to stand for 30 min. Absorbance of the resulting solution was measured at 517 nm by a UV-Visible spectrophotometer (UV-160A, Shimadzu, Kyoto, Japan). The antioxidant activity was calculated using the following equation:

$$\text{Total antioxidant activity (\%)} = \left(1 - \frac{A_{\text{Sample (517 nm)}}}{A_{\text{Control (517 nm)}}}\right) \times 100$$

Microbial quality. Microbiological quality was screened by total plate count on plate count agar (PCA, Merck) and by yeast and mold counts on potato dextrose agar (PDA, Merck) acidified with tartaric acid. Two packages were opened under hygienic conditions, and a 10-g sample was placed into a sterile stomacher bag with

90 mL of peptone water. Samples were homogenated for 5 min and serial dilutions were made in peptone water. Appropriate dilutions were plated onto duplicate plates of PCA and PDA medium. Plates were incubated at 37 °C for 2 d for total mesophilic aerobic bacteria and at 22 °C for 5 d for yeasts and molds. The results were presented as log CFU/g.

Sensory evaluation. Sensory evaluation of pomegranate arils was performed during storage by a sensory panel of 8 trained judges. Panelists were asked to evaluate color, freshness, taste, texture, and product acceptability using a 5-point scale. Scores of 3 and above were considered as acceptable for commercial purposes. Overall product acceptability was scored on a 5-point hedonic scale where 5 corresponded to extremely liked and 1 corresponded to extremely disliked.

Data analysis. Data were analyzed using the Statistical Analysis System software program, version 8.02 (SAS Inst., Cary, N.C. U.S.A.). The effects of applications, storage time and their interaction on the quality parameters were analyzed by PROC MIXED procedure of SAS (SAS, 2001) and Duncan's multiple range tests with examination for significant differences ($P \leq 0.05$).

Results and Discussion

Internal headspace gas composition

Figure 1 and 2 present headspace atmospheres, percent oxygen, and carbon dioxide, respectively. The results showed that the O₂ levels decreased and CO₂ increased within packages during storage as reported by Lopez-Rubira and others (2005). The oxygen concentration reached equilibrium for MAP2 (100% nitrogen) and MAP3 (5% O₂) after 6 d of storage; however, the O₂ concentration decreased and CO₂ values increased continuously under initially enriched oxygen atmosphere (MAP4). Although the initial oxygen level was 0% for MAP2 application with 100% nitrogen, the oxygen concentration increased inside the package to 2.7% at the storage day of 18 due to film permeability. For the passive MAP (MAP1), the oxygen concentration remained constant after 9 d of storage indicating that when an internal atmosphere was passively modified by product respiration, more time was necessary to reach the gas equilibrium. However, the steady state for O₂ and CO₂ (equilibrium) was not achieved for passively modified atmosphere packaged pomegranate arils during storage which was related to

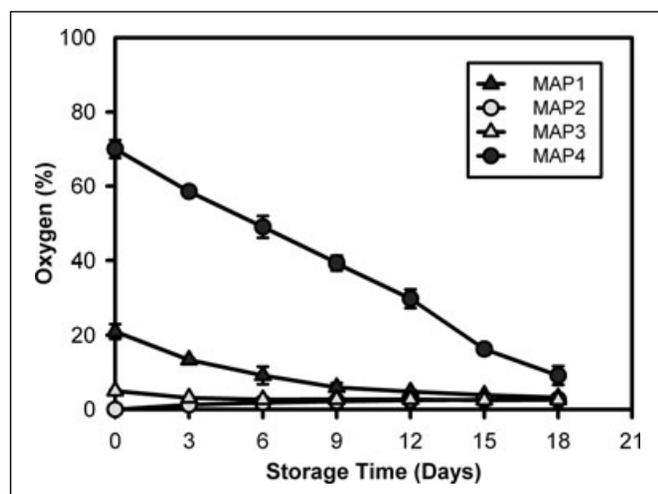


Figure 1—Effect of modified atmosphere packaging (MAP) on the headspace oxygen concentration (%) during storage. (MAP1: 21% O₂, 79% N₂; MAP2: 100% N₂; MAP3: 5% O₂, 10% CO₂, 85% N₂; MAP4: 70% O₂, 10% CO₂, 20% N₂. Points without error bars are smaller than symbols.)

variable respiration rates (RR) of the fruit by Lopez-Rubira and others (2005).

Carbon dioxide levels increased for all applications during storage; however, the increase was much higher for enriched oxygen application (MAP4) than the other MAP applications (Figure 2). At the end of the storage, carbon dioxide concentration reached 35% under enriched oxygen, 23%, 19%, and 23% under passive (MAP1), MAP with nitrogen (MAP2), and MAP with low oxygen applications (MAP3), respectively. These increases in CO₂ levels did not cause off-flavor development as perceived by sensory panelists. Although atmospheres enriched in CO₂ are suggested to prolong the commercial life of the seeds (Hess-Pierce and Kader 1997), a more permeable film to CO₂ could be preferred to avoid CO₂ accumulation in the package.

Physical quality

The color characteristics of pomegranate arils are presented by Table 1. In the overall analysis of variance (ANOVA) model, there was no significant effect of MAP or storage time ($P > 0.05$) on redness (a^*), which is used as an indicator of color stability. Similarly, the effects of MAP and storage time were insignificant on yellowness (b^*) values of pomegranate arils ($P > 0.05$). Although there were some small fluctuations, redness and yellowness values did not change much throughout the storage. There were significant effects of MAP application, storage day and MAP application \times storage day interaction for lightness (L^*) values ($P \leq 0.05$). The L^* value of the aril did not differ much during 18 d of storage under passive (MAP1) and active MAPs with low (MAP2 and MAP3) and high oxygen (MAP4) applications. Anthocyanins are responsible for the color of the pomegranate seeds. There were also no significant differences between MAP applications in terms of total anthocyanin content which is in agreement with the color measurements. A study by Artes and Tomas-Barberan (2000) showed that preservation of the seeds with passive and active MAP resulted in similar pigment stability.

There were significant effects of MAP application, storage day, and MAP applications \times storage day interaction on the texture (firmness) values ($P \leq 0.05$). While the firmness value was 157.6 N at day 0, it was significantly increased at 3 d of storage to 191.6, 183.4,

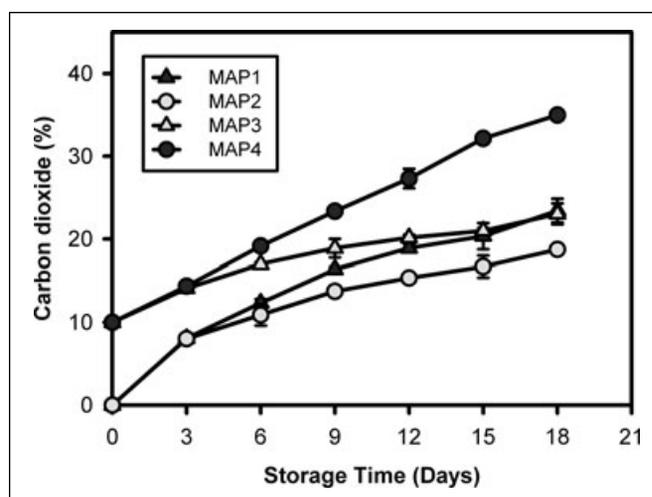


Figure 2—Effect of modified atmosphere packaging (MAP) on the headspace carbon dioxide concentration (%) during storage. (MAP1: 21% O₂, 79% N₂; MAP2: 100% N₂; MAP3: 5% O₂, 10% CO₂, 85% N₂; MAP4: 70% O₂, 10% CO₂, 20% N₂. Points without error bars are smaller than symbols.)

192, and 195.5 for MAP1, MAP2, MAP3, and MAP4, respectively (Table 1). There was no significant difference between MAP applications until 9 d of storage in terms of texture. However, the difference became significant between MAP 4 and the other applications after the storage time of 15 d. Firmness of pomegranate arils packaged under enriched oxygen (MAP4) was significantly higher than the other MAP applications after the 15th day of storage. Changes in firmness could be due to changes in water content during storage. However, this increase in firmness determined by the texture analyzer was not perceived by the sensory panelists where no significant difference was observed among MAP applications in terms of texture values.

Chemical quality

Table 2 and 3 present chemical parameters of minimally processed and modified atmosphere packaged arils during cold storage. Results showed that there was no significant effect of modified atmosphere on TSS until the storage time of 18 d ($P > 0.05$). At the 18 day of storage, TSS of pomegranate arils under passive MAP (MAP1) was slightly higher than the other MAP applications. For all MAP applications, the TSS remained unchanged for the storage of 9 d and started to decrease for the rest of the storage. Artes and others

(2000b) also reported that there was no significant but slight changes observed in TSS of pomegranate during cold storage.

In general, no significant difference was observed between atmospheres in terms of total titratable acidity (TTA; $P > 0.05$). However, storage time had significant effect on TTA at all applications ($P \leq 0.05$). TTA significantly decreased in all applications especially at the 3rd day ($P \leq 0.05$) and stayed almost unchanged for the rest of the storage. Decrease in acidity during storage is in agreement with the results of Artes and others (2000b). This could be related to metabolic activities of pomegranate during storage.

There was not much difference observed between MAP applications in terms of product pH during storage. The pH decreased from 3.30 to 3.27 at the end of storage for almost all applications. Although changes in pH were slight, it was found to be statistically significant ($P \leq 0.05$). Artes and others (2000b) reported that at the end of the shelf life, all treatments maintained or increased pH values, except pomegranate fruits in perforated PP at 5°C, which had slightly decreased pH values.

There were significant effects of MAP application, storage day and MAP application \times storage day interaction on the total phenol content ($P \leq 0.05$). In general, total phenolic content (milligrams gallic acid equivalent/liter of fruit juice) of pomegranate arils

Table 1—The effect of MAP on the physical parameters (color and texture) of pomegranate arils during cold storage.

		Color ¹ and texture (firmness) ²						
Application ³		Day 0	Day 3	Day 6	Day 9	Day 12	Day 15	Day 18
<i>L*</i>	MAP1	14.53 ± 1.76 ^A _{bc}	14.05 ± 1.18 ^B _{bc}	14.79 ± 1.85 ^A _{bc}	16.88 ± 2.69 ^A _a	14.86 ± 2.15 ^A _{bc}	15.49 ± 2.32 ^A _{ab}	13.13 ± 2.05 ^B _c
	MAP2	14.53 ± 1.76 ^A _{cd}	15.70 ± 1.39 ^A _{bc}	16.27 ± 2.44 ^A _{abc}	13.35 ± 1.59 ^B _{cd}	16.13 ± 2.57 ^A _{abc}	17.02 ± 2.17 ^A _{ab}	17.90 ± 1.66 ^A _a
	MAP3	14.53 ± 1.76 ^A _a	14.92 ± 1.79 ^{AB} _a	14.71 ± 2.82 ^A _a	12.13 ± 1.52 ^B _b	14.66 ± 2.83 ^A _a	16.40 ± 2.52 ^A _a	14.61 ± 2.65 ^B _a
	MAP4	14.53 ± 1.76 ^A _a	15.72 ± 1.56 ^A _a	14.55 ± 1.11 ^A _a	15.64 ± 2.08 ^A _a	14.21 ± 2.52 ^A _a	15.01 ± 2.26 ^A _a	14.89 ± 2.53 ^B _a
<i>a*</i>	MAP1	16.61 ± 3.30 ^A _a	17.16 ± 2.55 ^A _a	15.87 ± 4.20 ^A _a	17.26 ± 3.58 ^A _a	17.08 ± 2.76 ^A _a	17.74 ± 3.65 ^A _a	16.81 ± 3.25 ^A _a
	MAP2	16.61 ± 3.30 ^A _a	15.36 ± 3.77 ^A _a	16.58 ± 2.76 ^A _a	17.77 ± 3.73 ^A _a	15.22 ± 2.89 ^A _a	14.53 ± 3.75 ^A _a	16.27 ± 4.07 ^A _a
	MAP3	16.61 ± 3.30 ^A _{ab}	18.29 ± 3.00 ^A _a	14.60 ± 3.14 ^A _b	16.55 ± 2.89 ^A _{ab}	15.42 ± 3.20 ^A _{ab}	17.14 ± 3.73 ^A _{ab}	15.35 ± 3.60 ^A _{ab}
	MAP4	16.61 ± 3.30 ^A _a	16.07 ± 2.50 ^A _a	16.50 ± 2.38 ^A _a	17.18 ± 3.00 ^A _a	15.83 ± 2.77 ^A _a	16.38 ± 3.31 ^A _a	17.26 ± 3.53 ^A _a
<i>b*</i>	MAP1	5.22 ± 1.01 ^A _a	5.37 ± 1.06 ^A _a	4.89 ± 1.10 ^A _a	5.78 ± 0.61 ^A _a	5.63 ± 0.74 ^A _a	5.50 ± 1.10 ^A _a	4.83 ± 0.95 ^A _a
	MAP2	5.22 ± 1.01 ^A _{ab}	5.58 ± 0.92 ^A _a	4.65 ± 0.59 ^A _a	5.15 ± 0.72 ^{AB} _{ab}	4.93 ± 0.97 ^A _{ab}	4.82 ± 0.99 ^A _{ab}	5.12 ± 0.82 ^A _{ab}
	MAP3	5.22 ± 1.01 ^A _a	5.63 ± 1.29 ^A _a	4.90 ± 0.76 ^A _a	4.80 ± 1.06 ^B _a	4.91 ± 0.96 ^A _a	5.00 ± 0.92 ^A _a	5.05 ± 0.89 ^A _a
	MAP4	5.22 ± 1.01 ^A _a	4.99 ± 0.85 ^A _a	4.91 ± 0.85 ^A _a	5.33 ± 0.74 ^{AB} _a	5.14 ± 0.74 ^A _a	4.97 ± 0.79 ^A _a	5.59 ± 0.59 ^A _a
Firmness (N)	MAP1	157.6 ± 23.9 ^B _b	191.6 ± 16.7 ^A _a	191.4 ± 10.4 ^A _a	194.0 ± 8.8 ^A _a	198.6 ± 12.0 ^{AB} _{ab}	191.3 ± 10.7 ^B _{ab}	188.2 ± 7.8 ^B _a
	MAP2	157.6 ± 23.9 ^A _c	183.4 ± 10.6 ^A _b	185.2 ± 10.1 ^A _b	186.0 ± 5.4 ^B _b	205.0 ± 4.8 ^A _{ab}	186.9 ± 7.4 ^B _b	186.1 ± 7.5 ^B _b
	MAP3	157.6 ± 23.9 ^A _b	192.0 ± 25.4 ^A _a	189.8 ± 10.9 ^A _a	185.2 ± 8.6 ^B _a	189.5 ± 9.5 ^A _a	187.0 ± 9.6 ^B _a	183.1 ± 9.5 ^B _a
	MAP4	157.6 ± 23.9 ^A _d	195.5 ± 21.9 ^A _{bc}	182.5 ± 14.7 ^A _c	188.4 ± 8.2 ^{AB} _c	195.2 ± 11.8 ^B _{bc}	209.1 ± 6.2 ^A _{ab}	216.5 ± 14.9 ^A _a

¹ *L** = lightness; *a** = redness; *b** = yellowness.

² For each column, similar capital letters (superscript) are not significantly different at $P \leq 0.05$ among MAP treatments. For each parameter, similar small letters (subscript) in rows are not significantly different at $P \leq 0.05$ during storage.

³ MAP1: 21% O₂, 79% N₂; MAP2: 100% N₂; MAP3: 5% O₂, 10% CO₂, 85% N₂; MAP4: 70% O₂, 10% CO₂, 20% N₂.

Table 2—The effect of MAP on chemical parameters (pH, TTA, and TSS) of pomegranate arils during cold storage.

		pH, TTA, and TSS ¹						
Application ²		Day 0	Day 3	Day 6	Day 9	Day 12	Day 15	Day 18
pH	MAP 1	3.30 ± 0.04 ^A _a	3.31 ± 0.03 ^A _a	3.22 ± 0.04 ^A _d	3.23 ± 0.04 ^A _{cd}	3.22 ± 0.04 ^{AB} _d	3.25 ± 0.03 ^A _{bc}	3.27 ± 0.03 ^A _b
	MAP 2	3.30 ± 0.04 ^A _a	3.30 ± 0.03 ^A _a	3.25 ± 0.05 ^A _c	3.25 ± 0.03 ^A _c	3.24 ± 0.03 ^A _c	3.25 ± 0.03 ^A _c	3.27 ± 0.03 ^A _b
	MAP 3	3.30 ± 0.04 ^A _{ab}	3.31 ± 0.07 ^A _a	3.25 ± 0.03 ^A _c	3.24 ± 0.03 ^A _c	3.23 ± 0.03 ^A _c	3.24 ± 0.03 ^A _c	3.27 ± 0.03 ^A _{bd}
	MAP 4	3.30 ± 0.04 ^A _a	3.29 ± 0.03 ^A _a	3.22 ± 0.03 ^A _c	3.19 ± 0.05 ^B _d	3.20 ± 0.04 ^B _{cd}	3.20 ± 0.03 ^B _{cd}	3.26 ± 0.03 ^A _b
Total titratable acidity (%)	MAP 1	3.39 ± 0.29 ^A _a	1.69 ± 0.06 ^A _b	1.86 ± 0.16 ^A _b	1.99 ± 0.17 ^A _b	2.10 ± 0.27 ^{AB} _b	1.69 ± 0.11 ^B _b	1.99 ± 0.08 ^A _b
	MAP 2	3.39 ± 0.29 ^A _a	1.72 ± 0.06 ^A _{cd}	1.99 ± 0.12 ^A _c	1.81 ± 0.04 ^{AB} _{cd}	2.48 ± 0.15 ^A _b	1.69 ± 0.06 ^B _d	1.82 ± 0.11 ^A _{cd}
	MAP 3	3.39 ± 0.29 ^A _a	1.63 ± 0.10 ^A _d	1.92 ± 0.11 ^A _{bc}	1.72 ± 0.10 ^B _{cd}	2.14 ± 0.08 ^{AB} _b	1.91 ± 0.05 ^A _{bc}	1.84 ± 0.06 ^A _{cd}
	MAP 4	3.39 ± 0.29 ^A _a	1.76 ± 0.11 ^A _b	1.90 ± 0.16 ^A _b	1.94 ± 0.16 ^A _b	1.94 ± 0.14 ^B _b	1.91 ± 0.09 ^A _{ab}	1.99 ± 0.14 ^A _b
TSS (°Brix)	MAP 1	15.0 ± 0.1 ^A _a	14.5 ± 0.1 ^A _b	14.5 ± 0.1 ^A _b	14.5 ± 0.1 ^A _b			
	MAP 2	15.0 ± 0.1 ^A _a	15.0 ± 0.1 ^A _a	15.0 ± 0.2 ^A _a	15.0 ± 0.1 ^A _a	14.5 ± 0.1 ^A _b	14.5 ± 0.21 ^A _b	14.0 ± 0.2 ^B _c
	MAP 3	15.0 ± 0.1 ^A _a	14.5 ± 0.2 ^A _b	14.5 ± 0.1 ^A _b	14.0 ± 0.1 ^B _c			
	MAP 4	15.0 ± 0.1 ^A _a	14.5 ± 0.1 ^A _b	14.5 ± 0.1 ^A _b	14.0 ± 0.2 ^B _c			

¹ For each column, similar capital letters (superscript) are not significantly different at $P \leq 0.05$ among MAP treatments. For each parameter, similar small letters (subscript) in rows are not significantly different at $P \leq 0.05$ during storage.

² MAP1: 21% O₂, 79% N₂; MAP2: 100% N₂; MAP3: 5% O₂, 10% CO₂, 85% N₂; MAP4: 70% O₂, 10% CO₂, 20% N₂.

Table 3 – The effect of MAP on chemical parameters (total phenolic content, total anthocyanin content, and total antioxidant activity) of pomegranate arils during cold storage.

		Total phenolic content, total anthocyanin content, and total antioxidant activity ¹						
Application ²		Day 0	Day 3	Day 6	Day 9	Day 12	Day 15	Day 18
Total phenolic content	MAP1	1448.4 ± 21.3 ^A	1487.6 ± 54.7 ^{AB} cd	1507.5 ± 52.3 ^{cd}	1622.0 ± 77.3 ^A b	1695.6 ± 85.4 ^A	1628.1 ± 54.0 ^A b	1531.8 ± 89.2 ^A
	MAP2	1448.4 ± 21.3 ^A cd	1412.7 ± 9.3 ^C d	1473.8 ± 73.0 ^{bc}	1506.7 ± 79.2 ^B b	1575.0 ± 57.2 ^B bc	1502.1 ± 34.4 ^B bc	1468.8 ± 83.8 ^{AB} bc
	MAP3	1448.4 ± 21.3 ^A bc	1454.5 ± 42.0 ^{bc}	1472.6 ± 53.9 ^A bc	1387.0 ± 84.5 ^C	1610.3 ± 82.6 ^B	1517.7 ± 98.9 ^B	1504.2 ± 96.6 ^{AB} b
	MAP4	1448.4 ± 21.3 ^A bc	1520.4 ± 55.2 ^{bc}	1518.3 ± 48.2 ^b	1523.2 ± 96.0 ^B b	1644.2 ± 88.3 ^{AB}	1705.3 ± 95.1 ^A	1415.2 ± 111.4 ^B c
Total anthocyanin content	MAP1	311.4 ± 5.3 ^A	321.9 ± 3.4 ^A	318.3 ± 7.2 ^B	322.3 ± 6.4 ^A	299.6 ± 10.0 ^C	305.0 ± 5.6 ^A c	279.0 ± 7.5 ^A d
	MAP2	311.4 ± 5.3 ^A	268.8 ± 8.0 ^C	308.0 ± 18.0 ^B	287.2 ± 7.8 ^C b	293.4 ± 6.8 ^{AB} b	271.2 ± 30.5 ^B	265.3 ± 10.0 ^B
	MAP3	311.4 ± 5.3 ^A	296.9 ± 11.7 ^{ab}	308.8 ± 22.5 ^A	271.0 ± 11.3 ^D ab	288.5 ± 8.9 ^B bc	258.7 ± 26.6 ^E	279.2 ± 12.5 ^A cd
	MAP4	311.4 ± 5.3 ^A	310.3 ± 21.6 ^A	333.3 ± 13.3 ^A	308.0 ± 13.8 ^B b	299.2 ± 12.3 ^A bc	292.1 ± 3.3 ^A c	274.2 ± 9.8 ^{AB} d
Total antioxidant activity (%)	MAP1	43.74 ± 1.07 ^A c	42.23 ± 0.52 ^C d	46.42 ± 1.26 ^D b	47.90 ± 0.64 ^B a	45.79 ± 0.84 ^C	46.59 ± 0.85 ^B b	41.46 ± 2.18 ^C d
	MAP2	43.74 ± 1.07 ^A c	42.96 ± 0.88 ^C cd	48.55 ± 0.44 ^B a	49.06 ± 0.53 ^A a	46.49 ± 1.17 ^C b	45.79 ± 2.51 ^B b	42.11 ± 0.85 ^C d
	MAP3	43.74 ± 1.07 ^A d	45.18 ± 1.39 ^B c	47.76 ± 0.55 ^C a	46.50 ± 1.56 ^C b	47.70 ± 0.46 ^B ab	46.87 ± 2.12 ^B ab	43.51 ± 1.02 ^B d
	MAP4	43.74 ± 1.07 ^A e	47.48 ± 0.93 ^C	49.54 ± 0.44 ^A a	49.17 ± 0.83 ^A b	49.17 ± 0.94 ^A b	51.25 ± 0.60 ^A 2, a	46.16 ± 1.05 ^A d

¹For each column, similar capital letters (superscript) are not significantly different at $P \leq 0.05$ among MAP treatments. For each parameter, similar small letters (subscript) in rows are not significantly different at $P \leq 0.05$ during storage.

²MAP1: 2% O₂, 79% N₂; MAP2: 100% N₂; MAP3: 5% O₂, 10% CO₂, 85% N₂; MAP4: 70% O₂, 10% CO₂, 20% N₂.

increased slightly until the 12th d of storage, and then decreased for all the treatments. The diversity of the amounts of total phenolic content during storage was probably due to changes in total acidity and TSS content, which in return affected the total anthocyanin content and total antioxidant activity. Although there were some differences, the effect of MAP application on the total phenolic content was not as pronounced as the effect of storage time.

There were significant effects of MAP application, storage day, and MAP application × storage day interaction on the total anthocyanin content ($P \leq 0.05$). In general, total anthocyanin content decreased as the storage time increased for all treatments. Total anthocyanin content decreased from 311.3 mg cyanidin-3-glucoside equivalent/L of fruit juice at day 0 to 279, 265.3, 279.2, and 274.2 mg cyanidin-3-glucoside equivalent/L of fruit juice after 18 d of storage for MAP1, MAP2, MAP3, and MAP4, respectively. Pomegranate arils packaged in air (MAP1) and enriched oxygen (MAP4) had higher total anthocyanin contents than the samples packaged in nitrogen (MAP2) or low oxygen atmosphere (MAP3) during storage. No significant change in total anthocyanin content in arils (cultivar Mollar) during MAP storage at 1 °C up to 7 d was reported by Gil and others (1996). Lopez-Rubira and others (2005) also reported that there was no significant change in total anthocyanin content of early harvested pomegranates after 13 d of storage. Our results were in agreement with these studies.

Pomegranate exhibits good antioxidant capacity primarily due to its high levels of phenolic acids, flavonoids and other polyphenolic compounds (Aviram and others 2002; Kulkarni and Aradhya 2005). There was a significant effect of MAP, storage day, and MAP application × storage day interaction for the total antioxidant activity of pomegranate arils ($P \leq 0.05$). Total antioxidant activity increased until the 9 d of storage then decreased under passive (MAP1) and active MAPs with low or no oxygen (MAP2 and MAP3, respectively). On the other hand, the total antioxidant activity of pomegranate arils packaged under enriched O₂ atmosphere (MAP4) increased until the 15 d of storage then decreased, and its activity was significantly higher than the other MAP applications on the 18th day of storage (Table 3). While pomegranate arils packaged under MAP1 (21% oxygen) had the highest loss in antioxidant activity, there was an increase in antioxidant activity for the samples packaged under enriched oxygen (MAP4) compared to its initial level. There was a positive relationship between antioxidant activity (%) and total phenolic content indicating the effect of polyphenol content on antioxidant activity (Table 3). Lopez-Rubira and others (2005) reported that the antioxidant activity of pomegranate arils UV-C treated and packaged in PP trays sealed with BOPP film under passive MAP did not significantly change throughout the storage time of 15 d at 5 °C.

Microbial quality

Yeast and mold growth was under the limit of detection for all the treatments. The aerobic mesophilic bacteria were in the range of 2.30 to 4.51 log CFU/g at the end of the storage (Table 4). The lowest count was observed at high oxygen application as 2.3 log CFU/g and the highest count was observed at 100% nitrogen application as 4.51 log CFU/g. This level did not affect the sensory quality of pomegranate arils at the end of the storage time. Even the highest microbial count was under 7 log CFU/g, which was established as maximum limit for aerobic bacteria by the Spanish legislation (Lopez-Rubira and others 2005). High levels of O₂ has been found to be effective in inhibiting enzymatic discoloration, preventing anaerobic fermentation reactions, and inhibiting aerobic and anaerobic microbial growth. Among the most often used gases in MAP (O₂, CO₂, and N₂), only CO₂ has significant and direct

Table 4 – The effect of MAP on the microbial quality (total mesophilic aerobic count, log CFU/g).

Application ²	Total mesophilic aerobic bacterial count (log CFU/g) ¹						
	Day 0	Day 3	Day 6	Day 9	Day 12	Day 15	Day 18
MAP1	<1 ^A _b	<1 ^A _b	<1 ^A _b	<1 ^A _b	<1 ^C _b	4.36 ^A _a	4.48 ^A _a
MAP2	<1 ^A _d	<1 ^A _d	<1 ^A _d	<1 ^A _d	1.78 ^A _c	2.53 ^{BC} _b	4.51 ^A _a
MAP3	<1 ^A _d	<1 ^A _d	<1 ^A _d	<1 ^A _d	1.78 ^A _c	2.76 ^B _b	3.36 ^B _a
MAP4	<1 ^A _c	<1 ^A _c	1 ^A _c	1 ^A _c	1.63 ^B _b	2.25 ^C _a	2.30 ^C _a

¹For each column, similar capital letters (superscript) are not significantly different at $P \leq 0.05$ among MAP treatments. For each parameter, similar small letters (subscript) in rows are not significantly different at $P \leq 0.05$ during storage.

²MAP1: 21% O₂, 79% N₂; MAP2: 100% N₂; MAP3: 5% O₂, 10% CO₂, 85% N₂; MAP4: 70% O₂, 10% CO₂, 20% N₂.

Table 5 – The effect of MAP on the sensory attributes and acceptance of pomegranate arils during cold storage.

Application ²	Day 0	Day 3	Day 6	Day 9	Day 12	Day 15	Day 18	Panelist scores ¹							
								Color	Freshness	Taste	Texture	Overall acceptance			
Color	MAP1	4.67 ± 0.52 ^A _a	4.67 ± 0.52 ^A _a	4.83 ± 0.41 ^A _a	4.83 ± 0.41 ^A _a	4.67 ± 0.52 ^A _a	4.50 ± 0.55 ^A _{ab}	4.00 ± 0.63 ^A _b							
	MAP2	4.67 ± 0.52 ^A _a	4.50 ± 0.55 ^A _{ab}	4.83 ± 0.41 ^A _a	4.33 ± 0.82 ^A _{ab}	5.00 ± 0.06 ^A _a	3.83 ± 0.75 ^A _b	3.83 ± 0.75 ^A _b							
	MAP3	4.67 ± 0.52 ^A _{ab}	4.33 ± 0.82 ^A _{abc}	4.33 ± 0.52 ^A _{abc}	4.33 ± 0.82 ^A _{abc}	4.83 ± 0.41 ^A _a	3.83 ± 0.75 ^A _{bc}	3.50 ± 0.55 ^A _c							
	MAP4	4.67 ± 0.52 ^A _a	4.33 ± 0.52 ^A _a	4.67 ± 0.52 ^A _a	4.50 ± 0.84 ^A _a	4.67 ± 0.52 ^A _a	4.50 ± 0.84 ^A _a	4.00 ± 0.63 ^A _a							
Freshness	MAP1	4.83 ± 0.41 ^A _a	4.83 ± 0.41 ^A _a	4.67 ± 0.52 ^A _{ab}	4.83 ± 0.41 ^A _a	4.17 ± 0.75 ^A _{bc}	4.33 ± 0.52 ^A _{abc}	3.83 ± 0.41 ^A _c							
	MAP2	4.83 ± 0.41 ^A _a	4.50 ± 0.55 ^A _{ab}	4.83 ± 0.41 ^A _a	4.17 ± 0.75 ^A _{abc}	4.83 ± 0.41 ^A _a	3.83 ± 0.75 ^A _{bc}	3.67 ± 0.52 ^A _c							
	MAP3	4.83 ± 0.41 ^A _a	4.67 ± 0.52 ^A _a	4.33 ± 0.52 ^A _{ab}	4.50 ± 0.84 ^A _{ab}	4.83 ± 0.41 ^A _a	3.83 ± 0.75 ^A _{bc}	3.33 ± 0.82 ^A _c							
	MAP4	4.83 ± 0.41 ^A _a	4.50 ± 0.55 ^A _{ab}	4.33 ± 0.52 ^A _{ab}	4.50 ± 0.84 ^A _{ab}	4.50 ± 0.55 ^A _{ab}	3.83 ± 0.98 ^A _b	4.00 ± 0.89 ^A _{ab}							
Taste	MAP1	5.00 ± 0.04 ^A _a	4.83 ± 0.41 ^A _{ab}	4.17 ± 0.75 ^A _{cd}	4.83 ± 0.41 ^A _{ab}	4.67 ± 0.52 ^A _{abc}	4.33 ± 0.52 ^A _{bcd}	4.00 ± 0.63 ^A _d							
	MAP2	5.00 ± 0.04 ^A _a	4.67 ± 0.52 ^A _a	4.33 ± 0.82 ^A _a	4.17 ± 0.75 ^A _a	4.67 ± 0.52 ^A _a	3.33 ± 0.82 ^A _b	3.17 ± 0.75 ^A _b							
	MAP3	5.00 ± 0.04 ^A _a	4.50 ± 0.55 ^A _{ab}	4.00 ± 0.63 ^A _b	4.50 ± 0.55 ^A _{ab}	4.33 ± 0.82 ^A _{ab}	4.00 ± 0.63 ^A _{ab}	3.00 ± 1.26 ^A _c							
	MAP4	5.00 ± 0.04 ^A _a	4.33 ± 0.52 ^A _{ab}	4.33 ± 0.52 ^A _{ab}	4.83 ± 0.41 ^A _a	4.67 ± 0.52 ^A _{ab}	3.83 ± 1.33 ^A _{bc}	3.33 ± 0.82 ^A _c							
Texture	MAP1	5.00 ± 0.05 ^A _a	5.00 ± 0.00 ^A _a	4.67 ± 0.82 ^A _{ab}	4.83 ± 0.41 ^A _a	4.50 ± 0.55 ^A _{abc}	4.00 ± 0.89 ^A _{bc}	3.83 ± 0.41 ^A _c							
	MAP2	5.00 ± 0.05 ^A _a	4.33 ± 0.52 ^B _a	4.67 ± 0.52 ^A _a	4.67 ± 0.52 ^A _a	4.83 ± 0.41 ^A _a	3.33 ± 0.82 ^A _b	3.50 ± 1.05 ^A _b							
	MAP3	5.00 ± 0.05 ^A _a	4.67 ± 0.52 ^{AB} _{ab}	4.33 ± 0.52 ^A _{ab}	4.67 ± 0.82 ^A _{ab}	4.50 ± 0.55 ^A _{ab}	4.00 ± 0.63 ^A _{bc}	3.50 ± 1.05 ^A _c							
	MAP4	5.00 ± 0.05 ^A _a	4.83 ± 0.41 ^{AB} _a	4.33 ± 0.52 ^A _{ab}	4.67 ± 0.52 ^A _{ab}	4.50 ± 0.55 ^A _a	3.67 ± 0.82 ^A _{bc}	3.50 ± 1.05 ^A _c							
Overall acceptance	MAP1	5.00 ± 0.06 ^A _a	4.83 ± 0.41 ^A _{ab}	4.33 ± 0.52 ^{AB} _{bcd}	4.83 ± 0.41 ^A _{ab}	4.50 ± 0.55 ^A _{abc}	4.00 ± 0.63 ^A _{cd}	3.83 ± 0.41 ^A _d							
	MAP2	5.00 ± 0.06 ^A _a	4.33 ± 0.52 ^A _a	4.83 ± 0.41 ^A _a	4.50 ± 0.55 ^A _a	4.83 ± 0.41 ^A _a	3.50 ± 1.05 ^A _b	3.33 ± 0.82 ^A _b							
	MAP3	5.00 ± 0.06 ^A _a	4.67 ± 0.52 ^A _{ab}	4.00 ± 0.63 ^B _b	4.50 ± 0.84 ^A _{ab}	4.50 ± 0.84 ^A _{ab}	3.83 ± 0.75 ^A _b	2.83 ± 1.17 ^A _c							
	MAP4	5.00 ± 0.06 ^A _a	4.83 ± 0.41 ^A _a	4.33 ± 0.52 ^{AB} _a	4.67 ± 0.52 ^A _a	4.67 ± 0.52 ^A _a	3.50 ± 1.05 ^A _b	3.50 ± 1.05 ^A _b							

¹For each column, similar capital letters (superscript) are not significantly different at $P \leq 0.05$ among MAP treatments. For each parameter, similar small letters (subscript) in rows are not significantly different at $P \leq 0.05$ during storage. Scores of 3 and above were considered as acceptable.

²MAP1: 21% O₂, 79% N₂; MAP2: 100% N₂; MAP3: 5% O₂, 10% CO₂, 85% N₂; MAP4: 70% O₂, 10% CO₂, 20% N₂.

antimicrobial activity due to alteration of cell membrane function including effects on nutrient uptake and absorption, direct inhibition of enzymes, or decreases in the rate of enzyme reactions, penetration of bacterial membranes leading to intracellular pH changes and changes to the physicochemical properties of proteins (Farber 1991).

Lopez-Rubira and others (2005) reported that microbial counts of minimally fresh processed arils increased throughout shelf life at 5 °C. Even though they treated the arils with UV-C, the shelf life was limited to 10 to 12 d due to the microbial growth. In our study, the arils were treated with chlorine.

Sensory quality

Table 5 shows the effects of MAP on the sensory attributes and acceptance of pomegranate arils during cold storage. The minimally processed pomegranate arils were acceptable (the sensory score of 3 and above out of 5) in terms of product attributes such as aril color, freshness, taste, and texture at all atmospheres until the end of the storage time. Overall, the pomegranate arils packed with air, nitrogen, and enriched oxygen were acceptable by the sensory panelists on the 18th day; however, it was limited to 15 d for the low oxygen atmosphere. The overall acceptance score of pomegranate arils packaged under low oxygen atmosphere was 2.8, which was lower than the acceptable level (score 3) on the storage day of 18. According to Lopez-Rubira and others (2005), the limit of visual quality by consumers was 14 d for early harvested (October) and 10 d for late harvested (December) pomegranate arils.

Conclusions

As a general trend, slight or no significant changes were detected in chemical, physical and sensory quality during refrigerated storage. The shelf life of pomegranate arils of Hicaznar was suggested as 18 d under air, nitrogen, and enriched oxygen atmospheres, and 15 d under the low oxygen atmosphere with the package type of PP tray with BOPP film at 5 °C storage. The selected packaging material combined with modified atmosphere at low storage temperature provided commercially acceptable shelf life of 18 d, quality, and convenience for pomegranate arils sanitized with chlorine.

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