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Active-Modified Atmosphere Packaging of Ready-to-Eat Pomegranate (*Punica granatum* L.) Arils at Ambient Temperature for Extending Shelf-Life

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Abstract: Modified atmosphere packaging (MAP) has been widely applied to extend the shelf-life of fresh-cut produces, such as ready-to-eat pomegranate arils. However, many studies used MAP to extend the shelf-life of arils at 3, 4, and 15 °C. The evidence suggested that MAP can extend the shelf-life of arils at an ambient temperature. Therefore, we attempted to extend the shelf-life of ready-to-eat pomegranate arils using active MAP at an ambient temperature and evaluated its chemical, quality, and microbial properties. Samples were flushed with specific gas compositions of oxygen (O_2) and carbon dioxide (CO_2) at an ambient temperature and 60 ± 2% relative humidity (RH). The findings demonstrated that active MAP increased the titratable acidity (0.32–3.64%), anthocyanins (0.27–0.42 g/L), reducing sugars (1.06–10.13%), and total soluble solids (15–19 °Brix), while it decreased the pH (3.20–3.54) and hardness (4.60–9.40 N) with a microbial load of $\leq$5 log CFU/g at ambient temperature for 5 days. Ultimately, active MAP could be an effective technique to be used in ready-to-eat produce industries and has the potential to guarantee a longer shelf-life at ambient temperature without the use of refrigerator facilities.

Keywords: ready-to-eat pomegranate arils; modified atmosphere packaging; gas composition; microbial load; shelf-life

1. Introduction

Pomegranate (*Punica granatum* L.; Punicaceae) is an ancient and well-known edible fruit and has been associated with several ancient cultures of the world. There has been a rapid increase in the commercial production of pomegranate, with a major contribution of 90% from the Northern Hemisphere countries, such as India, China, Iran, and Turkey, in which India and Iran are the dominant exporters of the fruit [1]. Pomegranates are intensively grown for fresh consumption of arils with the interior network of membranes, which contains 60% of total fruit weight, 80% juice (85% water and 10% total sugars), and 20% seeds [2]. However, extracting the arils from the leathery husk of pomegranate is a labor-intensive and tedious process, which limits its consumption as a fresh fruit for fast-paced consumer lifestyles.

Over the years, ready-to-eat or fresh-cut produce has reached tremendous growth due to its freshness, safe, nutritious, and health-beneficial properties [3]. This trend in selection of fresh-cut produce further continues to be a popular choice for many consumers to have meals away from home and on-the-go fresh options [2]. Therefore, preparation of pomegranate arils in ready-to-eat form would be a convenient alternative to increasingly busy consumer lifestyles in order to fulfill their needs with lower food safety risks. Nonetheless, maintaining the nutritional value of ready-to-eat pomegranate arils is facing new challenges, such as microbiological hazards and foodborne illnesses, which may cause decay as well as deterioration in the visual and nutritional quality, causing adverse impacts on human health [4]. Hence, it is imperative to reduce the higher microbiological risks associated with ready-to-eat pomegranate arils. To overcome this, modified atmosphere packaging (MAP) has been extensively used to preserve the quality and extend the shelf-life of ready-to-eat fresh fruit and vegetables during storage and marketing with a high consumer acceptability [5].

MAP, active or passive, is a package influencing a process and the transfer of gasses through the packaging material [6]. A study by Palma et al. [2] extended the shelf-life of ready-to-eat arils up to 10 days at 5 °C and 90% RH using a polypropylene film under passive MAP. Another study by Banda et al. [7] used a low barrier bi-axially oriented polyester film under both active and passive MAP to prolong the shelf-life of ready-to-eat pomegranate arils up to 12 and 9 days at 5 °C, respectively. These two independent studies concluded the high organoleptic properties of ready-to-eat arils with a reduced microbial load and food safety risks. Likewise, studies were conducted to improve the pomegranate fruit quality by short-term hypobaric treatment combined with MAP [8] and pre-cooling as well as MAP [9]. A more recent review highlighted the need of MAP to reduce postharvest losses and to improve quality of the fresh-cut pomegranates [10,11]. Over the years, many studies evolved to extend the shelf-life of arils at 3, 4, and 15 °C [12–14]. However, these studies were highly centered to extend the shelf-life of arils at storage temperatures of 3–15 °C and have failed to address the application of active MAP under optimum storage temperature. This represented the need for in-depth understanding of active MAP to prolong the shelf-life of ready-to-eat arils at an ambient temperature without significant loss in quality attributes. The emergence of extended shelf-life at ambient temperature would encourage low- and middle-income consumers, who lack refrigerator facilities, in decision-making, and purchasing behavior of ready-to-eat pomegranate arils.

Generally, gas composition, such as oxygen (O_2) and carbon dioxide (CO_2), plays a vital role in extending the shelf-life of fresh-cut produces. A review by Caleb et al. [15] highlighted the different composition of optimized O_2 (3–19%) and carbon dioxide CO_2 (1–23.28%) for wide variety of fruits and vegetables. Likewise, other studies recommended the gas composition of 5% O_2 and 0–5% CO_2 at 5 °C and 3–5% O_2 and 5–10% CO_2 at 5 °C during MAP [15]. Another study recommended the 10% O_2 + 15% CO_2 to extend the shelf-life of ready-to-eat pomegranate arils [16]. However, the use of gas composition is purely depending on the type of produce, packaging material, and storage temperature. Up until now, the evidence for the shelf-life and storage of arils under an active-MAP at ambient temperature has remained unclear and inconclusive. Hence, we hypothesized that the active MAP can be a suitable quality preservation technique to prolong the shelf-life of ready-to-eat pomegranate arils at an ambient temperature.

Therefore, the present study investigated the extending of the shelf-life of ready-to-eat pomegranate arils using active MAP at an ambient temperature and evaluated its chemical, quality, and microbial properties.

2. Materials and Methods

2.1. Sample Collection

Freshly harvested and high-quality pomegranate (*Punica granatum* L.; Indian variety: Bhagwa) fruits were obtained from the local commercial market in Pulivendula, YSR Kadapa, Andhra Pradesh, India. The freshly picked pomegranates were covered with a

foam net and then packed in a corrugated box (35 × 33 × 18 cm) containing shredded paper strips, and then transported to the food processing laboratory within 15 min. The fruit samples were washed thoroughly with tap water, rinsed with distilled water, and then drained with a blotting paper to remove the surface water. All the samples were stored in the refrigerator at 4 °C until further use (no longer than 2 days).

2.2. Sample Processing, Packaging, and Treatment

The fruit samples were placed on a cutting board (35 × 20 cm) and rolled twice to loosen the skin and seeds. Then, the longitudinal cut was performed along the equatorial zone to open into two halves. The extracted arils (juice-enclosed seeds) were manually separated from the peel and other non-edible parts of the fruit. The extracted arils were gently washed with water containing chlorine (100 ppm), rinsed with drinking water, and then softly patted on bibulous paper without causing any mechanical damage [2]. Samples (170 g) were packed in transparent high-density polyethylene (HDPE) food-grade pouches (15 × 20 cm). The oxygen permeability of HDPE was 100 cc/m^2/day at 25 °C and 0–5% relative humidity (RH) with a thickness of 60 µm. The packed samples were hermetically heat-sealed by a chamber sealer (Vac-Star Verpackungsmaschinen AG, Sugiez, Switzerland) and were flushed with specific gas compositions of O_2 and CO_2 at an ambient temperature (25 °C) and 60 ± 2% RH, as shown in Table 1. The heat-sealed sample without treatment containing a normal atmospheric gas composition was considered as a control. All the samples were then stored in the laboratory at an ambient temperature (25 °C) for 5 days and 60 ± 2% RH. A total of 51 packages were evaluated for chemical, quality, and microbial properties on days 0 and 5 at 25 °C and 60 ± 2% RH. Packaging material, storage temperature, storage time, and other experimental conditions were optimized during pre-trials. Samples were coded as T1, T2, T3 . . . T16, and control, as shown in Table 1. All the treatment conditions consisted of three replications.

Table 1. The initial flushed gas compositions of CO_2 and O_2 for modified atmosphere packaging at an ambient temperature with a RH of 60 ± 2% [1].

Treatment Code	Headspace Gas Composition (%)	
	O_2	CO_2
Control	21 †	0.03 †
T1	0.2	6.3
T2	0.1	9.5
T3	0.6	12
T4	2.5	13
T5	2.9	3.4
T6	4.9	2.9
T7	7.0	2.8
T8	1.0	2.8
T9	2.8	5.8
T10	3.5	5.8
T11	11	5.9
T12	4.6	11
T13	2.5	8.3
T14	4.7	8.3
T15	6.3	8.7
T16	7.0	12

[1] The remaining gas composition, other than oxygen (O_2,%) and carbon dioxide (CO_2,%), was nitrogen (N_2,%). † Normal earth's atmospheric gas composition. RH is relative humidity (%).

2.3. Chemical Analysis

2.3.1. Titratable Acidity and pH

Titratable acidity (TA) of samples was measured according to the methodology described by Liu et al. [17]. The results were calculated according to Equation (1) and reported

on the basis of % citric acid. The pH of samples was measured by a digital pH-meter (Model-12320, Dewsil Scientific Pvt. Ltd., Delhi, India).

$$\text{Titratable acidity } (\%) = \left[\left(\frac{M_c \times (V_1 - V_2) \times K}{\text{Volume of sample (mL)}}\right) \times \left(\frac{50}{\text{Mass of solute (g)}}\right) \times 100\right] \quad (1)$$

where M_c = molar concentration of NaOH; V_1 = volume of NaOH consumed in sample (mL); V_2 = volume of NaOH consumed in blank (mL); K = conversion coefficient, 0.067.

2.3.2. Anthocyanins

The anthocyanin content of samples was determined according to Flores-Martínez et al. [18]. Briefly, samples (200 μL) were thoroughly mixed with ethanol–HCl (10 mL; 85:15, *v/v*) under a stirring at 200 rpm for 30 min. The reaction mixture was centrifuged (vs-03, Bioline Technologies, Maharashtra, India) at 100× *g* for 15 min. The absorbance was measured at 535 nm using a UV–VIS spectrophotometer (Evolution™ 260 Bio, Thermo Fisher Scientific Inc., Waltham, MA, USA). The results were calculated as grams of cyanidin-3-glucoside (C3G) per liter of sample (g C3G/L) using Equation (2).

$$\text{Anthocyanins (g/L)} = \left[\left(\frac{A}{\varepsilon}\right) \times \left(\frac{V}{10^6}\right)(\text{MW}) \times \left(\frac{1}{\text{Sample weight (g)}}\right) \times \left(10^6\right)\right] \quad (2)$$

where A = absorbance of sample; ε = molar absorptivity (cyanidin-3-glucoside = 26,965 L mol^{-1} cm^{-1}); V = volume of sample (mL); MW = molecular weight of cyanidin-3-glucoside (449.20 g mol^{-1}).

2.3.3. Ascorbic Acid

The ascorbic acid content was determined according to the method described by Liu, Li, Xu, Fu, Liao, Shi and Chen [17]. The ascorbic acid content of samples was calculated as shown in Equation (3) and was expressed as g/100 g.

$$\text{Ascorbic acid (g/100 g)} = \left[\left(\frac{(V - V_0) \times \text{ titer value}}{\text{Sample weight (g)}}\right) \times 100\right] \quad (3)$$

where V = volume of 2,6-dichloroindophenol solution consumed (mL); V_0 = volume of 2,6-dichloroindophenol consumed in blank sample (mL).

2.3.4. Reducing Sugars

Reducing sugars were determined according to Choi et al. [19] on the basis of the D-glucose standard curve and expressed as a percentage.

2.4. Quality Analysis

2.4.1. Weight Loss

The initial weight of samples was measured using an electronic balance (ATN120, Scaletec Mechatronics Pvt. Ltd., Gujarat, India). The weight loss of samples was recorded and calculated as shown in Equation (4) according to Liu, Li, Xu, Fu, Liao, Shi and Chen [17] and expressed as percentage weight loss.

$$\text{Weight loss } (\%) = \left[\left(\frac{\text{Initial weight (g)} - \text{final weight at testing (g)}}{\text{Initial weight (g)}}\right) \times 100\right] \quad (4)$$

2.4.2. Hardness

Hardness of individual samples was measured using a texture analyzer (CT3™ Texture Analyser, Brookfield AMETEK®, Lab Unlimited, Surrey, United Kingdom) by compression using a 35 mm cylindrical probe. Samples were compressed to 60% of the original height using a crosshead speed of 1 and 10 mm/s for test and post-test, respectively.

The mean value of samples (n = 10) was measured for each treatment, and the hardness was expressed as maximum compression force in newtons (N).

2.4.3. Total Soluble Solids (TSS) Content

The TSS of samples was determined by using a traditional hand-held refractometer (Atago Co., Ltd., Tokyo, Japan). Briefly, samples were crushed manually to collect the juice. Then, a few drops of juice samples were placed on the prism surface of the refractometer, and the results were expressed as °Brix.

2.5. Package Headspace Gas Composition (O_2 and CO_2)

The headspace gas concentration of O_2 and CO_2 in samples were measured in each package using a gas analyzer (Check Point II, PBI Dansensor, DWN Instrumentation Ltd., Cork, Ireland), which was previously calibrated with air O_2 and CO_2 percentages [20]. The gas composition signals were directly displayed on the digital panel of the gas analyzer, and the composition of O_2 and CO_2 was expressed as a percentage.

2.6. Microbiological Analysis

Microbial analysis (yeast and mold counts) was determined according to the method described by Sheikhi et al. [21]. Briefly, samples (10 g) were thoroughly mixed with 1% sterile peptone water (90 mL) for 15 min, and the serial dilutions were performed with the 1% sterile peptone water. Aliquots (200 μL) were plated on Petri plates containing solidified potato dextrose agar and incubated in a bacteriological incubator at 25 °C for 5 days. The numbers of colonies were counted with a digital colony counter, and the results were expressed as log colony-forming units per gram (log CFU/g).

2.7. Statistical Analysis

All experiments were conducted at least in triplicate ($n \geq 3$) and reported as mean ± standard deviation (SD). The differences in the mean values of each experiment were statistically analyzed using one-way analysis of variance (ANOVA) in IBM® SPSS® version 16.0 (IBM Ltd., Chicago, IL, USA). Mean values were differentiated using Duncan's multiple range test at 95% confidence intervals (α = 0.05). The Microsoft Excel® version 2019 (Microsoft Co., Ltd., Redmond, WA, USA) was used to construct the graphs and determine mean values as well as standard deviation among the samples.

3. Results and Discussion

3.1. Chemical Analysis

3.1.1. Titratable Acidity and pH

On day 0, titratable acidity of samples ranged from 0.80 to 1.20% and 1.20%, respectively, for samples (T1–T16) and control, which fluctuated in the range of 0.32–3.64% and 1.86%, respectively, for samples and control at the end of the storage on day 5 (Figure 1A). The trend in titratable acidity was comparable to the study conducted by Martínez-Romero et al. [22] and Banda, Caleb, Jacobs and Opara [7], where the authors reported an increased and fluctuated titratable acidity for pomegranate arils during storage. We assumed that the variations in gas composition and increase of respiration rate might have contributed to the fluctuations in titratable acidity. Other possible explanations could be related to the pre-treatment of pomegranate arils before storage and other metabolic reactions caused by the abiotic stress under active MAP at an ambient temperature [7,13].

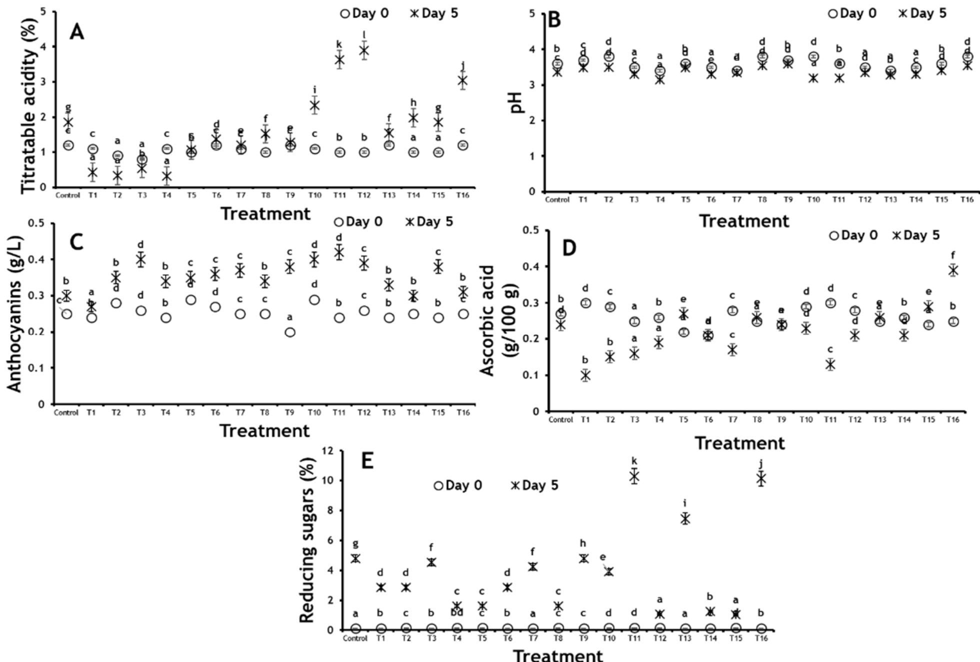

Figure 1. Chemical analysis of minimally processed pomegranate arils stored under active MAP at ambient temperature. Titratable acidity (**A**), pH (**B**), anthocyanins (**C**), ascorbic acid (**D**), and reducing sugars (**E**). Error bar represents the standard deviation from the mean of three independent replicates. The mean values followed by lowercase alphabets (a–l) among the treatments (T1–T16 and control) were significantly different ($p < 0.05$) on the basis of Duncan's multiple range test. MAP = modified atmosphere packaging. For treatment codes, refer to Table 1.

On the other hand, the pH value of pomegranate arils showed fluctuations between 3.40 to 3.80 and 3.20 to 3.54 on days 0 and 5, respectively, for all treatment samples (Figure 1B). The results further indicated the lower pH values (3.20 to 3.54) for samples under active MAP over the end of storage period, which was relatively 1.07 times lower compared to pH values on day 0 (3.40 to 3.80), which support the data reported by Banda, Caleb, Jacobs and Opara [7], who showed significantly ($p < 0.05$) decreased pH values at an average of 2.90 for all active MAP-treated pomegranate arils. The decrease in pH could be attributed to the gas composition (low O_2 and production of CO_2), especially production of CO_2 in active MAP, which solubilizes in water molecules around the package, resulting in the formation of carbonic acid in the aril tissues [23]. Another study by Sarhadi and Sadeghizadeh [24] concluded the variation in pH values could be related to the permeability of CO_2 and production of organic acids during MAP. The alteration in generation of CO_2 could be the consequences of different treatments that contributed to the variation in pH values. Therefore, these factors should be considered in order to meet the fresh-cut produce regulatory requirements and for consumer acceptability.

3.1.2. Anthocyanins

The anthocyanin content was slightly increased in samples (T1–T16) under the active-MAP compared to that of the control samples, which was 0.845 times higher than the control (0.30 g/L) at the end of the storage period on day 5 (Figure 1C). The variation in anthocyanins content was observed throughout the storage period in all treatments, which ranged from 0.20 to 0.42 g/L at the end of the storage period. The increased anthocyanin content in samples were corroborates with the findings documented by Moradinezhad, Ansarifar and Mohammadian Moghaddam [13], wherein the authors showed the increased anthocyanins for the pomegranate arils packed under active and passive MAP. Similarly, Banda, Caleb, Jacobs and Opara [7] and Ayhan and Eştürk [23] reported an increase in anthocyanins content of pomegranate arils under active MAP. Palma et al. [2] highlighted the

variations in the levels of anthocyanins content due to presence of organic acids (e.g., ascorbic acid) and titratable acidity, which can provide carbon skeleton for the synthesis of secondary metabolites (e.g., anthocyanins) during storage.

The increased anthocyanin content can be attributed to the alterations in metabolic activity during storage, progress in pigment biosynthesis after harvest, interaction of arils with gas composition, and increase in phenylalanine ammonia lyase activity, as well as biochemistry and stability of individual anthocyanins [2,13,25]. Overall, active MAP improved the anthocyanin content in pomegranate arils and can be used as an effective technique to preserve anthocyanins in pomegranate arils stored at an ambient temperature.

3.1.3. Ascorbic Acid

On day 0, control and samples T1–T16 exhibited the ascorbic acid content of 0.22–0.30 g/100 g, which fluctuated and reached to 0.10–0.39 g/100 g in all samples at the end of the storage period on day 5 (Figure 1D). This confirmed the dissimilarity in effect of active-MAP on the ascorbic acid content of pomegranate arils. Similar results were reported in pomegranate arils under cold storage at 1, 4, and 8 °C at 95% RH for 14 days, wherein a declined ascorbic acid content was observed [26]. In another study, fluctuations in ascorbic acid content of fresh-cut produce was observed throughout the storage period [27]. It is known that various factors, such as gas composition (elevated CO_2) and storage temperature, may cause fast degradation of ascorbic acid or delayed ascorbic acid biosynthesis [20,28]. The reduction in ascorbic acid can be explained by metabolic changes in the arils by the use of organic acids in the respiratory process. These findings further concluded that the active MAP could alter the ascorbic acid content in pomegranate arils and thus influencing factors in active-MAP should be considered in order to protect against degradation of ascorbic acid in pomegranate arils.

3.1.4. Reducing Sugars

On day 0, reducing sugars content ranged from 0.12 to 0.17% in all samples, which was changed to 1.02 to 10.13% on day 5 (Figure 1E). On day 5, both control and samples T1–T16 exhibited a fluctuation in reducing sugars content, which was 26.57 to 40 times higher reducing sugars content than samples T1–T6 and control of day 0. In accordance with the present findings, a previous study demonstrated significantly increased carbohydrates from ≈141 (0 day) to ≈144.33 g/kg (day 7) for freeze-dried pomegranate arils at 1, 4, and 8 °C [26]. Similarly, Özdemir and Gökmen [29] and Palma et al. [2] reported the two reducing sugars (glucose and fructose) as the main carbohydrates present in pomegranate arils stored at 5 °C for 21 days and 30 or 60 days, respectively. Patanè, Malvuccio, Saita, Rizzarelli, Siracusa, Rizzo and Muratore [27] reported the temperatures of 26 °C and 30 °C likely to increase the sugar levels as a result of alterations in carbohydrate biosynthetic enzyme activity and metabolism of sugar compounds during the storage. Likewise, we assumed that the storage temperature (25 °C) and gas composition might have influenced the carbohydrate biosynthesis of pomegranate arils. The variations in gas composition (increase and/or decrease in O_2 or CO_2) might have hydrolyzed polysaccharides into sugars. The results revealed that the active MAP presented high reducing sugars over the end of storage, which could influence the flavor, appearance, chemical, and sensory characteristics of the pomegranate arils, maintaining the quality during storage.

3.2. Quality Analysis

3.2.1. Weight Loss

Active MAP influenced the weight loss of pomegranate arils, being more noticeable in the control (10.18%) compared to samples T1–T16 (1.13–7.35%) at the end of the storage period (Figure 2A). This indicated that the control samples exhibited almost 4.24 times higher weight loss than samples under active MAP during the storage period, which was also confirmed by visual observation, wherein control samples exhibited high water droplet formation on the inner walls of the packaging compared to samples under active MAP.

Generally, under an ambient temperature, transpirational water loss and loss of carbon reserves due to respiration were high for pomegranate arils (i.e., need of water), which increased the weight loss of pomegranate arils. Similar findings were documented by Caleb, Mahajan, Manley and Opara [14], in which authors highlighted the increased weight loss of arils (1.90 to 6.20%) stored at 5–15 °C for 14 days. The weight loss could be associated with packed material and RH, which might have enhanced the water uptake and promoted the condensation [30]. The active MAP could efficiently maintain the moisture content of pomegranate arils and allowed the packed material to form a suitable atmosphere that could reduce samples respiration and transpiration due to reduced water consumption and evaporation. The variation in weight loss among the different treatments could be related to the variations in respiration and transpiration rates of samples during active MAP, which are affected by the differences in the driving force and skin diffusion resistance of samples [31]. In the same study, Khorshidi, Davarynejad, Tehranifar and Fallahi [31], the authors highlighted the effect of surface/volume ratio of samples on the change in weight loss. The variations in gas composition may contribute to the change in weight loss due to continuous water uptake and other physiological processes such as respiration and transpiration. Moreover, package material in active MAP can act as a good mechanical barrier and maintain the suitable environmental conditions for samples, which collectively affected the reduction in weight loss of samples. Moreover, package material in active MAP can act as a good mechanical barrier and maintain the suitable environmental conditions for samples, which collectively affected the less reduction in weight loss of samples [32]. Overall, these findings suggested that active MAP could be useful to control product appearance during storage, which might be important physiological characterization in the consumer market.

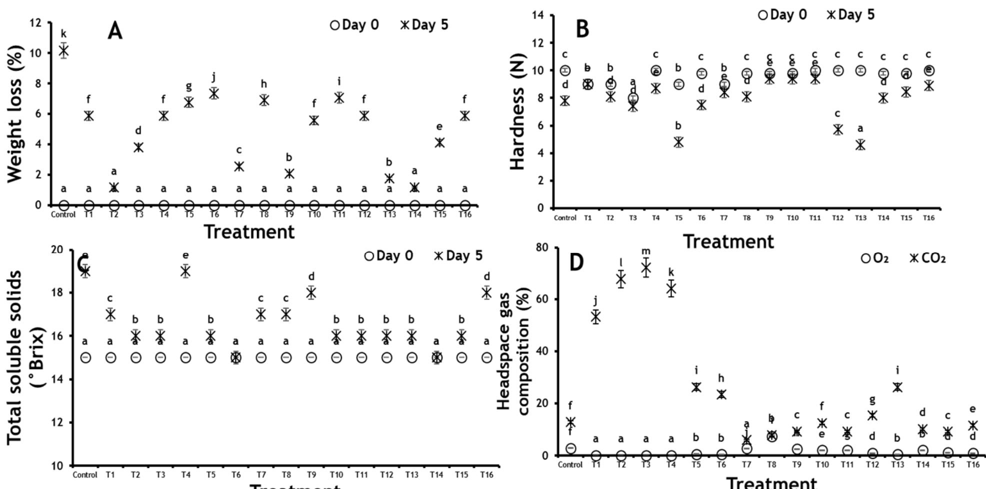

Figure 2. Quality analysis and gas composition of minimally processed pomegranate arils stored under active MAP at ambient temperature. Weight loss (**A**), hardness (**B**), total soluble solids (**C**), and headspace gas composition (**D**). Error bar represents the standard deviation from the mean of three independent replicates. In Figure 2D, headspace gas composition was reported on day 5. The mean values followed by lowercase letters (a–m) among the treatments (T1–T16 and control) were significantly different ($p < 0.05$) on the basis of Duncan's multiple range test. MAP = modified atmosphere packaging. For treatment codes, refer to Table 1.

3.2.2. Hardness

The decrease in hardness for samples T5, T12, and T13 was significant ($p < 0.05$) compared to other samples (T1–T4, T6–T11, and T14–16) and the control, as shown in Figure 2B. Although the differences in hardness of samples over storage was higher, the findings further reported active MAP caused less structural changes in sample hardness at the end of the storage. Similar findings were documented in a previous study, wherein MAP

prolonged the storage life of strawberries up to 2 and 4 days at 10 and 20 °C, respectively, with decreased hardness from 5 to 2 N [32]. These findings agreed with the observations on the hardness of passive MAP-treated pomegranate arils, wherein the authors reported decreased hardness from 76.10 to 67.50 N at 5–15 °C for 14 days [14]. These changes in sample hardness were likely due to the decreased vapor pressure, degradation of cell wall polysaccharides, and selection of packaging material [32]. Thus, these findings suggested the active MAP could result in maintaining the better structure, lower tissue damage, and improved shelf-life quality of pomegranate arils over storage.

3.2.3. Total Soluble Solids (TSS)

On day 0, the TSS content of samples (T1–T16) was found to be 15 °Brix, ranging from 15 to 19 °Brix over the end of storage period at an ambient temperature (Figure 2C). Samples T6–T14 showed similar effect with active MAP, while other samples exhibited a significant ($p < 0.05$) change in TSS content of pomegranate arils over days 0 and 5. A similar trend in TSS content was reported by O'Grady, Sigge, Caleb and Opara [26] for pomegranate arils stored at 1 °C after 14 days. Moreover, another study noticed higher changes in TSS content in pomegranate arils under active and passive MAP stored at 5 °C for 12 days [7]. Likewise, Caleb, Mahajan, Manley and Opara [14] demonstrated a change in TSS content on day 3 and then unchanged over a storage period of 14 days; however, drastic change in TSS content was observed on day 7 of pomegranate arils under MAP at 5, 10, and 15 °C. The variations in TSS content could be ascribed to the presence of higher CO_2 content in the active MAP, accumulation/degradation of sugars in arils [13,14], and increase in reducing sugars content. Another study by Erkan et al. [33] observed the variations in TSS as affected by the gas composition of MAP-treated mango over 9 months of storage. Accordingly, it can be concluded that the samples reported a fluctuation in TSS content of pomegranate arils, which was closely related to the impact of active MAP.

3.3. Package Headspace Gas Composition (O_2 and CO_2)

Samples under active MAP showed the decreased O_2 and relatively increased CO_2 (except for samples T8−T10 and T14−16) compared to control (Figure 2D). This was also confirmed by the variations in O_2 and CO_2 concentrations over the storage period compared to initial concentrations of O_2 and CO_2 on day 0 (Table 1). These variations could be attributed to the respiration of pomegranate arils undergoing ripening process and also affects the metabolism of samples [20]. This behavior agreed with a study reported by Liu, Li, Xu, Fu, Liao, Shi and Chen [17], wherein the gas composition of decreased O_2 and increased CO_2 positively influenced the product quality with a reduced respiration rate. Another study by Hussein, Caleb, Jacobs, Manley and Opara [30] documented the increased CO_2 for fresh pomegranate arils after 3 days of storage at 5 °C. However, the extreme increased CO_2 may deteriorate the quality attributes, such as increased weight loss, nutritional loss, and sensorial attributes of the final product. Thus, previous studies recommended gas composition of 5% O_2 and 0–5% CO_2 at 5 °C, 3–5% O_2 and 5–10% CO_2 at 5 °C, and 10% O_2 + 15% CO_2 to extend the shelf-life of ready-to-eat fresh-cut fruits and vegetables [15,16]. Therefore, we concluded that the concentrations of O_2 and CO_2 remained under desired gas composition of the storage atmosphere and maintained the freshness of the pomegranate arils.

3.4. Microbiological Analysis

On day 0, yeast and mold counts were below the detection limit (<1 log CFU/g) in all samples (T1–T16 and control); however, the microbial count significantly ($p < 0.05$) increased by the end of storage period on day 5 (Table 2), which was found to be in the range of 3.50 to 5 log CFU/g. Compared to control, samples under active MAP significantly ($p < 0.05$) influenced the growth of microorganisms, which was 1.30 to 1.86 times lower than control (6.50 log CFU/g). The highest microbial count (5.50 log CFU/g) on pomegranate arils was observed in T2, T4, T8, and T14, while the lowest (3.50 log CFU/g) was observed

in T1 and T3. One study by Caleb et al. [34] documented the yeast and mold counts in the range of 0.36 to 2.17 log CFU/g and 1.76 to 2.59 log CFU/g, respectively, for two pomegranate aril cultivars under MAP stored at 5 °C for 14 days. The variation in the results could be related to gas composition and storage temperature, where we used an ambient temperature. However, another study conducted by the same research group reported the total count of yeast and mold were in the range of 2.15 to 5.62 log CFU/g, which agreed with our findings [35]. There was a relationship between the active MAP used and the reduction in microorganisms, as documented in earlier studies. For example, the gas composition in active MAP was able to reduce the microbial growth on pomegranate arils, which altered the microbial cell membrane and caused the inactivation of microorganisms in pomegranate arils [35]. According to food safety standards implemented in New Zealand and Australia, microbial count should be within the range of <3 CFU/g (satisfactory) to 100 CFU/g (marginal) for ready-to-eat foods [36]. Likewise, Canada also categorized the fresh-cut fruit and vegetable microbiological analysis as categories 1, 2A, and 2B to ensure the safe production of fresh-cut fruits and vegetables. However, each country has its own microbiological regulatory standards for fresh-cut fruits and vegetables. In brief, the findings reported less microbial count and were in line with the microbiological criteria set by different food safety agencies for fresh-cut fruits and vegetables. Thus, samples presented a suitable microbiological profile for consumption.

Table 2. Microbiological analysis of pomegranate arils stored under active MAP at ambient temperature [1].

Treatment	Yeast and Mold Counts (log CFU/g)	
	Storage Time (Days)	
	0	5
Control	<1 ^{a,A}	6.50 ± 0.34 ^{f,B}
T1	<1 ^{a,A}	3.50 ± 0.01 ^{a,B}
T2	<1 ^{a,A}	5.00 ± 0.24 ^{e,B}
T3	<1 ^{a,A}	3.50 ± 0.02 ^{a,B}
T4	<1 ^{a,A}	5.00 ± 0.04 ^{e,B}
T5	<1 ^{a,A}	4.00 ± 0.14 ^{b,B}
T6	<1 ^{a,A}	4.50 ± 0.20 ^{c,B}
T7	<1 ^{a,A}	4.00 ± 0.18 ^{b,B}
T8	<1 ^{a,A}	5.00 ± 0.22 ^{d,B}
T9	<1 ^{a,A}	4.50 ± 0.37 ^{c,B}
T10	<1 ^{a,A}	4.00 ± 0.15 ^{b,B}
T11	<1 ^{a,A}	4.50 ± 0.03 ^{c,B}
T12	<1 ^{a,A}	4.50± 0.16 ^{c,B}
T13	<1 ^{a,A}	4.50 ± 0.28 ^{c,B}
T14	<1 ^{a,A}	5.00 ± 0.28 ^{e,B}
T15	<1 ^{a,A}	4.50 ± 0.25 ^{c,B}
T16	<1 ^{a,A}	4.50 ± 0.05 ^{c,B}

[1] Results are presented as mean ± standard deviation ($n \geq 3$). Mean values with different lowercase superscripts (a–f) within columns among the treatments (T1–T16 and control) and uppercase superscripts (A and B) within rows (between storage time) were statistically different at $p < 0.05$ on the basis of Duncan's multiple range test. All values were rounded to the nearest decimal digit. MAP = modified atmosphere packaging, CFU = colony-forming unit. For treatment codes (T1–T16 and control), refer to Table 1.

4. Conclusions

The findings highlighted the use of active MAP at an ambient temperature and prolonged the shelf-life of pomegranate arils for 5 days. The overall results indicated that the active MAP had an effect on chemical and quality attributes of pomegranate arils by a microbial load of ≤5 log CFU/g at an ambient temperature. Thus, the findings recommend the use of active MAP as a preservation technique to maintain the quality of pomegranate arils by increasing their shelf-life limited to 5 days at an ambient temperature. Hence, the findings have enormous potential in many ready-to-eat produce industries,

retail shops, and supermarkets to guarantee longer shelf-life without the use of refrigerator facilities. Moreover, this motivates consumers from low- and middle-income countries in decision-making and purchasing behavior of ready-to-eat pomegranate arils.

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