



Effect of perforation-mediated modified atmosphere packaging and storage duration on physicochemical properties and microbial quality of fresh minimally processed 'Acco' pomegranate arils



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ABSTRACT

This study investigated the effects of number of perforations (P-0, -3, -6 and -9 per 160.1 cm²) and storage duration on the physicochemical quality attributes and microbiological quality of fresh minimally processed pomegranate (*Punica granatum* L., cv. Acco) arils stored at 5 °C for 15 days. Arils were analysed for physicochemical quality attributes on day 0, 3, 6, 9, 12 and 15. Microbial analyses for aerobic mesophilic bacteria, yeast and moulds were made on 0, 6, 10 and 14 day, and the presence of *Escherichia coli* was tested before and at the end of storage. Headspace gas composition was significantly influenced by number of perforation. Highest CO₂ accumulation was observed in non-perforated MAP, while O₂ concentration increased with increase in number of perforations in PM-MAP. Highest decrease in total soluble solids (TSS) from 15.4 to 13.1°Brix was observed in P-9 PM-MAP arils. Highest counts of aerobic mesophilic bacteria (5.5 log CFU g⁻¹) and yeast and moulds (5.3 log CFU g⁻¹) were observed in P-0 and P-9 PM-MAP. No *E. coli* were detected before and at the end of storage. Overall, P-3 and P-6 PM-MAPs better maintained quality attributes of pomegranate arils than P-0 and P-9.

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1. Introduction

Pomegranate (*Punica granatum* L.) fruit consumption is growing due in part to its unique sensory and nutritional properties coupled with medicinal benefits attributed to high content of phytonutrients and antioxidant properties (Hassan, El-Halwagi, & Sayed, 2012; Opara, Al-Ani, & Al-Shuaibi, 2008). Minimally processed ready-to-eat pomegranate arils have high economic importance due to their convenience, healthiness and their desirable sensory characteristics as compared to whole produce, which poses difficulties in extracting the arils (Artés, Gomez, & Artés-Hernandez,

2007; Caleb, Opara, & Witthuhn, 2012; Defilippi, Whitaker, Hess-Pierce, & Kader, 2006). Nevertheless, shelf-life of pomegranate arils is shorter than whole fruit. While the latter can be stored for 3–4 months at temperatures below 10 °C (Artés & Tomas-Barberan, 2000; Fawole & Opara, 2013; Ghafir, Ibrahim, & Zaied, 2010; Nanda, Rao, & Krishnamurthy, 2001) pomegranate arils can last for a period of 1–2 weeks when stored under 5 °C (Caleb, Mahajan, Manley, & Opara, 2013; Lopez-Rubira, Conesa, Allende, & Artes, 2005). Fresh-cuts or minimally processed produce are highly susceptible to microbial growth (via the cut surfaces and juice exudates), enzymatic disorders and physiological response, which limit the shelf-life (Farber et al., 2003; Ragaert, Devlieghere, & Debevere, 2007).

Modified atmosphere packaging (MAP) is a postharvest tool used to preserve quality of various fresh whole and minimally processed fruit and vegetables (Sepulveda, Galletti, Sanez, & Tapia, 2000). MAP relies on the dynamic process of alteration of gaseous

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composition inside a package determined by permeability of packaging film and produce respiration (Mahajan, Oliveira, Montanez, & Frias, 2007). However, barrier properties to gases (O_2 and CO_2) and water vapour limit MAP applicability of many commercial polymeric films (Mangaraj, Goswami, & Mahajan, 2009). Conventional (passive MAP) that uses high barrier polymeric films are characterized by the generation of unsuitable in-package gas composition, condensation of water vapour and subsequent microbial growth causing loss of quality and impaired shelf-life (Lucera, Costa, Mastromatteo, Conte, & DelNobile, 2010; Mistriotis, Giannoulis, Giannopoulos, & Briassoulis, 2011).

Hence, perforation-mediated modified atmosphere packaging (PM-MAP) offers the possibility of optimising polymeric films in order to compensate for barrier limitations. Studies have reported successful use of PM-MAP on quality preservation and extension of shelf-life of various fresh whole and minimally processed fruit and vegetables such as mandarin (Del-Valle, Hernández-Munoz, Catalá, & Gavara, 2009), fresh-cut apple slices (Cliff, Toivonen, Forney, Liu, & Lu, 2010), litchi (De Reuck, Sivakumar, & Korsten, 2009), strawberries (Kartal, Aday & Caner, 2011), fresh sliced mushroom (Oliveira, Sousa-Gallaghera, Mahajan, & Teixeira, 2012) and broccoli (Fernández-León et al., 2013). Thus, the purpose of this study was to investigate the effects of PM-MAP technology on the postharvest physicochemical quality attributes and microbial safety of minimally processed pomegranate fruit (cv. Acco).

2. Materials and methods

2.1. Plant material and sample preparation

Pomegranate fruit (cv. Acco) grown in a commercial orchard located in Porterville, Wellington area (33°38'S 18°59'E), Western Cape, South Africa. They were manually harvested at commercial maturity based on external colour of the fruit peel, total soluble solids (TSS), titratable acidity (TA) and TSS:TA ratio. Fruit were sorted manually to get rid of damaged ones, and healthy fruit were washed in sodium hypochlorite (NaOCl) solution (0.2 g L^{-1}). Sterilized whole fruit were aseptically hand processed by carefully removing arils (without crushing) under cool temperature ($<8 \text{ }^\circ\text{C}$). Extracted arils were not subjected to any further postharvest treatments such as washing in chlorinated or organic solutions before packaging, in order maintain standard commercial practice. Approximately 100 g of fresh arils were packaged in polypropylene (PP) trays ($15.1 \times 10.6 \times 2.5 \text{ cm}^3$) and heat sealed using a semi-automated machine (Food Packaging Equipments, Cape Town, South Africa) with a non-perforated polymeric film POLYLID® 107 polyethylene (thickness $55 \text{ }\mu\text{m}$; permeance rate to CO_2 , $2.8\text{--}3.3 \times 10^{-12} \text{ mol m}^{-2} \text{ s}^{-1} \text{ Pa}^{-1}$; O_2 , $6.1\text{--}7.0 \times 10^{-13} \text{ mol m}^{-2} \text{ s}^{-1} \text{ Pa}^{-1}$ and water vapour, $10.4\text{--}9.4 \times 10^{-5} \text{ mol m}^{-2} \text{ s}^{-1} \text{ Pa}^{-1}$ at $25 \text{ }^\circ\text{C}$, 50% relative humidity) (Barkai Polyon Ltd, Kibbutz Barkai, Israel). The selected polymeric film has a high barrier property for gases and water vapour transmission, which could result in excessive decrease in O_2 and accumulation of CO_2 inside packaged produce as shown by Caleb et al. (2013). Heat sealed films were manually perforated using a sterilized needle (0.8 mm diameter (\emptyset)) with any of the perforations 3, 6 and 9 number of to obtain P-3, P-6 and P-9 on film surface of 160.1 cm^2 . The numbers of perforations were selected for this study based on experimental design analysis using statistical software (Statistica 12.0, Statsoft, USA). Non-perforated film (P-0) was used as a control while clamshell tray was included to simulate traditional packaging of fresh pomegranate arils in the South African market. Labels of $7.0 \times 3.8 \text{ cm}^2$ area were placed onto each package film to simulate retail conditions, without covering any of the perforations. At the processing facility, packaged products were cooled down to $2 \text{ }^\circ\text{C}$, and packed in sterile

cooler boxes with dry ice to maintain low temperature during transportation from the processing facility to the Postharvest Technology Research Laboratory at Stellenbosch University. Boxes were fitted with data loggers (Gemini Data Loggers, West Sussex, UK) to monitor the cold chain. On arrival, the packaged samples were stored at $5 \text{ }^\circ\text{C}$ and $95 \pm 2\% \text{ RH}$ for 15 days. A baseline analysis to investigate the microbial and physicochemical properties of pomegranate fruit samples was conducted on fresh arils prior to storage. Sampling for further physicochemical analyses was taken at 3 day intervals until the end of storage period, while microbial analysis included tests for *Escherichia coli*, aerobic mesophilic bacteria, yeast and moulds on days 0, 6, 10 and 14. Three packages were analysed for each experimental condition on each day.

2.2. Changes in gas composition during storage

Before opening the package on each sampling day, gas (CO_2 and O_2) composition inside the packages was determined using a gas analyser with an accuracy of 0.5 kPa (Checkmate 3, PBI Dansensor, Ringstead, Denmark). Levels of CO_2 and O_2 were presented as kPa. Immediately after gas analysis, each package was reweighed before the physicochemical properties of aril were analysed.

2.3. Analysis of physicochemical properties

2.3.1. Weight loss

Weight of each pack of arils was measured using an electronic weighing balance (ML3002.E, Mettler Toledo, Switzerland) with an accuracy of $\pm 0.01 \text{ g}$. Using the initial weight of sample recorded prior to storage, weight loss (WL) was obtained using the following equation:

$$WL = \frac{W_o - W_f}{W_o} \times 100 \quad (1)$$

where, WL is the weight loss (%), W_o is the initial weight (g) and W_f is the final weight (g) prior to package analysis.

2.3.2. Texture

Firmness of individual arils was measured using texture analyser (TA-XT Plus, Stable Micro Systems, England, UK) by compression using a 35 mm diameter cylindrical probe, set at compression strain of 60% and 9.5 mm distance to rupture the aril membrane. The probe was set at a speed of 1.0 and 10.0 mm s^{-1} for test and post-test, respectively. Averages of 15–20 arils were tested for each treatment. Firmness was expressed as maximum compression force in Newton (N).

2.3.3. Colour

Colour of arils was determined on basis of CIE $L^*a^*b^*$ colour system by Commission International del' Eclairage (CIE) measured using a digital colour meter (Minolta Chroma Meter, CR-400, Japan). Calibration of the colour meter was performed against a white tile background (Illuminants C: $Y = 89.53$, $x = 0.3247$, $y = 0.3198$) prior to each measurement. Arils were spread to cover a petri dish and colour measurements were taken from 5 different points of the dish. Colour parameters, lightness (L^*), redness/greenness (a^*) and yellowness/blueness (b^*) were measured and means of all measurements were determined for each package type. Using software Exponent v.4 (Stable MicroSystem Ltd., Godalming, UK), colour measurement data were interpreted and the results presented as the mean (\pm standard error (S.E)) of the number of measurement taken. Chroma (C^*) values, which indicate the quantitative attribute of colour intensity and hue angle (h°) which is considered as the qualitative attribute of colour of samples were

calculated using Equations (2) and (3), respectively (Pathare, Opara, & Al-Said, 2013).

$$C^* = \sqrt{a^{*2} + b^{*2}} \quad (2)$$

$$h^\circ = \tan^{-1} \left(\frac{b^*}{a^*} \right) \quad (3)$$

2.3.4. Titratable acidity, pH, and total soluble solids

Pomegranate juice was prepared from arils of each pack separately using LiquaFresh juice extractor (Mellerware, South Africa). Pomegranate juice pH and total soluble solid (TSS) were measured using a pH meter (BASIC 20+, Crison, Barcelona, Spain) and digital refractometer expressed as °Brix (Atago, Tokyo, Japan), respectively. Titratable acidity (TA) of aqueous pomegranate juice was determined potentiometrically by titration with 0.1 mol L⁻¹ NaOH to an end point of pH 8.2 using a Metrohm 862 compact auto titrosampler (Herisau, Switzerland). The TA value was expressed as grams of citric acid (CA) per litres of crude pomegranate juice (g L⁻¹ citric acid).

2.3.5. Analysis of microbial quality

Microbiological stability of pomegranate arils was analysed according to methods described by Caleb et al. (2013), for aerobic mesophilic bacteria and yeast and moulds. Plate count agar (PCA) was used for aerobic mesophilic bacteria, while yeast and mould counts was characterised using potato dextrose agar (PDA) acidified with tartaric acid (100 g L⁻¹). For indicator microorganisms *E. coli* were analysed using tryptone bile x-glucuronide (TBX) agar. Packages for this purpose were opened under sterile conditions, and 10 g of pomegranate arils from each sample was obtained aseptically and homogenised with 100 mL of physiological sterile solution (PSS) (8.5 g L⁻¹). The three-fold dilutions were prepared using 1 mL of homogenised sample into 9.0 mL of PSS. To enumerate microbial loads of samples, 1 mL of each dilution was pour-plated in triplicate onto appropriate prepared media, PCA, PDA and TBX agars for aerobic mesophilic bacteria yeast and mould counts and *E. coli*, respectively. Plates for aerobic mesophilic bacteria and *E. coli* were incubated for 2 day at 37 °C and 35 °C, respectively, while yeast and moulds plates were incubated at 26 °C for 5 day. After incubation, colonies grown on plates were counted (only between 30 and 300 colonies), and results presented as log of colony-forming units per gram (log CFU g⁻¹) of sample arils.

2.4. Chemicals

Sodium hydroxide was obtained from Merck (Pty) Ltd. (Modderfontein, South Africa). Double distilled water used deionized using Direct 8 Milli-Q deionization system and filtered through a 0.45 mm membrane filter (Montpellier, France). Plate count agar, potato dextrose agar and tryptone bile x-glucuronide agar were obtained from Merck (Pty) Ltd. (Modderfontein, South Africa).

2.5. Statistical analysis

All experimental data obtained were subjected to factorial analysis of variance (ANOVA) at 95% confidence interval using Statistical software (Statistica 12.0, Statsoft, USA). Main effects (number of perforations and storage duration) and interaction effects (number of perforations × storage duration) were assessed using Pareto analysis at 95% confidence interval. Post-hoc test (Duncan's Multiple Range Test) was used to test for statistical

significance such that observed differences at $p < 0.05$ were considered significant.

3. Results and discussion

3.1. Weight loss

Slight percentage increase in weight was observed for arils packaged in P-0 MAP after day 3 and in clamshell trays up to day 9, due to water vapour condensation. Water vapour condensation was observed on the film surface of packaged arils during storage (Fig. 1). Generally, weight loss of packaged arils increased progressively over storage time (Fig. 2). Similar results was reported by Caleb et al. (2013), who observed an initial increase in weight of MAP packaged pomegranate arils (cvs. Acco and Herskawitz) stored at 5 and 10 °C. At the end of storage, changes in weight were higher in perforated packages, where 1.9, 4.4 and 6.2% weight loss were recorded in P-3, P-6 and P-9 PM-MAP packaged arils, respectively (Fig. 2). However, previous findings reported by Caleb et al. (2013) revealed 0.53% weight loss of fresh MAP packaged arils (cv. Acco) after 14 day of storage at 5 °C. Progressive decrease in weight of arils packaged in PM-MAP (P-3, P-6 and P-9) could be attributed to moisture loss in arils through perforations. As reported by Lucera et al. (2010), high water vapour permeability enhanced by perforations of MAP film enhance water uptake from packaged produce by evaporation, resulting into weight loss. Bhatia, Asrey, Jha, Singh, and Kannaujia (2013) observed higher physiological loss in weight of minimally processed 'Mridula' pomegranate arils packaged in low density polyethylene (LDPE) and Cryovac bags of higher water vapour permeability over those in polypropylene bags.

3.2. Gas compositions within packages

During cold storage, gas composition changed significantly in all packages ($p < 0.05$) as shown in Table 1. In non-perforated (P-0) packages, O₂ concentration decreased progressively below critical level (2 kPa) by day 15. This level of O₂ is detrimental to produce as it favours fermentative anaerobic reactions leading to off odours and off flavours (Almenar et al., 2007; De Reuck et al., 2009). Similar results were reported by Almenar et al. (2007) who observed critical level of O₂ (>5 kPa) in strawberries packaged in MAP cups without micro-perforations after 6 d of storage. Clamshell trays had the highest level of O₂ (19.2 kPa) while perforated packages P-3, P-6 and P-9 PM-MAP maintained higher level of O₂ of 17.6 kPa, 18.6 kPa and 18.4 kPa, respectively, at the end of storage. Poor hermetic seal of clamshell trays enhanced non-resistant flow of O₂ in trays while perforations in PM-MAP influenced permeation and depletion of O₂. Changes in O₂ in PM-MAP were consistent with previous report by Almenar et al. (2007) who observed higher O₂ concentration (5–18 kPa) in 125 mL-capacity MAP cups of strawberries with one and three perforations (~100 μm Ø). On the contrary, the composition of CO₂ increased in all packages after 3 day of storage, with P-0 packages having the highest CO₂ accumulation and 9.6 kPa and 34.0 kPa on day 3 and 15, respectively. The level of CO₂ in perforated packages decreased significantly at the end of 15 day in storage with increase in number of perforations, from 1.3 kPa for P-3 to 0.6 kPa and 0.7 kPa for P-6 and P-9, respectively. Perforations enhanced the permeability of the polymeric film and prevented the accumulation of CO₂ in PM-MAP. Similar observations were reported on MAP of semi-permeable films of minimally processed 'Wonderful' pomegranate (Sepulveda et al., 2000).

3.3. Colour

The colour of pomegranate arils and juice is an important

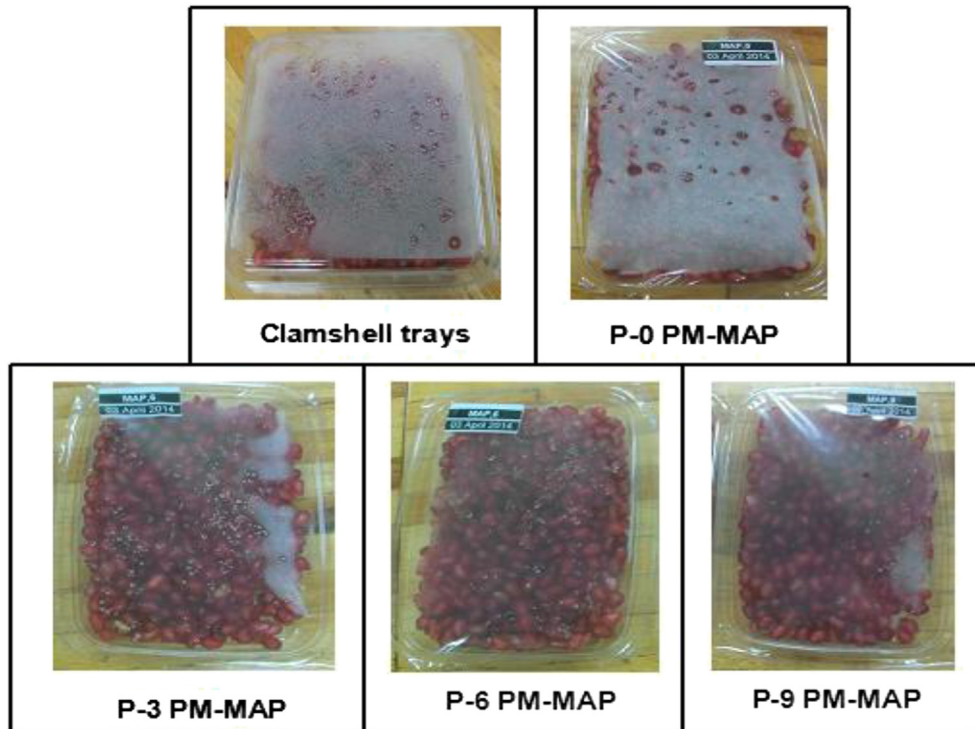


Fig. 1. Visual observation of water vapour (moisture) condensation on the film surface of the perforation-mediated modified atmosphere (PM-MAP) and clamshell package at the end of 15 day storage for minimally processed 'Acco' pomegranate arils stored at 5 °C. P is numbers of perforations: P-0 (non-perforated), P-3 (three perforations), P-6 (six perforations) and P-9 (nine perforations).

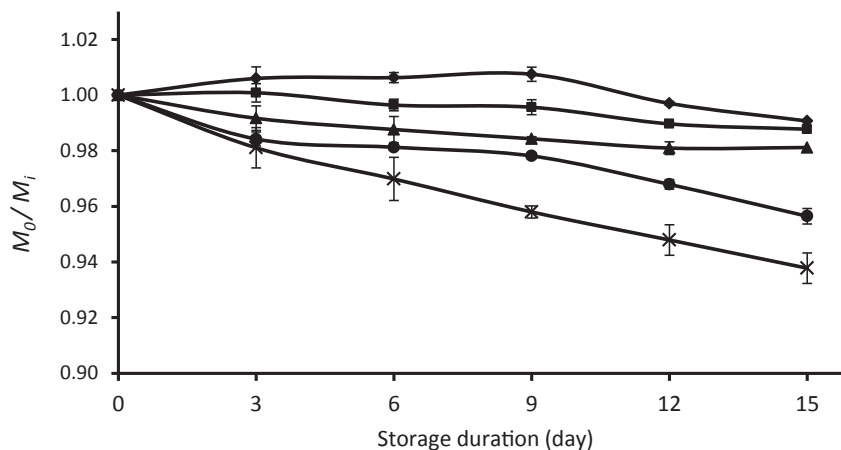


Fig. 2. Change in weight of perforation-mediated modified atmosphere and clamshell packaged pomegranate arils cv. 'Acco' stored at 5 °C for 15 days (normalised weight values with respect to the initial weight of pomegranate arils (M_i in g)). P is numbers of perforations: P-0 (non-perforated), P-3 (three perforations), P-6 (six perforations) and P-9 (nine perforations); ◆ = clamshell trays; ■ = P-0; ▲ = P-3; ● = P-6; x = P-9. Error bars indicate standard deviation from mean value ($n = 3$).

quality attribute perceived by consumers (Fawole & Opara, 2013). Minimal change in chroma (C^*) and hue angle (h°) values were observed across all packaging treatments over storage duration (Table 2). Arils packaged in P-0 had an increase in C^* from 9.8 ± 0.43 on day 0– 10.4 ± 0.17 after 14 day. This is in agreement with the effects observed by Almenar et al. (2007) in fresh strawberry fruit packaged in non-perforated high- CO_2 atmospheres of MAP using cups. Slight significant decrease in C^* was observed in arils packaged in clamshell trays and PM-MAP end of storage from 9.8 to 8.4, 9.7, 9.4 and 9.6 for clamshell, P-3, P-9 and P-9, respectively. Almenar et al. (2007) observed lower chroma values in strawberry fruits stored in high-permeable MAP films with 7 micro-perforations

(50 μm \emptyset) and flexible semi-permeable PVC bags. Hue angle (h°) declined slightly across all packages from 25.5 ± 0.24 on day 0–25.1, 24.6, 23.2, 22.2 and 24.52 for clamshell, P-0, P-3, P-6 and P-9 packaged arils, respectively, at the end of storage.

These results are in agreement with the observation reported by O'Grady, Sigge, Caleb, and Opara (2014). The authors reported a general decrease in individual colour parameters of 'Arakta', 'Bahgwa', and 'Ruby' pomegranate arils by day 7 of storage at 1 °C, 4 °C and 8 °C. Similarly, De Reuck et al. (2009) who reported decrease in C^* and h° angle during 21 days of cold storage at 2 °C, in both perforated (with 10 and 4 perforations of 0.6 mm \emptyset), and non-perforated MAP packaged fresh litchi (cvs. Mauritius and McLean's

Table 1

Changes in package headspace gas composition of packaged fresh pomegranate arils stored at 5 °C for 15 days (mean values ± standard error, $n = 3$), values presented are approximated to nearest significant digit. P is numbers of perforations: P-0 (non-perforated), P-3 (three perforations), P-6 (six perforations) and P-9 (nine perforations).

| Gas composition (kPa) | Packaging | Storage duration (day) | | | | | |
|-----------------------|-----------|---------------------------|------------------------------|-----------------------------|----------------------------|-----------------------------|----------------------------|
| | | 0 | 3 | 6 | 9 | 12 | 15 |
| O ₂ | Clamshell | 19.6 ± 0.1 ^a | 19.1 ± 0.03 ^{bcd} | 19.1 ± 0.05 ^{bcd} | 19.0 ± 0.03 ^{cde} | 19 ± 0.11 ^{cde} | 19.2 ± 0.05 ^{abc} |
| | P-0 | 19.5 ± 0.02 ^{ab} | 8.7 ± 0.1 ^l | 5.9 ± 0.1 ^k | 2.7 ± 0.1 ⁱ | 1.7 ± 0.1 ^m | 1.3 ± 0.3 ^m |
| | P-3 | 19.5 ± 0.05 ^a | 18.7 ± 0.1 ^{defg} | 18.4 ± 0.2 ^{gh} | 18.1 ± 0.1 ^h | 17.5 ± 0.41 ⁱ | 17.6 ± 0.2 ⁱ |
| | P-6 | 19.6 ± 0.01 ^a | 18.8 ± 0.01 ^{cdefg} | 18.5 ± 0.1 ^{fgh} | 18.5 ± 0.2 ^{gh} | 18.6 ± 0.03 ^{defg} | 18.6 ± 0.1 ^{efg} |
| | P-9 | 19.5 ± 0.1 ^a | 19.0 ± 0.1 ^{cde} | 18.7 ± 0.1 ^{cdefg} | 19 ± 0.03 ^{cdef} | 18.6 ± 0.1 ^{defg} | 18.4 ± 0.3 ^{gh} |
| CO ₂ | Clamshell | 0.04 ± 0.0 ^h | 0.1 ± 0.03 ^h | 0.2 ± 0.03 ^h | 0.2 ± 0.03 ^h | 0.3 ± 0.14 ^h | 0.1 ± 0.1 ^h |
| | P-0 | 0.05 ± 0.0 ^h | 9.6 ± 0.14 ^e | 13.7 ± 0.2 ^d | 21.1 ± 0.9 ^c | 27.9 ± 0.9 ^b | 34 ± 0.2 ^a |
| | P-3 | 0.05 ± 0.01 ^h | 0.5 ± 0.03 ^{gh} | 0.9 ± 0.1 ^{gh} | 1.3 ± 0.03 ^{fg} | 1.96 ± 0.7 ^f | 1.3 ± 0.1 ^{fg} |
| | P-6 | 0.04 ± 0.0 ^h | 0.5 ± 0.1 ^{gh} | 0.6 ± 0.05 ^{gh} | 0.6 ± 0.02 ^{gh} | 0.7 ± 0.03 ^{gh} | 0.6 ± 0.1 ^{gh} |
| | P-9 | 0.04 ± 0.01 ^h | 0.2 ± 0.03 ^h | 0.5 ± 0.03 ^{gh} | 0.6 ± 0.01 ^{gh} | 0.8 ± 0.2 ^{gh} | 0.7 ± 0.05 ^{gh} |

Mean values in the same column and along the rows with different letters are significantly different ($p < 0.05$).

Table 2

Effects of perforation-mediated modified atmosphere and clamshell packaging on colour attributes of minimally processed 'Acco' pomegranate arils stored at 5 °C for 15 days (mean values ± standard error, $n = 5$). Values presented are approximated to nearest significant digit. P is numbers of perforations: P-0 (non-perforated), P-3 (three perforations), P-6 (six perforations) and P-9 (nine perforations).

| Colour attributes | Packaging | Storage duration (day) | | | | | |
|-------------------|-----------|---------------------------|----------------------------|----------------------------|----------------------------|----------------------------|----------------------------|
| | | 0 | 3 | 6 | 9 | 12 | 15 |
| L* | Clamshell | 10.3 ± 0.43 ^{cd} | 13.2 ± 1.22 ^{abc} | 13.6 ± 0.41 ^{abc} | 12.7 ± 1.01 ^{abc} | 13.5 ± 0.09 ^{abc} | 13.7 ± 1.07 ^{abc} |
| | P-0 | 10.3 ± 0.43 ^{cd} | 11.5 ± 1.51 ^{bc} | 13 ± 0.51 ^{abc} | 11.6 ± 0.50 ^{abc} | 12.7 ± 0.29 ^{abc} | 13.6 ± 0.25 ^{abc} |
| | P-3 | 10.3 ± 0.43 ^{cd} | 13 ± 0.73 ^{abc} | 13.2 ± 0.64 ^{abc} | 13.5 ± 0.63 ^{abc} | 12.5 ± 0.59 ^{abc} | 12.4 ± 0.18 ^{abc} |
| | P-6 | 10.3 ± 0.43 ^{cd} | 13.6 ± 0.44 ^{abc} | 13.2 ± 0.63 ^c | 13.6 ± 13 ^{abc} | 12.9 ± 0.18 ^{abc} | 13.5 ± 0.45 ^{abc} |
| | P-9 | 10.3 ± 0.43 ^{cd} | 13.4 ± 0.43 ^{abc} | 12.5 ± 0.74 ^{abc} | 13.9 ± 0.82 ^{abc} | 12.2 ± 0.74 ^{abc} | 12.9 ± 0.19 ^{abc} |
| a* | Clamshell | 8.4 ± 0.47 ^{ac} | 9.7 ± 1.11 ^{abc} | 9.1 ± 0.62 ^{abc} | 8.2 ± 0.52 ^{abc} | 7.9 ± 0.89 ^{bc} | 7.5 ± 1.02 ^{bc} |
| | P-0 | 8.4 ± 0.47 ^{ac} | 8 ± 1.91 ^{abc} | 8.6 ± 0.41 ^{abc} | 8.5 ± 0.40 ^{abc} | 8.5 ± 0.04 ^{abc} | 8.2 ± 1.00 ^{abc} |
| | P-3 | 8.4 ± 0.47 ^{ac} | 9.3 ± 0.61 ^{abc} | 9.7 ± 0.51 ^{abc} | 9.6 ± 0.55 ^{abc} | 8.5 ± 0.78 ^{abc} | 8.8 ± 1.04 ^{abc} |
| | P-6 | 8.4 ± 0.47 ^{ac} | 8.8 ± 0.33 ^{abc} | 9.1 ± 0.65 ^{abc} | 9.7 ± 0.70 ^{abc} | 9.8 ± 0.13 ^{ab} | 10.5 ± 0.91 ^a |
| | P-9 | 8.4 ± 0.47 ^{ac} | 8.9 ± 0.52 ^{abc} | 8.6 ± 0.40 ^{abc} | 9.2 ± 0.08 ^{abc} | 9.2 ± 0.06 ^{abc} | 10.2 ± 1.03 ^a |
| b* | Clamshell | 3.8 ± 0.11 ^{bc} | 4 ± 0.42 ^{ab} | 3.8 ± 0.31 ^{ab} | 3.9 ± 0.12 ^{ab} | 4.2 ± 0.53 ^{ab} | 3.6 ± 0.32 ^{ab} |
| | P-0 | 3.8 ± 0.11 ^{bc} | 3.8 ± 0.48 ^{ab} | 3.9 ± 0.23 ^{ab} | 3.7 ± 0.32 ^{ab} | 3.9 ± 0.73 ^{ab} | 3.7 ± 0.33 ^{ab} |
| | P-3 | 3.8 ± 0.11 ^{bc} | 3.5 ± 0.16 ^{ab} | 4.2 ± 0.12 ^{ab} | 4.1 ± 0.52 ^{ab} | 3.6 ± 0.14 ^{ab} | 3.6 ± 0.43 ^{ab} |
| | P-6 | 3.8 ± 0.11 ^{bc} | 4 ± 0.34 ^{ab} | 3.7 ± 0.72 ^{ab} | 4.3 ± 0.32 ^{ab} | 3.7 ± 0.20 ^{ab} | 4.3 ± 0.24 ^a |
| | P-9 | 3.8 ± 0.11 ^{bc} | 4.1 ± 0.20 ^{ab} | 4.1 ± 0.13 ^{ab} | 3.8 ± 0.33 ^{ab} | 3.6 ± 0.23 ^{ab} | 4.3 ± 0.31 ^a |
| C* | Clamshell | 9.8 ± 0.43 ^{ae} | 9.3 ± 0.12 ^{cde} | 9.2 ± 0.16 ^{fgh} | 8.9 ± 0.15 ^{c-h} | 8.7 ± 0.52 ^{bc} | 8.4 ± 0.33 ^{e-h} |
| | P-0 | 9.8 ± 0.43 ^{ae} | 9.2 ± 0.40 ^{c-f} | 9.2 ± 0.31 ^{cdef} | 9 ± 0.34 ^{d-h} | 9.9 ± 0.51 ^{ab} | 10.4 ± 0.17 ^a |
| | P-3 | 9.8 ± 0.43 ^{ae} | 9.8 ± 0.22 ^h | 9.6 ± 0.33 ^{bc} | 9.5 ± 0.11 ^{bcd} | 9.6 ± 0.17 ^{bc} | 9.7 ± 0.10 ^{gh} |
| | P-6 | 9.8 ± 0.43 ^{ae} | 9.7 ± 0.22 ^{gh} | 9.4 ± 0.43 ^{cde} | 9.4 ± 0.12 ^{cde} | 9.4 ± 0.42 ^{cde} | 9.4 ± 0.15 ^{cde} |
| | P-9 | 9.8 ± 0.43 ^{ae} | 9.3 ± 0.20 ^{fgh} | 9.3 ± 0.25 ^{fgh} | 9.5 ± 0.43 ^{bcd} | 9.6 ± 0.72 ^{bcd} | 9.6 ± 0.21 ^{bcd} |
| h° | Clamshell | 25.5 ± 0.24 ^{ac} | 25.2 ± 0.40 ^{abc} | 25.2 ± 0.30 ^{abc} | 25.2 ± 0.46 ^{abc} | 25.2 ± 0.92 ^{abc} | 25.1 ± 0.33 ^{a-d} |
| | P-0 | 25.5 ± 0.24 ^{ac} | 25.5 ± 0.30 ^{abc} | 24.6 ± 0.44 ^{bcd} | 24.4 ± 0.14 ^{bcd} | 24.4 ± 0.04 ^{c-f} | 24.6 ± 0.31 ^{bcd} |
| | P-3 | 25.5 ± 0.24 ^{ac} | 25.1 ± 0.64 ^a | 24.9 ± 0.51 ^{a-d} | 23.7 ± 0.32 ^{d-g} | 23.3 ± 0.44 ^{e-h} | 23.2 ± 0.51 ^{d-g} |
| | P-6 | 25.5 ± 0.24 ^{ac} | 24.4 ± 0.55 ^{cde} | 24.4 ± 0.45 ^{abc} | 23.2 ± 0.54 ^{fgh} | 23.1 ± 0.20 ^{fgh} | 22.2 ± 0.14 ^h |
| | P-9 | 25.5 ± 0.24 ^{ac} | 25.2 ± 0.33 ^{abc} | 25.2 ± 0.3 ^{abc} | 24.8 ± 0.5 ^{bcd} | 24.5 ± 0.40 ^{cd} | 24.5 ± 0.32 ^{abc} |

Mean values in the same column with different superscript lowercase letters are significantly different ($p < 0.05$) among package treatment, and mean values in the same row with different superscript lowercase letters are significantly different ($p < 0.05$) along storage duration.

Red). The authors related decline in h° values to high CO₂ injury in non-perforated MAP, but no clear reasons were given for decrease in h° observed in perforated packages. The decrease in h° values in this study could possibly be attributed to browning of the arils (Meighani, Ghasemnezhad, & Bakhshi, 2014).

3.4. Total soluble solids, titratable acidity and pH

The results in Table 3 show that number of perforations in PM-MAP and storage duration had significant effects on TSS of 'Acco' pomegranate juice ($p < 0.05$). It was observed that at the end of storage, clamshell trays and P-0 had slightly higher TSS value (14.7 ± 0.14 and 14.3 ± 0.13 °Brix, respectively) than PM-MAP. Perforated MAP (P-3, P-6 and P-9) resulted in gradual but progressive decrease in TSS until the end of the 15 days of storage period. The decrease in TSS could be attributed to increased metabolic activities of pomegranate arils during storage such as

conversion of soluble sugars into other organic acids such as citric, malic, oxalic and succinic accelerated by high concentration of O₂ in these packages (Bhatia et al., 2013). Similar trend of decrease in TSS of pomegranate arils packaged in MAP of high permeable LDPE and Cryovac films was reported by Bhatia et al. (2013). However, contrary to these results, other studies showed that there was an increase in TSS of fresh processed 'Wonderful' pomegranate arils packaged in MAP of perforated oriented polypropylene (POPP) film (with 33 perforations of 2 mm Ø in 9 × 12 cm² area). Increase in TSS was related to loss of water due to high dehydration observed in these packages (Gil, Martinez, & Artés, 1996).

Furthermore, prior to storage (day 0), measure TA was 11 ± 0.1 g L⁻¹ of citric acid in pomegranate juice. The TA values in this study were higher compared to values reported by O'Grady et al. (2014). This could be attributed to cultivar differences as 'Acco' pomegranate is more soar compared to 'Ruby', 'Bahgwa' and 'Arakta'. After day 3 of storage, arils packaged in non-perforated (P-

Table 3
Changes in physicochemical properties of minimally processed 'Acco' pomegranate arils packaged under perforation-mediated modified atmosphere and in clamshell and stored at 5 °C for 15 days (mean values ± standard error, $n = 3$). Values presented are approximated to nearest significant digit. P is numbers of perforations: P-0 (non-perforated), P-3 (three perforations), P-6 (six perforations) and P-9 (nine perforations).

| Physicochemical properties | Packaging | Storage duration (day) | | | | | |
|---------------------------------------|-----------|--------------------------|---------------------------|---------------------------|---------------------------|---------------------------|--------------------------|
| | | 0 | 3 | 6 | 9 | 12 | 15 |
| pH | Clamshell | 3.6 ± 0.01 ^a | 3.4 ± 0.1 ^{ab} | 3.9 ± 0.1 ^a | 3.9 ± 0.03 ^a | 4.0 ± 0.02 ^a | 3.9 ± 0.01 ^a |
| | P-0 | 3.6 ± 0.01 ^a | 3.5 ± 0.02 ^d | 3.8 ± 0.01 ^b | 3.4 ± 0.01 ^c | 4.2 ± 0.02 ^a | 3.7 ± 0.01 ^b |
| | P-3 | 3.6 ± 0.01 ^a | 3.3 ± 0.03 ^b | 3.8 ± 0.01 ^b | 3.4 ± 0.03 ^d | 4.0 ± 0.02 ^b | 3.6 ± 0.1 ^{bc} |
| | P-6 | 3.6 ± 0.01 ^a | 3.2 ± 0.1 ^b | 3.9 ± 0.02 ^a | 3.6 ± 0.05 ^b | 4.0 ± 0.14 ^d | 3.6 ± 0.02 ^b |
| | P-9 | 3.6 ± 0.01 ^a | 3.4 ± 0.01 ^a | 3.8 ± 0.03 ^a | 3.9 ± 0.02 ^d | 3.8 ± 0.11 ^d | 3.5 ± 0.02 ^d |
| TA (citric acid) (g L ⁻¹) | Clamshell | 11 ± 0.1 ^a | 6 ± 0.4 ^b | 6 ± 0.3 ^b | 5 ± 0.1 ^c | 5 ± 0.1 ^b | 4 ± 0.1 ^d |
| | P-0 | 11 ± 0.1 ^a | 7 ± 0.1 ^b | 7 ± 0.3 ^b | 7 ± 0.1 ^b | 7 ± 0.5 ^b | 7 ± 0.2 ^b |
| | P-3 | 11 ± 0.1 ^a | 7 ± 0.3 ^a | 7 ± 0.3 ^a | 6 ± 0.1 ^b | 5 ± 0.2 ^b | 5 ± 0.5 ^b |
| | P-6 | 11 ± 0.1 ^a | 6 ± 0.2 ^b | 6 ± 0.1 ^b | 5 ± 0.2 ^c | 4 ± 0.2 ^d | 4 ± 0.1 ^d |
| | P-9 | 11 ± 0.1 ^a | 6 ± 0.1 ^b | 5 ± 0.1 ^c | 4 ± 0.1 ^d | 4 ± 0.1 ^c | 4 ± 0.3 ^c |
| TSS (°Brix) | Clamshell | 15.4 ± 0.02 ^a | 15.6 ± 0.05 ^a | 16 ± 0.05 ^a | 15 ± 0.05 ^a | 14.7 ± 0.5 ^a | 14.7 ± 0.14 ^a |
| | P-0 | 15.4 ± 0.02 ^a | 15.4 ± 0.42 ^{ab} | 15 ± 0.2 ^{bc} | 14.6 ± 0.2 ^{cd} | 14.1 ± 0.03 ^b | 14.3 ± 0.13 ^b |
| | P-3 | 15.4 ± 0.02 ^a | 15.5 ± 0.30 ^{ab} | 14.1 ± 0.22 ^{bc} | 14.3 ± 0.14 ^{bc} | 14.0 ± 0.05 ^c | 13.9 ± 0.22 ^b |
| | P-6 | 15.4 ± 0.02 ^a | 15.0 ± 0.21 ^{bc} | 14.8 ± 0.33 ^{bc} | 14.4 ± 0.1 ^c | 13.9 ± 0.33 ^{bc} | 13.5 ± 0.14 ^d |
| | P-9 | 15.4 ± 0.02 ^a | 14.8 ± 0.12 ^c | 14.6 ± 0.15 ^c | 14.3 ± 0.03 ^c | 13.8 ± 0.05 ^d | 13.1 ± 0.04 ^d |
| Firmness (N) | Clamshell | 7.8 ± 0.03 ^a | 7.8 ± 0.18 ^a | 7.2 ± 0.44 ^{ab} | 7 ± 0.11 ^{bc} | 6.6 ± 0.32 ^a | 5.4 ± 0.20 ^d |
| | P-0 | 7.8 ± 0.03 ^a | 6.7 ± 0.21 ^{bc} | 7.8 ± 0.22 ^a | 6.8 ± 0.10 ^b | 6.3 ± 0.31 ^{ab} | 5.6 ± 0.12 ^{bc} |
| | P-3 | 7.8 ± 0.03 ^a | 6.6 ± 0.31 ^{bc} | 7.1 ± 0.20 ^b | 6.9 ± 0.32 ^a | 6.3 ± 0.14 ^a | 6.2 ± 0.33 ^a |
| | P-6 | 7.8 ± 0.03 ^a | 6.8 ± 0.22 ^b | 7.6 ± 0.34 ^a | 7 ± 0.23 ^b | 6 ± 0.12 ^b | 5.8 ± 0.13 ^{ab} |
| | P-9 | 7.8 ± 0.03 ^a | 6.9 ± 0.13 ^b | 6.8 ± 0.25 ^{bc} | 6.3 ± 0.24 ^a | 6.1 ± 0.44 ^a | 3.8 ± 0.22 ^d |

Mean values in the same column with different subscript uppercase letters are significantly different ($p < 0.05$) among package treatment, and mean values in the same row with different superscript lowercase letters are significantly different ($p < 0.05$) along storage duration.

0) MAP had the lowest decrease in TA (7 ± 0.1 g L⁻¹ of citric acid) which remained unchanged for the rest of the storage period. These results are in agreement with the effects observed by Caleb et al. (2013) in fresh 'Acco' pomegranate arils packaged under passive-MAP of non-perforated biaxial oriented-PP film. Observed decrease in TA was attributed to initial response and metabolic activities of the packaged arils during storage. TA values decreased for pomegranate arils in perforated packages could be attributed to increase in metabolic activities due to high O₂ concentrations in observed these packages, in which citric acid was used as substrate. Amoros et al. (2008) reported that micro-perforated polyethylene MAP bags reduced malic acid content of loquat fruit (cv. Algerie) by ~30% after 6 weeks of cold storage (6 °C). In agreement to the effects of PM-MAP on TA observed in our study, metabolic activities resulted from O₂ (16–18 kPa) and lower CO₂ (2–4 kPa) MAP composition resulted to decrease in malic acid which was used as substrate.

Generally, the pH values of aril juice varied across all treatments throughout the storage duration (Table 3). The changes observed in pH showed a fluctuation trend of slight decrease and/or increase which was statistically significant ($p < 0.05$). The fluctuating trend in pH was observed in the rest of the packages. This fluctuation in pH of arils can be explained by the differences in CO₂ accumulated in the PM-MAP during storage. A similar trend was observed in 'Hicaznar' pomegranate arils during cold storage under passive MAP (Ayhan & Estürk, 2009).

3.5. Firmness of arils

In this study, firmness of arils decreased with increase in the number of perforations over the duration of storage (Table 3). The interaction of number of perforations on package and storage duration had a significant effect on the firmness of arils ($p < 0.05$). Aril firmness was best maintained under P-3 (6.2 ± 0.33 N), while the highest loss in firmness were observed in P-9 (3.8 ± 0.22 N) after 14 days of storage. Decrease in firmness of arils packaged in PM-MAP may be attributed to loss in moisture influenced by perforation. Our results corroborated with previous findings

reported by Ayhan and Estürk (2009), who found that variation in the firmness of 'Hicaznar' pomegranate arils stored under passive MAP of PP film was related to water loss through the packages during 18 days of storage at 5 °C. Similarly, Bhatia et al. (2013) reported that after 15 days of cold storage (5 ± 2 °C) of 'Mridula' pomegranate arils packaged under passive MAP, highest loss in firmness was observed in arils packaged in LDPE and Cryovac based-laminate film which had highest water vapour permeability among the studied packages. According to Gimenez et al. (2003), high O₂ concentration may also contribute to loss of texture in arils packaged in PM-MAP.

3.6. Microbial quality

Microbial counts on minimally processed pomegranate arils increased significantly with storage duration (Fig. 3). Both storage duration and packaging type significantly influenced the growth of all analysed microorganisms ($p < 0.05$). On day 0, initial aerobic mesophilic bacteria and yeast and mould counts on fresh arils were below 1 log CFU g⁻¹. There was no *E. coli* detected in all packages after processing and packaging of arils and at the end of storage. Lowest aerobic mesophilic bacteria count was observed in P-0 (4.1 ± 0.02 log CFU g⁻¹), and highest counts in P-9 PM-MAP packaged arils (5.5 ± 0.01 log CFU g⁻¹). In contrast, lowest yeast and moulds counts were observed in P-6 MAP (5 ± 0.03), while the highest counts was in P-0 MAP (5.3 ± 0.01 log CFU g⁻¹) at the end of storage. Higher counts of yeasts and moulds count found in this study collaborates with the report by Caleb et al. (2013) who observed that aerobic mesophilic bacteria counts were lower than yeast and moulds in minimally processed fresh 'Herskawitz' and 'Acco' pomegranate arils under passive MAP stored at 5 °C. This may be attributed to the low pH values of packaged arils, which favours the growth of yeast and moulds in comparison with aerobic mesophilic bacteria (Caleb et al., 2013). Similarly, the higher yeast and mould counts observed in P-0 MAP packaged arils in comparison to P-9 PM-MAP could be attributed to higher level of CO₂ recorded in P-0 MAP samples. Studies have suggested that higher yeast and moulds counts in packaged produce can be attributed to

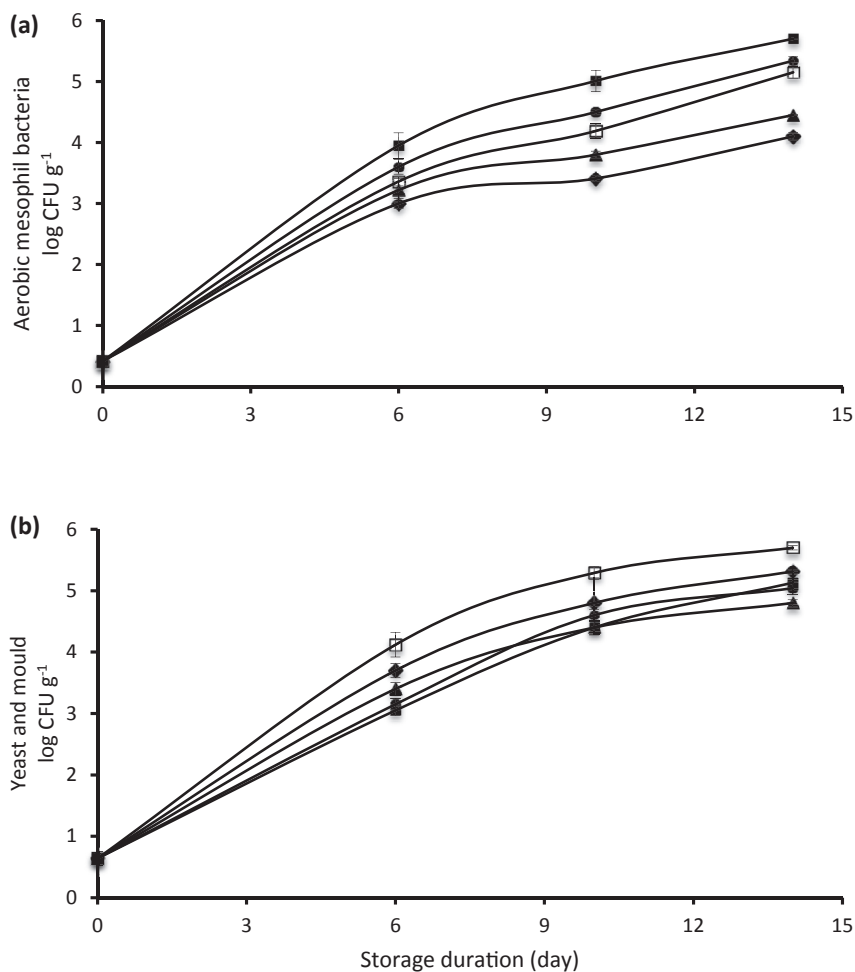


Fig. 3. Effect of perforation-mediated modified atmosphere and clamshell packaging on the growth of aerobic mesophilic bacteria (a), and yeast and mould (b) in minimally processed 'Acco' pomegranate arils stored at 5 °C for 15 days. P is numbers of perforations: P-0 (non-perforated), P-3 (three perforations), P-6 (six perforations) and P-9 (nine perforations); □ = clamshell trays; ◆ = P-0; ▲ = P-3; ● = P-6; ■ = P-9. Error bars indicate standard deviation from mean value ($n = 3$).

increase in or accumulation of CO₂ inside packages (Lopez-Rubira et al., 2005).

After 14 days of storage, aerobic mesophilic bacteria counts were below the maximum (7 log CFU g⁻¹) acceptable counts for raw and fresh-cut fruits imposed by South African regulation (FCD, Act 54 1979). On the other hand, yeast and mould counts in P-3 and P-6 PM-MAP packaged arils did not exceed maximum acceptable limit for raw and fresh-cut fruits (5 log CFU g⁻¹) (FCD, Act 54 1979) at the end of storage. In general, higher microbial counts were found in this study in comparison to previous findings on MAP of fresh pomegranate arils (Ayhan & Estürk, 2009). This variation may be attributed in part to the presence of perforations on MAP which provide points of entry for microorganisms during storage. The trend of progressive increase in aerobic mesophilic counts with perforations of packages may be a basis of this explanation. Also possible difference in cultivars investigated, maturity of arils and storage conditions. *E. coli* is a hygienic criterion that indicates level of fecal contamination during the manufacturing processes (Abadias, Usall, Anguera, Solsona, & Viñas, 2008). The absence of *E. coli* in the investigated packaged arils shows that preparation of samples were performed in a highly hygienic facility and the presence of perforations on packaged fresh could be microbiological safe.

4. Conclusion

Perforations on modified atmosphere packaging prevented build-up of CO₂ in the headspace atmosphere during 15 days of cold storage. In this study, water vapour condensation was observed in clamshell trays and non-perforated (P-0) MAP due to poor permeability. As such, arils in these packages had poor visual quality and signs of decay. Thus, MAP with the appropriate number of perforations (in this study 3 or 6 perforation per 160.1 cm²) could be a useful tool to avoid excessive CO₂ accumulation or anoxic state, thereby overall quality of minimally processed pomegranate arils. Perforated packages showed a potential to prevent the growth of aerobic mesophilic bacteria, yeast and moulds for 15 days of storage. Yeast and moulds did not exceed maximum acceptable limit in industry (5 log CFU g⁻¹) in P-3 and P-6 MAPs and no growth of *E. coli* was detected throughout the 15 day storage period. Thus, good processing practices, optimal cold chain and contamination free environment should be ensured during commercial post-harvest practices, handling and storage of perforated MA-packaged fresh produce. These results showed the industrial relevance of using perforation as a feasible low-cost approach to optimise film permeability and guarantee longer shelf-life of fresh produce.

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