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Bioactive compounds and quality attributes of pomegranate arils (*Punica granatum* L.) processed after long-term storage

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ABSTRACT

This study investigated the effects of packaging on physicochemical properties, bioactive components (anthocyanins, ascorbic acid and β-carotene) and shelf life of pomegranate arils (cvs. Arakta, Bahgwa and Ruby) obtained from fruit stored for a long-term (between 10 and 14 weeks). Headspace-volume-to-mass ratio of packaged arils had a significant impact on gas composition inside the packages, O_2 concentrations was not below the critical limit (2%). Arils weight loss did not exceed 0.2%, while juice leakage ranged from 0.17 to 4.17 mL 100 g^{-1} across all treatments. Physicochemical parameters were not significantly affected by packaging. However, there were significant differences in bioactive component amongst the cultivars, with cv. Bahgwa having the highest level of anthocyanin (112.50 mg L^{-1}) and β-carotene (6.20 mg L^{-1}). Based on visual quality and development of off-odour, shelf life of pomegranate arils was limited to day 7 for 'Arakta' and 'Bahgwa', and 5 d for 'Ruby'. This study provides a useful guide for postharvest handling and storage of packaged pomegranate arils.

1. Introduction

Pomegranate fruit (*Punica granatum* L.) has recaptured consumer interest globally, due to its health promoting benefits (Holland & Bar-Ya'akov, 2008). Pomegranate fruit have been reported to possess high antioxidant capacity and anti-mutagenic, anti-inflammatory, anti-hypertension and anti-atherosclerotic activities (Viuda-Martos, Fernández-López, & Pérez-Álvarez, 2010). Additionally, pomegranate is a good source of micro- and maro-nutrients, organic acids and bioactive compounds (Opara, Al-Ani, & Al-Shuaibi, 2009). Minimally processed and ready-to-eat pomegranate arils offer an appealing product compared to the whole fruit and increases the prospect of production and consumption (Artés, Villaescusa, & Tudela, 2000; Caleb, Opara, & Witthuhn, 2012). Pomegranate fruit (*P. granatum* L.) is a non-climacteric fruit, with relatively low respiration rate (RR) and produces trace

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amounts of ethylene (Caleb et al., 2012). Average RR of fresh arils varies with pomegranate fruit cultivar: López-Rubira, Conesa, Allende, & Artés (2005) reported RR of 1.15 mL kg h^{-1} for cv. 'Mollar Elche' stored at 5 °C, and Ersan, Gunes, & Zor (2010) reported RR of 1.5 mL kg h^{-1} for cv. 'Hicaz' under modified atmospheric condition (2% O_2 + 10% CO_2).

Packaging plays an important role in maintaining the nutritional and microbial quality of fresh or fresh-cut produce (Opara & Mditshwa, 2013). Packaging protects the food products, serves as an alternative measure for controlling diseases and provides structural support for convenient storage and transportation purposes. This plays a significant role in extending shelf life of food products and reduces the risk of foodborne pathogens (Opara & Mditshwa, 2013; Riudavets, Castañé, Alomar, María, & Gabarra, 2009; Smith, Zagory, & Ramaswamy, 2005). Various storage and packaging applications have been studied for pomegranate arils this include heat sealed trays with oriented polypropylene film, rigid polystyrene vessels, perforated polyethelene bags, ethyl vinyl acetate films, polypropylene trays and bi-axially oriented polypropylene films (Ayhan & Eştürk, 2009; Ergun & Ergun, 2009; Gil, Martínez, & Artés, 1996; Sepúlveda, Galletti, Sáenz, & Tapia, 2000; Sepúlveda et al., 2001).

Modified atmosphere packaging offers a dynamic process of varying the gas composition inside a give package. It relies on the interplay of various factors such as headspace gas composition, the respiration rate of the fresh produce, weight of packaged product, gas permeability through packaging material, film thickness and surface area, and storage temperature (Fonseca, Oliveira, & Brecht, 2002; Mahajan, Oliveira, Montanez, & Frias, 2007; Sousa-Gallagher & Mahajan, 2013). Evaluation of package engineering design parameters has been conducted for various fresh-cut produce (Caleb, Mahajan, Manley, & Opara, 2013; Oliveira, Sousa-Gallagher, Mahajan, & Teixeira, 2012). Ayhan and Eştürk (2009) investigated the effect of modified atmosphere packaging on the overall quality of processed pomegranate arils in cold storage at 5 °C. They observed no significant change in chemical and physical attributes of the stored arils. The effect of different types of semi-permeable films and antioxidant solutions on the quality of minimally processed pomegranate arils was investigated during storage at 4 °C for 14 days by Sepúlveda et al. (2000). The authors observed no colour changes in arils, pH and titratable acids and microbial counts were lowest in the antioxidant treated and packaged samples. All these studies have shown that packaging plays an important role in the keeping quality of pomegranate arils to preserve the quality of the fruit by reducing shrivelling, weight loss and microbial load.

Due to increasing consumer demand, commercial pomegranate production is globally increasing (Caleb et al., 2012). Long-distance shipping is required for international markets, and the fruit is exposed to various postharvest handling processes. Pomegranate fruit has a long storage life ranging from 2 to 7 months, depending on the cultivar and storage conditions. However, prolonged storage causes physiological disorders and promotes decay (Caleb et al., 2012). There is limited information in literature on the effect of long-term storage prior to processing and packaging on the bioactive components and physicochemical properties of pomegranate arils. This study investigated the effects of packaging on physicochemical properties, bioactive components (anthocyanins, ascorbic acid and β-carotene) and shelf life of pomegranate arils (cvs. Arakta, Bahgwa and Ruby) obtained from fruit stored for a long-term (between 10 and 14 weeks).

2. Materials and methods

2.1. Plant material and processing

Pomegranate fruit (*P. granatum* L.) cvs. 'Arakta', 'Bhagwa' and 'Ruby', harvested manually at commercial maturity were obtained from Houtconstant farm in Western Cape (33°01′00″ S, 18°58′59″ E), South Africa. Fruit with good quality attributes, without bruises, splits or other quality defects were carefully selected and transported in a well-ventilated air-conditioned vehicle for about 120 km the same day to the Postharvest Technology and Research Laboratory, Stellenbosch University, South Africa. Fruit were stored 10–14 weeks at 7 °C and 95% RH to minic shipping and long-term storage practices ('Arakta', 'Bhagwa' and 'Ruby' for 10, 12 and 14 weeks, respectively) prior to processing and packaging. After long-term storage fruit were sorted to exclude any fruit with disorders, which may have developed during storage. Each fruit was rinsed with sterile distilled water and manually peeled in a disinfected processing room below 10 °C, using a sharp knife and care was taken to avoid damaging the arils. Each fruit yielded approximately 50% of total fruit weight as arils. Arils were packaged according to the volume to weight ratio ranging from 2.1 to 2.6 g mL^{-1} due to slight variation in package designs with PET1 > PET2 > PP. Packages used within this study are summarized in (Table 1). After packaging, samples were stored for 14 d at 5 °C and 95% RH and sampling were carried out on day 0, 7 and 14. All physicochemical, proximate composition and selected bioactive component analyses were performed in triplicate on sampling days.

2.2. Headspace gas composition analysis

Before opening the packages, internal headspace gas composition of oxygen (O_2) and carbon dioxide (CO_2) was determined using a headspace gas analyser (Checkmate 3, PBI Dansensor, Ringstead, Denmark). Results were expressed as O_2% and CO_2%, respectively. After the headspace gas composition analysis was taken, a qualitative evaluation was performed on the arils inside the packages to quantify any the incidence of decay and development of off-odour. No incidence of browning was observed. Therefore, browning index was not reported during this study.

2.3. Weight loss and juice leakage

Weight loss of arils was measured by taking the initial and final weight of each packaged sample using an electronic weighing balance (ML 3002.E, Mettler Toledo, Switzerland). The punnets were weighed at day 0, 7 and 14 to assess the percentage weight loss of arils over time. Juice leakage (mL 100 g^{-1}) was determined after removing all the arils from the

Table 1 – Description of packaging trays and clamshell used in this study.

Package description	Volume (mL)	Thickness (mm)	Material
Clamshell	330	0.30	Polyethylene terephthalate (PET1) Higher barrier property (HBP)
Rectangular tub and lid	180	0.35	Polyethylene terephthalate (PET2) (HBP)
Round tub	320	0.70	Polypropylene (PP)
Round lid		0.40	Polypropylene (PP) (HBP)

punnets by tilting the punnets at a 20° angle for 5 min and measuring the liquid with a 5 mL syringe.

2.4. Colour

About 15 mL of the pomegranate arils juice sample was used for colour analyses. Colour was measured in terms of Commission International del' Eclairage (CIE) L^*, a^*, b^* colour coordinates using a chroma metre (Model CR-400, Konica Minolta sensing Inc., Osaka, Japan). Coordinates L^*, a^* and b^* measure colour in terms of lightness/brightness (L^*), red/green (a^*) and blue/yellow (b^*). Hue angle (H^*) and C^*, was calculated from Eqs. (1) and (2), respectively (Alighourchi & Barzegar, 2009; Pathare, Opara, & Al-Said, 2013):

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

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

2.5. Total soluble solids, titratable acidity and pH

Pomegranate arils inside each package were juiced separately using LiquaFresh juice extractor (Mellerware, Cape Twon, South Africa). Juice was directly used to measure total soluble solids (TSS), titratable acidity (TA) and pH. TSS was measured using a hand refractometer (Atago PR-32α, Tokyo, Japan) and expressed as °Brix. TA was measured based on the AOAC method 965.30 (AOAC, 2006) using an automated Metrohm 862 compact titrosampler (Metrohm, Herisau, Switzerland). Titrosampler potentiometrically titrated the juice sample with 0.1 N NaOH to an end point of pH 8.1 and expressed as TA (g citric acid 100 mL^{-1}) of juice sample. Ratio of TSS: TA of the pomegranate juice was calculated as one of the indices of maturity. For pH measurement, a pH metre (BASIC 20 + Model, Crison, Barcelona, Spain) was used after calibrating with pH buffers 4 and 7.

2.6. Analyses of selected bioactive components

2.6.1. Anthocyanin

Anthocyanin content of pomegranate juice was determined via pH-differential method using two buffer systems comprising of 0.025 M potassium chloride (pH 1) and 0.4 M sodium acetate (pH 4.5). One millilitre of sample juice was mixed with 9 mL of the buffer solution. The absorbance (A) was measured at wavelength reading of 510 and 700 nm using a Helis Omega UV–vis spectrophotometer (Thermo Fisher Scientific, Madison, WI, USA), after 10 min incubation of the mixture in a dark cabinet. The difference in absorbance was calculated using the following equation ($A = (A_{510} - A_{700})_{pH\ 1.0} - (A_{510} - A_{700})_{pH\ 4.5}$). Total monomeric anthocyanin pigments (TMAP) were calculated as follows:

$$TMAP = \left[\frac{A \times MW \times DF \times 100}{\varepsilon \times L}\right] \quad (3)$$

where A = Absorbance; MW = molecular weight of cyanidin-3-glucoside (449.2 g mol^{-1}); DF = dilution factor; ε = 26,900 molar absorptive coefficient and L = path length in cm. Results were expressed as mg cyanidin-3-glucoside per L of juice (Fawole, Opara, & Theron, 2012).

2.6.2. Ascorbic acid

Ascorbic acid was determined spectrophotometrically against a standard curve using 0.0025% 2,6-dichlorophenolindophenol (DCP) dye and 1% metaphosphoric acid (MPA) (Barros, Ferreira, Queiros, Ferreira, & Baptista, 2007). Combination of blue coloured DCP dye and colourless MPA resulted in a pink coloured solution, which was decolourised/reduced in the presence of ascorbic acid. Ascorbic acid of unknown concentrations in pomegranate juice samples was quantified using a standard curve of known concentrations from a stock solution (1 mg mL^{-1}) L-ascorbic acid. Both the stock solution and the juice samples were diluted with a DCP-MPA solution and absorbance was measured at 515 nm wavelength. Pomegranate juice samples were thawed at room temperature, diluted with MPA, vortexed (Model nr. G560E, Scientific Industries, USA) and sonicated (Ultrasonic Cleaner DC400H, MRC Ltd., Holon, Israel) for 3 min in cold water to extract the ascorbic acid present in the juice. Extract was centrifuged at 10,000 rpm at 4 °C to obtain a clear homogenous solution, diluted with DCP dye and kept in a dark cabinet for 10 min. To correct for the natural pink colour of pomegranate juice, another set of centrifuged extract samples were taken and diluted with distilled water instead of MPA. The absorbance of the samples (MPA and water diluted extracts) and standard curve was read at 510 nm wavelength. Ascorbic acid values were extrapolated from a standard curve with $R^2 > 0.90$. Ascorbic acid content was expressed as mg ascorbic acid per L pomegranate juice (mg L^{-1}).

2.6.3. Total carotenoids

Total content of carotenoids was determined colometrically against a β-carotene standard curve (Quiro's & Costa, 2006). Pomegranate juice was thawed and diluted with ethanol: hexane (1:1) and butylhydroxy toluene (BHT). Samples were vortexed (Model nr. G560E, Scientific Industries, USA), sonicated (Ultrasonic Cleaner DC400H, MRC Ltd., Holon, Israel) in cold water for 10 min and centrifuged at 5000 rpm, 4 °C for 5 min. Absorbance of the samples as well as the standard curve was measured at 470 nm wavelength. Results were

extrapolated from a standard curve with $R^2 > 0.90$ and expressed as mg β-carotene per L pomegranate juice (mg L^{-1}).

2.7. Statistical analysis

Statistical analyses were carried out using Statistica software version 10 (Statsoft, Tulsa, USA), to evaluate the effects that packaging has on various quality attributes of individual pomegranate cultivars. Experimental data obtained were subjected to analyses of variance analysis (ANOVA) at 95% confident interval, and significant difference of the mean values was further determined using Fisher's least significant difference (L.S.D.) multiple comparison test. Pearson test was used to show correlation between experimental data sets ($p < 0.01$). All physico-chemical, proximate composition and selected bioactive component analyses were performed in triplicate ($n = 3$) on sampling days.

3. Results and discussion

3.1. Headspace gas composition

Headspace gas composition of pomegranate arils showed a continuous increase in CO_2 levels and decrease in O_2 levels over 14 days of storage, similar to findings reported by other authors (Ayhan & Eştürk, 2009; Caleb, Mahajan, et al., 2013). Package type was observed to have significant effect on headspace gas composition, while cultivar differences had no influence on change in headspace gas composition (Fig. 1). The

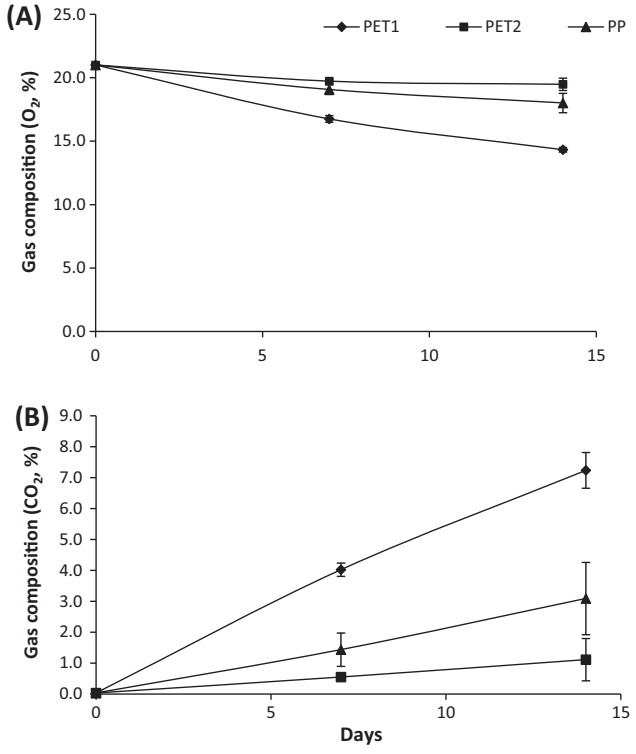

Fig. 1 – Changes in % gas composition within packages during storage, (A) O_2 and (B) CO_2. ◆ = PET1; ■ = PET2; ▲ = PP.

observed variation in gas composition among the different types of packaging could be attributed to the volume-to-mass of arils packed. Studies have shown that the headspace volume-to-mass of packaged pomegranate arils have an influence on the change in gas composition (Caleb, Mahajan, et al., 2013). Additionally, the slow rate of change in gas composition observed in this study could be attributed to the lack of hermetic sealing of the packages. CO_2 in PET1 was about 4% on day 7 and doubled to approximately 8% by day 14 in all cultivars.

3.2. Juice leakage and weight loss

Juice leakage of arils ranged from 0.17–4.17 mL 100 g^{-1} across all packages and pomegranate cultivars. Amongst the three cultivars, juice leakage was most prominent in cv. Ruby arils in comparison to cvs. Arakta and Bahgwa. Weight loss of pomegranate arils did not exceed 0.25% across all packages, with highest (0.25%) and least (0.05%) weight loss occurring in PET1 and PP trays, respectively. Similar observation was reported for pomegranate arils packed in PET clamshell punnets by Caleb, Mahajan, et al. (2013). Weight loss in this study was lower than weight loss (0.6–0.8%) reported in arils packed in oriented polypropylene heat-sealed pouches stored at 4 °C for 7 days (Gil et al., 1996). Lower weight loss observed in this study could be attributed to high barrier properties of the packages to the movement of water vapour, which increases the level of relative humidity within the package and enhanced the moisture condensation (Caleb, Mahajan, et al., 2013). This highlight on the importance of optimizing the water vapour transmission rate of packaging material, in order to prevent condensation and extend produce shelf life

3.3. Change in colour

Packaging type and storage duration had a minimal influence on the changes in chromatic parameters, while cultivar differences was observed with average L^* value ranging from 35.0 to 35.6 for cv. Arakata, 34.0–35.1 for cv. Bahgwa and 35.9–37.2 for cv. Ruby. Lightness of 'Arakta' aril juice was slightly higher when stored in PET1 and PET2 but remained unchanged in PP on day 14 compared to day 0. Redness (a^*) value ranged from 14.2–15.5 for cv. 'Arakta', 12.7–14.0 for cv. Bahgwa and 12.8–15.5 for cv. Ruby pomegranate arils. Colour intensity (C^*) values were within the average range of 13.5–16.7 across all samples, cv. Arakta had the highest chroma values, while 'Bahgwa' had the lowest. Hue angle (H^*) values range from 20.3 to 23.8 across all samples, with a slight increase over the storage period (Table 2). Packaging with storage duration had a significant influence on the colour intensity. Chromatic values in this study differ from those reported in literature for pomegranate arils (Ayhan and Eştürk, 2009; Caleb, Mahajan, et al., 2013). This can be attributed to differences in cultivar and direct colour measurement of arils instead of juice in this study.

3.4. Chemical analyses

Table 3 summarizes the changes in chemical parameters of minimally processed and packaged pomegranate arils for the

Table 2 – Comparison of the effect of packaging type, storage duration and cultivar difference on colour parameter (mean ± SD) of pomegranate arils.

Days	Package	Chroma (C^*)			Hue (H^*)		
		'Arakta'	'Bahgwa'	'Ruby'	'Arakta'	'Bahgwa'	'Ruby'
0		$15.4 \pm 0.40^{A}{}_{b}$	$13.5 \pm 1.33^{B}{}_{ab}$	$16.6 \pm 1.16^{A}{}_{a}$	$21.2 \pm 0.13^{A}{}_{d}$	$20.3 \pm 0.47^{B}{}_{c}$	$20.9 \pm 0.24^{AB}{}_{b}$
	PET1	$16.7 \pm 0.87^{A}{}_{a}$	$13.7 \pm 0.49^{B}{}_{b}$	$15.8 \pm 0.28^{A}{}_{a}$	$21.6 \pm 0.11^{A}{}_{c}$	$20.4 \pm 0.29^{B}{}_{c}$	$20.9 \pm 0.27^{B}{}_{b}$
7	PET2	$16.2 \pm 0.62^{A}{}_{ab}$	$13.9 \pm 0.04^{B}{}_{b}$	$15.4 \pm 0.49^{A}{}_{a}$	$21.3 \pm 0.21^{A}{}_{cd}$	$20.6 \pm 0.20^{B}{}_{c}$	$21.1 \pm 0.15^{A}{}_{b}$
	PP	$16.0 \pm 0.64^{A}{}_{ab}$	$14.3 \pm 0.82^{B}{}_{ab}$	$15.2 \pm 0.95^{AB}{}_{a}$	$21.2 \pm 0.13^{A}{}_{d}$	$20.8 \pm 0.24^{B}{}_{c}$	$20.9 \pm 0.49^{AB}{}_{b}$
	PET1	$15.3 \pm 0.95^{A}{}_{ab}$	$14.2 \pm 0.42^{B}{}_{ab}$	$15.4 \pm 0.39^{A}{}_{a}$	$22.2 \pm 0.09^{B}{}_{b}$	$21.4 \pm 0.16^{C}{}_{b}$	$23.1 \pm 0.79^{A}{}_{a}$
14	PET2	$15.7 \pm 1.29^{A}{}_{ab}$	$15.2 \pm 1.46^{A}{}_{ab}$	15.9 ± 0.90^{Aa}	$22.6 \pm 0.30^{AB}{}_{a}$	$22.3 \pm 0.27^{B}{}_{a}$	$23.4 \pm 0.71^{A}{}_{a}$
	PP	$15.9 \pm 1.20^{A}{}_{ab}$	$15.0 \pm 0.66^{A}{}_{a}$	$14.0 \pm 1.59^{A}{}_{a}$	$22.4 \pm 0.14^{A}{}_{ab}$	$22.1 \pm 0.18^{A}{}_{a}$	$23.8 \pm 0.58^{A}{}_{a}$

Different letters indicate significant difference ($p < 0.05$) according to the multiple LSD test.
* For each column similar lower case letters in subscripts are not significant, while parameters in rows with similar upper case superscript letters are not significantly different.

three cultivars during storage. A comparison amongst the cultivars, cv. Arakta had a slightly higher TA, while TSS/TA was highest in cv. Ruby. Result shows that there was no significant effect of packaging and storage on TA, TSS and pH of pomegranate arils juice, with exception of cv. Ruby with slight fluctuations. This result was in agreement with other studies. Peña et al. (2013) observed no significant change in TA for sustained deficit irrigated pomegranate fruit during storage from the initial value of 0.25 g citric acid mL^{-1}. Sepúlveda et al. (2001) reported that packaging had no significant effect on TA of three pomegranate cultivars during storage. The non-significant effect of packaging applications on chemical attributes corroborates pervious study reported by Ayhan and Eştürk (2009) and Caleb, Mahajan, et al. (2013). Variability in pH, TA and TSS values in the studies could be attributed to cultivar differences and the effect of increased CO_2 solubility inside the packages.

3.5. *Selected bioactive components*

Total anthocyanin, β-carotene and ascorbic acid content of pomegranate arils juice on day 0 were approximately 92.10, 4.17 and 24.80 mg L^{-1} for cv. Arakta; 112.50, 6.20 and 24.20 mg L^{-1} for cv. Bahgwa; and, 73.80, 3.34 and 20.50 mg L^{-1} 1 for cv. Ruby (Table 4). There was a significant difference in the selected bioactive component across the three cultivars, with 'Bahgwa' having the highest level of anthocyanin and β-carotene. A low level of β-carotene was found in all pomegranate cultivars compared to anthocyanin and ascorbic acid content and this level declined throughout storage. Carotenoids are more labile for degradation when extracted from their relatively stable natural state (Coultate, 2007). A general trend of decrease in anthocyanin, β-carotene and ascorbic acid was observed as the storage time increased for all treatment, however, for anthocyanin and ascorbic acid a

Table 3 – Effect of packaging and storage duration on the change in chemical attributes of three pomegranate cultivars.

Cultivars	Days	Package(s)	TA (g 100 mL^{-1})	pH	TSS (°Brix)	TSS/TA
'Arakta'	0		0.33 ± 0.03^{a}	3.29 ± 0.00^{d}	15.4 ± 0.47^{abc}	47.6 ± 5.73^{b}
	7	PET1	0.28 ± 0.02^{bc}	3.30 ± 0.03^{d}	15.1 ± 0.35^{c}	53.2 ± 1.67^{ab}
		PET2	0.31 ± 0.04^{abc}	3.37 ± 0.01^{b}	15.5 ± 0.12^{abc}	50.1 ± 6.28^{ab}
		PP	0.28 ± 0.01^{c}	3.38 ± 0.02^{ab}	15.2 ± 0.25^{bc}	55.1 ± 0.68^{a}
	14	PET1	0.32 ± 0.01^{ab}	3.33 ± 0.02^{c}	15.6 ± 0.21^{abc}	48.9 ± 2.03^{ab}
		PET2	0.31 ± 0.00^{abc}	3.39 ± 0.01^{ab}	15.9 ± 0.15^{a}	51.2 ± 0.49^{ab}
		PP	0.30 ± 0.01^{abc}	3.40 ± 0.01^{a}	15.8 ± 0.26^{ab}	53.3 ± 0.81^{ab}
'Bahgwa'	0		0.28 ± 0.01^{a}	3.24 ± 0.01^{c}	15.70 ± 0.10^{ab}	55.4 ± 1.17^{ab}
	7	PET1	0.28 ± 0.01^{a}	3.25 ± 0.01^{c}	15.93 ± 0.21^{a}	56.3 ± 1.17^{ab}
		PET2	0.28 ± 0.00^{a}	3.25 ± 0.02^{c}	15.70 ± 0.17^{ab}	56.1 ± 0.62^{ab}
		PP	0.28 ± 0.00^{a}	3.26 ± 0.01^{c}	15.57 ± 0.25^{b}	55.6 ± 0.90^{ab}
	14	PET1	0.28 ± 0.00^{a}	3.32 ± 0.02^{b}	15.97 ± 0.15^{a}	57.0 ± 0.55^{a}
		PET2	0.28 ± 0.00^{a}	3.36 ± 0.00^{a}	15.83 ± 0.21^{ab}	56.6 ± 0.74^{ab}
		PP	0.29 ± 0.01^{a}	3.35 ± 0.02^{a}	15.80 ± 0.17^{ab}	55.1 ± 0.51^{b}
'Ruby'	0		0.26 ± 0.01^{a}	3.35 ± 0.01^{d}	15.8 ± 0.10^{a}	60.0 ± 1.36^{e}
	7	PET1	0.23 ± 0.01^{b}	3.43 ± 0.01^{c}	15.5 ± 0.15^{abc}	66.3 ± 1.63^{d}
		PET2	0.22 ± 0.01^{c}	3.43 ± 0.01^{c}	15.3 ± 0.29^{c}	68.4 ± 0.47^{bc}
		PP	0.22 ± 0.01^{c}	3.47 ± 0.02^{b}	15.4 ± 0.40^{bc}	70.9 ± 0.02^{a}
	14	PET1	0.22 ± 0.01^{c}	3.55 ± 0.01^{a}	15.7 ± 0.25^{ab}	70.2 ± 1.13^{ab}
		PET2	0.23 ± 0.01^{b}	3.56 ± 0.02^{a}	15.7 ± 0.10^{ab}	67.3 ± 1.70^{cd}
		PP	0.22 ± 0.00^{c}	3.57 ± 0.01^{a}	15.7 ± 0.00^{ab}	71.4 ± 0.00^{a}

Different letters indicate significant difference ($p < 0.05$) according to the multiple LSD test.
Comparison amongst the three cultivars was not analyzed.

Table 4 – Effect of package type and storage duration on change in selected bioactive components of the three pomegranate cultivars.

Days	Package	Ascorbic acid content (mg L^{-1})			β-carotene (mg L^{-1})			Total anthocyanin content (mg L^{-1})		
		'Arakta'	'Bahgwa'	'Ruby'	'Arakta'	'Bahgwa'	'Ruby'	'Arakta'	'Bahgwa'	'Ruby'
0		$24.80 \pm 2.92^{A}{}_{d}$	$24.20 \pm 1.94^{A}{}_{e}$	$20.50 \pm 1.21^{B}{}_{e}$	$4.17 \pm 0.32^{B}{}_{a}$	$6.20 \pm 0.70^{A}{}_{a}$	$3.34 \pm 0.78^{B}{}_{a}$	$92.10 \pm 1.83^{B}{}_{e}$	$112.50 \pm 2.09^{A}{}_{d}$	$73.80 \pm 2.82^{A}{}_{abc}$
	PET1	$40.80 \pm 3.66^{C}{}_{b}$	$47.20 \pm 2.38^{B}{}_{b}$	$48.80 \pm 0.98^{A}{}_{b}$	$4.17 \pm 0.38^{A}{}_{a}$	$5.63 \pm 1.08^{A}{}_{ab}$	$0.63 \pm 0.68^{C}{}_{c}$	$98.20 \pm 3.36^{B}{}_{d}$	$132.50 \pm 2.64^{A}{}_{a}$	$70.60 \pm 1.64^{C}{}_{cd}$
7	PET2	$45.40 \pm 3.81^{B}{}_{a}$	$49.70 \pm 1.34^{B}{}_{a}$	$56.50 \pm 2.94^{A}{}_{a}$	$4.26 \pm 1.25^{A}{}_{a}$	$5.21 \pm 2.19^{A}{}_{ab}$	$2.76 \pm 2.15^{A}{}_{ab}$	$99.20 \pm 1.54^{B}{}_{d}$	$127.30 \pm 1.54^{A}{}_{ab}$	$69.60 \pm 3.78^{C}{}_{d}$
	PP	$33.90 \pm 1.26^{C}{}_{c}$	$48.60 \pm 2.60^{B}{}_{ab}$	$56.80 \pm 2.07^{A}{}_{a}$	$2.73 \pm 0.50^{A}{}_{b}$	$2.45 \pm 0.72^{A}{}_{d}$	$2.55 \pm 1.24^{A}{}_{ab}$	$101.80 \pm 3.16^{B}{}_{d}$	$124.80 \pm 4.79^{A}{}_{b}$	$62.0 \pm 2.24^{C}{}_{e}$
	PET1	$12.0 \pm 1.50^{C}{}_{e}$	$34.50 \pm 1.90^{B}{}_{cd}$	$42.60 \pm 1.37^{A}{}_{d}$	$0.65 \pm 0.49^{B}{}_{c}$	$4.03 \pm 0.79^{A}{}_{c}$	$0.91 \pm 0.35^{B}{}_{c}$	$117.60 \pm 4.72^{A}{}_{b}$	$127.20 \pm 9.70^{A}{}_{ab}$	$75.90 \pm 5.72^{B}{}_{a}$
14	PET2	$39.50 \pm 0.28^{B}{}_{b}$	$33.10 \pm 2.29^{C}{}_{d}$	$45.50 \pm 0.86^{A}{}_{c}$	$1.31 \pm 0.64^{B}{}_{c}$	$4.58 \pm 0.43^{A}{}_{bc}$	$1.66 \pm 0.38^{B}{}_{bc}$	$108.30 \pm 11.36^{B}{}_{c}$	$128.50 \pm 7.41^{A}{}_{ab}$	$74.40 \pm 4.26^{C}{}_{ab}$
	PP	$38.70 \pm 1.85^{B}{}_{b}$	$35.70 \pm 1.13^{C}{}_{c}$	$44.80 \pm 0.99^{A}{}_{c}$	$0.93 \pm 0.47^{B}{}_{c}$	$3.96 \pm 0.52^{A}{}_{c}$	$1.13 \pm 0.44^{B}{}_{c}$	$123.90 \pm 2.53^{A}{}_{a}$	$118.70 \pm 1.40^{A}{}_{c}$	$71.20 \pm 1.73^{B}{}_{bcd}$

Different letters along the column indicate significant difference ($p < 0.05$) according to the multiple LSD test.
For each column similar lower case letters in subscripts are not significant, while parameters in rows with similar upper case superscript letters are not significantly different.

slight increase were recorded on day 7 for all the cultivars. Anthocyanin levels of 'Bahgwa' and 'Ruby' arils were slightly lower in PP after day 7 and 14. Observed decrease in total anthocyanin content is in agreement with previous studies on the effect of packaging and storage duration on pomegranate arils (Ayhan & Eştürk, 2009; Caleb, Mahajan, et al., 2013). This shows that appropriate packaging could help maintain nutritional qualities of fresh-cut pomegranate. But inherent cultivar differences have an influence on the presence and quantity of bioactive content in pomegranate fruit (Legua et al., 2012).

3.6. *Qualitative evaluation*

Incidence of decay and development of off-odour was monitored throughout the storage period. The cv. Ruby was the most susceptible of the three cultivars with mould growth observed from day 7 of storage as well as the development of off-odour, while mould growth was observed only in PET2 for cv. Bahgwa arils on day 7 (Table 5). Correlation matrix summarized in Table 6, showed a significant ($p < 0.01$) positive correlation between mould incidence, CO_2 production and O_2 consumption in the different types of packages. Susceptibility of cv. 'Ruby' could be due to the longer cold room storage of 14 weeks and its lower TA levels in comparison to cv. 'Arakta' or 'Bahgwa'. Fruit physicochemical attribute such as pH and TA have been shown to have a significant effect on microbial shelf life of minimally processed fruit (Soliva-Fortuny & Martín-Belloso, 2003). However, visual quality of all cultivars deteriorated further from day 7 to 14, due to mould growth. A detailed microbial analysis is recommended to confirm the safety of packaged pomegranate arils stored under cold temperature.

Base on the qualitative evaluation the shelf of packaged pomegranate arils in this study was limited to 7 d (cv. Arakta or Bahgwa) and less than 7 d for cv. Ruby. This is consistent with literature that the development of off-odours could serve as an indicator of postharvest shelf life (Caleb, Opara, et al., 2013). The shelf life of arils in this current study is shorter in comparison to related studies in literature. Development of off-odour was reported to limit the shelf life pomegranate arils 'Acco' and 'Herskawitz' at day 10 (Caleb, Mahajan, et al., 2013). López-Rubira et al. (2005) reported that the shelf life of

Table 5 – Estimated mould incidence on pomegranate arils after 7 and 14 days in 'Arakta', 'Bahgwa' and 'Ruby' arils.

Cultivar	Package	Visual mould estimation (%)		
		Day 0	Day 7	Day 14
Arakta	PET1	0	0	0–25%
	PP	0	0	25–50%
	PET2	0	0	50–75%
Bahgwa	PET1	0	0	<25%
	PP	0	0	0–25%
	PET2	0	<25%	25%
Ruby	PET1	0	<25%	0–25%
	PP	0	<25%	25–75%
	PET2	0	<25%	75%

Table 6 – Correlation between percentage O_2, CO_2 and mould incidence in different punnet varieties.

Punnet	Variable (%)	CO_2 (%)	O_2 (%)	Mould incidence (%)
PET1	CO_2	1.00		
	O_2	−0.97*	1.00	
	Mould incidence	0.75*	−0.66*	1.00
PET2	CO_2	1.00		
	O_2	−0.97*	1.00	
	Mould incidence	0.74	−0.61*	1.00
PP	CO_2	1.00		
	O_2	−0.99*	1.00	
	Mould incidence	0.68*	−0.59*	1.00

* Correlations were significant at $p \leq 0.01$ level.

pomegranate arils cv. Mollar Elche treated with UV-C radiation was influenced by the harvested dates (earlier or late harvest), and the effect of UV-C on microbial load was inconclusive. This could be attributed to the long-term storage of pomegranate whole fruit prior extraction and packaging of arils, which enhances fruit decay (Palou, Crisosto, & Garner, 2007).

4. Conclusions

Headspace gas concentration was significant influenced by the headspace-volume-to-mass ratio of packaged arils and O_2 concentrations inside the packages remain above the critical level (>2%) throughout the duration of storage. Cultivar differences showed no significant influence on observed change in gas composition. The use of clamshell packaging reduces aril weight loss and minimized juice leakage compared to oriented polypropylene packaging; however, it enhances microbial decay. Physicochemical parameters investigate in this study were not significantly affected by packaging; but, there were significant differences amongst cultivars. Furthermore, there were significant differences in selected bioactive components across the three cultivars, with 'Bahgwa' having the highest level of anthocyanin and β-carotene. A low level of β-carotene was found in all pomegranate cultivars compared to anthocyanin and ascorbic acid content, which declined throughout storage. Based on visual quality and development of off-odour inside the packages. The shelf life of pomegranate arils was limited to day 7 for cvs. 'Arakta' and 'Bahgwa' (stored for 10–12 weeks), while cv. 'Ruby' (stored for 14 weeks) had a shorter shelf life of 5 days. This study provides a useful guide towards improved postharvest handling, packaging and storage of minimally processed pomegranate arils.

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REFERENCES

Alighourchi, H., & Barzegar, M. (2009). Some physicochemical characteristics and degradation kinetic of anthocyanin of reconstituted pomegranate juice during storage. *Journal of Food Engineering, 90*, 179–185.

AOAC. (2006). *Official methods of analysis of AOAC International*. Gaithersburg, Md: AOAC International.

Artés, F., Villaescusa, R., & Tudela, J. A. (2000). Modified atmosphere packaging of pomegranate. *Journal of Food Science, 65*, 1112–1116.

Ayhan, Z., & Eştürk, O. (2009). Overall quality and shelf life of minimally processed and modified atmosphere packaged "ready-to-eat" pomegranate arils. *Journal of Food Science, 74*, C399–C405.

Barros, L., Ferreira, M., Queiros, B., Ferreira, I. C. F. R. , & Baptista, P. (2007). Total phenols, ascorbic acid, β-carotene and lycopene in Portuguese wild edible mushrooms and their antioxidant activities. *Food Chemistry, 103*, 413–419.

Caleb, O. J., Mahajan, P. V., Manley, M., & Opara, U. L. (2013a). Evaluation of parameters affecting modified atmosphere packaging engineering design for pomegranate arils. *International Journal of Food Science and Technology, 48*, 2315–2323.

Caleb, O. J., Opara, U. L., Mahajan, P. V., Manley, M., Mokwena, L., & Tredoux, A. G. J. (2013b). Effect of modified atmosphere packaging and storage temperature on volatile composition and postharvest life of minimally processed pomegranate arils (cvs. Acco and Herskawitz). *Postharvest Biology and Technology, 79*, 54–61.

Caleb, O. J., Opara, U. L., & Witthuhn, C. R. (2012). Modified atmosphere packaging of pomegranate fruit and arils: A review. *Food and Bioprocess Technology, 5*, 15–30.

Coultate, T. P. (2007). *Food the chemistry of its components*. UK: RSC Publishing.

Ergun, M., & Ergun, N. (2009). Maintaining quality of minimally processed pomegranate arils by honey treatments. *British Food Journal, 111*, 396–406.

Ersan, S., Gunes, G., & Zor, A. O. (2010). Respiration rate of pomegranate arils as affected by O_2 and CO_2, and design of modified atmosphere packaging. *Acta Horticulturae, 876*, 189–196.

Fawole, O. A., Opara, U. L., & Theron, K. I. (2012). Chemical and phytochemical properties and antioxidant activities of three pomegranate cultivars grown in South Africa. *Food and Bioprocess Technology, 5*, 425–444.

Fonseca, S. C., Oliveira, F. A. R., & Brecht, J. K. (2002). Modelling respiration rate of fresh fruits and vegetables for modified atmosphere packages: A review. *Journal of Food Engineering, 52*, 99–119.

Gil, M. I., Martínez, J. A., & Artés, F. (1996). Minimally processed pomegranate seeds. *Lebensmittel-Wissenschaft und-Technologie, 29*, 708–713.

Holland, D., & Bar-Ya'akov, I. (2008). The pomegranate: New interest in an ancient fruit. *Chronica Horticulturae, 48*, 12–15.

Legua, P., Melgarejo, P., Abdelmajid, H., Martínez, J. J., Martínez, R., Ilham, H., et al. (2012). Total phenols and antioxidant capacity in 10 Moroccan pomegranate varieties. *Journal of Food Science, 71*, C115–C120.

López-Rubira, V., Conesa, A., Allende, A., & Artés, F. (2005). Shelf life and overall quality of minimally processed pomegranate arils modified atmosphere packaged and treated with UV-C. *Postharvest Biology and Technology, 37*, 74–185.

Mahajan, P. V., Oliveira, F. A. R., Montanez, J. C., & Frias, J. (2007). Development of user-friendly software for design of modified atmosphere packaging for fresh and fresh-cut produce. *Innovative Food Science and Emerging Technologies, 8*, 84–92.

Oliveira, F., Sousa-Gallagher, M. J., Mahajan, P. V., & Teixeira, J. A. (2012). Evaluation of MAP engineering design parameters on quality of fresh-sliced mushrooms. *Journal of Food Engineering, 108*, 507–514.

Opara, U. L., Al-Ani, M. R., & Al-Shuaibi, Y. S. (2009). Physicochemical properties, vitamin C content, and antimicrobial properties of pomegranate fruit (*Punica granatum* L.). *Food and Bioprocess Technology, 2*, 315–321.

Opara, U. L., & Mditshwa, A. (2013). A review on the role of packaging in securing food system: Adding value to food products and reducing losses and waste. *African Journal of Agricultural Research, 8*, 2621–2630.

Palou, L., Crisosto, C. H., & Garner, D. (2007). Combination of postharvest antifungal chemical treatments and controlled atmosphere storage to control grey mould and improve storability of 'Wonderful' pomegranate. *Postharvest Biology and Technology, 43*, 133–142.

Pathare, P. B., Opara, U. L., & Al-Said, F. A. (2013). Colour measurement and analysis in fresh and processed foods: A review. *Food and Bioprocess Technology, 6*, 36–60.

Peña, M. E., Artés-Hernández, F., Aguayo, E., Martínez-Hernández, G. B., Galindo, A., Artés, F., et al. (2013). Effect of sustained deficit irrigation on physicochemical properties, bioactive compounds and postharvest life of pomegranate fruit (cv. 'Mollar de Elche'). *Postharvest Biology and Technology, 86*, 171–180.

Quiro's, A. R., & Costa, H. S. (2006). Analysis of carotenoids in vegetable and plasma samples: A review. *Journal of Food Composition and Analysis, 19*, 97–111.

Riudavets, J., Castañé, C., Alomar, O., María, J. P., & Gabarra, R. (2009). Modified atmosphere packaging (MAP) as an alternative measure for controlling ten pests that attack processed food products. *Journal of Stored Products Research, 45*, 91–96.

Sepúlveda, E., Galletti, l., Sáenz, C., & Tapia, M. (2000). Minimal processing of pomegranate var. Wonderful. *Symposium on production, processing and marketing of pomegranate in the Mediterranean region: Advances in research and technology* (pp. 237–242).

Sepúlveda, E., Sáenz, C., Berger, H., Galletti, l., Valladares, C., & Botti, C. (2001). Minimal processing of pomegranate cv. Española: Effect of three package materials. *Acta Horticulturae, 553*, 711–712.

Smith, J. P., Zagory, D., & Ramaswamy, H. S. (2005). Packaging of fruits and vegetables. In D. M. Barrett, L. Somogyi, & H. Ramaswamy (Eds.), *Processing fruits* (p. 358). Boca Raton, FL: CRC Press, Inc.

Soliva-Fortuny, R. C., & Martín-Belloso, O. (2003). New advances in extending shelf life of fresh-cut fruit: A review. *Trends in Food Science and Technology, 14*, 341–353.

Sousa-Gallagher, M. J., & Mahajan, P. V. (2013). Integrative mathematical modelling for MAP design of fresh-produce: Theoretical analysis and experimental validation. *Food Control, 29*, 444–450.

Viuda-Martos, M., Fernández-López, J., & Pérez-Álvarez, J. A. (2010). Pomegranate and its many functional components as related to human health: A review. *Comprehensive Reviews in Food Science and Food Safety, 9*, 635–665.