Effect of modified atmosphere packaging and storage temperature on volatile composition and postharvest life of minimally-processed pomegranate arils (cvs. ‘Acco’ and ‘Herskowitz’)

Oluwafemi J. Caleb\textsuperscript{a,b}, Umezurike Linus Opara\textsuperscript{a,*}, Pramod V. Mahajan\textsuperscript{c}, Marena Manley\textsuperscript{b}, Lucky Mokwena\textsuperscript{d}, Andreas G.J. Tredoux\textsuperscript{e}

\textsuperscript{a} Postharvest Technology Research Laboratory, South African Research Chair in Postharvest Technology, Faculty of AgricSciences, Stellenbosch University, Private Bag X1, Stellenbosch 7602, South Africa
\textsuperscript{b} Department of Food Science, Faculty of AgricSciences, Stellenbosch University, Private Bag X1, Stellenbosch 7602, South Africa
\textsuperscript{c} INRA Research, Montpellier, France
\textsuperscript{d} Central Analytical Facilities, Mass Spectrometry Unit, Stellenbosch University, Private Bag X1, Stellenbosch 7602, South Africa
\textsuperscript{e} Institute for Wine Biotechnology, Department of Viticulture and Oenology, Faculty of AgricSciences, Stellenbosch University, Private Bag X1, Stellenbosch 7602, South Africa

\textbf{ARTICLE INFO}

Article history:
Received 9 December 2012
Accepted 13 January 2013

\textbf{ABSTRACT}

This study investigated the effects of passive modified atmosphere packaging (MAP), storage temperature (5, 10 and 15 °C) and duration of 14 days on the postharvest quality attributes, compositional change in flavour attributes and microbiological quality of minimally processed pomegranate arils (Punica granatum L.), cvs ‘Acco’ and ‘Herskowitz’. Volatile compounds were extracted via headspace solid phase micro-extraction (HS-SPME) and analyzed by gas chromatography–mass spectrometry (GC–MS). A total of 17 and 18 volatiles were detected and identified in the headspace of pomegranate juices of ‘Acco’ and ‘Herskowitz’, respectively. Based on the physicochemical attributes and microbial evaluation, the postharvest life of MA-packaged ‘Acco’ and ‘Herskowitz’ was limited to 10 days due to fungal growth ≥2 log CFU g\textsuperscript{-1} at 5 °C. However, the concentration (%) and compositional changes in volatile compounds indicated that the flavour/aroma life (7 days) was shorter than the postharvest shelf-life based on appearance and other physicochemical (10 days) for both cultivars.

© 2013 Elsevier B.V. All rights reserved.

\section{1. Introduction}

Assessment of postharvest shelf-life of fresh-cut or minimally processed packaged fruit and vegetables is often based on changes/stability in physical attributes such as colour, firmness, juiciness, absence of decay, and chemical attributes such as total soluble sugars (TSS), pH and titratable acidity (TA). These attributes reflect visual acceptance and physicochemical properties associated with produce quality; however, they neglect the significance of flavour or aroma quality (Pelayo et al., 2003; Kader, 2008). The development of desired characteristic flavour in packaged fresh-cut products plays a crucial role in consumer preference and this influences future decisions to purchase the produce. Furthermore, identification of characteristic aroma during storage life of packaged fresh-cut material can serve as an indicator of product shelf-life (Kader, 2008). Thus, the understanding of volatile development should be incorporated into the postharvest life concept for fresh-cut fruit and vegetables. Additionally, temperature control is essential to maintaining flavour and quality of fresh produce (Tietel et al., 2012).

During the last decade, there has been increased global production and consumption of pomegranates, due to health benefits and enriched bioactive phytochemicals of this fruit (Holland et al., 2009; Viuda-Martos et al., 2010). Current research on aroma and flavour of pomegranates has focused on the identification of unique volatiles produced by ripe fruit (Calín-Sánchez et al., 2011; Melgarejo et al., 2011; Mayuoni-Kirshinbaum et al., 2012). Using the headspace solid-phase micro-extraction (HS-SPME) and gas chromatography–mass spectrometry (GC–MS), Calín-Sánchez et al. (2011) and Melgarejo et al. (2011) identified 18 and 21 aroma volatiles, respectively, in juices of nine different Spanish pomegranate cultivars. Mayuoni-Kirshinbaum et al. (2012) identified aroma-active compounds using solvent-assisted flavour evaporation (SAFE) and head space solid phase microextraction (HS-SPME) methods in ‘Wonderful’ pomegranates. The identifications included various classes such as aldehydes, monoterpenes, alcohols, esters, furans and acids, and the most prominent volatiles were ethyl-2-methylbutanoate, hexenal, limonene, trans-2-hexenal, cis-3-hexenol, cis-2-heptenal, β-pinene and β-caryophyllene.

\textsuperscript{*} Corresponding author. Tel.: +27 21 808 4064; fax: +27 21 808 3743.
\textit{E-mail addresses:} opara@sun.ac.za, umunam@yahoo.co.uk, phemikhaleb@yahoo.com (U.L. Opara).

0925-5214/5 – see front matter © 2013 Elsevier B.V. All rights reserved.

http://dx.doi.org/10.1016/j.postharvbio.2013.01.006
Furthermore, Calín-Sánchez et al. (2011) and Melgarejo et al. (2011) suggested that consumer liking of pomegranate juices could be linked to high levels of monoterpene. This observation was corroborated by a report of Mayuoni-Kirshinbaum et al. (2012), where 5 out of 12 detected ‘Wonderful’ pomegranate aroma-active compounds by the GC-O sniffing panellists were terpenes, which suggests that this class of aroma compounds and concentration plays a role in cultivar preference for pomegranate (Melgarejo et al., 2011). However, increased interest in minimally processed and fresh-cut pomegranate arils with high nutritional value and improved aril quality has highlighted the limited knowledge of factors that affect flavour development in modified atmosphere packaged pomegranate arils.

Modified atmosphere packaging (MAP) is a dynamic process of altering gaseous composition inside a package. It relies on the interaction between the respiration rate (RR) of the produce, and the transfer of gases through the packaging material, with no further control exerted over the initial gas composition (Caleb et al., 2012). MAP has been reported to extend the shelf-life of minimally processed arils (Sepúlveda et al., 2000; López-Rubira et al., 2005; Ayhan and Eştürk, 2009). Sepúlveda et al. (2000) observed that minimally processed pomegranate arils cv. ‘Wonderful’ were storable for 14 days at 4 °C ± 0.5 in semi-permeable films. This study was focused on the effect of different types of semi-permeable and antioxidant solutions on aril quality. López-Rubira et al. (2005) investigated the shelf-life and overall quality of minimally processed pomegranate arils cv. ‘Mollar Elche’ treated with UV-C and packaged under passive-MAP in polypropylene (PP) baskets sealed with BOPP film and stored at 5 °C. They observed that the shelf-life of arils was influenced by the harvest dates (earlier or late harvest). The report on the effect of UV-C radiation on microbial growth was inconclusive, with the microbial counts not systematically reduced. Ayhan and Eştürk (2009) studied the effect of various gas compositions in active-MAP on the shelf-life and overall quality of minimally processed pomegranate arils stored at 5 °C. They observed no significant change in physicochemical attributes of arils during cold storage, while aerobic mesophilic bacteria were in the range of 2.30–4.51 log CFU g⁻¹. However, none of these studies provided information on the development of flavour in MAP packaged pomegranate arils.

This study investigated the postharvest shelf-life of two pomegranate cultivars during storage under passive MAP at 5, 10 and 15 °C based on physicochemical properties, microbial stability and on changes in concentration and composition of volatile compounds. Our goal was to evaluate the potential of using changes in volatile composition as indicators of shelf-life.

2.2. Fruit processing and packaging procedures

Fruit were manually sorted to remove those with blemishes after which the outer skins (husk) of healthy whole fruit were washed in sterilized water with 200 μL⁻¹ of sodium hypochlorite (NaOCl) solution. Fruit husks were mechanically processed for aril extraction using a commercial pomegranate aril extraction unit (Arilsystems, Juran Metal Works, Israel). The extracted arils were collected on a sterile conveyor belt in order to air dry and manually remove damaged arils. Each cultivar was processed separately and all processing was conducted at temperatures below 10 °C. Arils were mixed to ensure uniformity and portions of 125 g arils were weighed into polypropylene (PP) trays which had been previously sterilized with ethylene oxide. PP trays were sealed with POLYLID films using a semi-automated heat sealing machine (Food Processing Equipment, South Africa). A label of 7.0 × 3.8 cm² area was placed onto each package film to simulate the labels found in the retail market packages. At the pack-house, packaged products were cooled down to 2 °C and transported in ice-packed cooler boxes fitted with data loggers (Gemini Data Loggers, United Kingdom) to the postharvest research laboratory. On arrival, temperature inside the cooler boxes ranged between 3 and 4.5 °C. Packaged samples were stored at 5, 10 and 15 °C and 95 ± 2% RH for 14 days, and sampling was carried out on 0, 3, 7, 10, and 14 days of storage. Two packs were analyzed for each experimental condition on each sampling day. A full factorial experimental design was used and were replicated six times (n = 6).

2.3. Headspace gas analysis

Before packages were opened on sampling days, the gas composition inside the packages was determined using a gas analyzer with an accuracy of 0.5% (Checkmate 3, PBI Dansensor, Ringstead, Denmark). Immediately after taking the gas analysis, packages were opened and used for microbial, physicochemical and volatile analyses.

2.4. Weight loss

Initial and final weight of each packaged arils was measured using an electronic weighing balance (ML3002.E, Mettler Toledo, Switzerland). Weight loss was calculated according to the following equation:

\[
WL = \frac{W_o - W_f}{W_o} \times 100
\]

where WL is the weight loss (%), W₀ is the initial weight (g) and W_f is the final weight (g) prior to package analysis.

2.5. Texture

Firmness of arils was measured using texture analyzer (TA-XT Plus, Stable Micro Systems, Surrey, England), with a 35 mm diameter cylindrical probe. Firmness was expressed as maximum compression force (N) required to rupture the arils. A test speed of 1.0 mm s⁻¹ and distance of 9.5 mm were used. An average of 10 arils was measured individually for each experimental condition.

2.6. pH, Total soluble solids, titratable acidity, and total anthocyanin content

Arils for each pack were juiced separately using a LiquaFresh juice extractor (Mellerware, South Africa) and the juice was directly used for pH and total soluble solid (TSS) measurements using a pH metre (Crisom, Barcelona, Spain) and digital refractometer expressed as Brix (Atago, Tokyo, Japan), respectively. Titratable
acidity (TA) was measured by titration to an end point of pH 8.2 using a Metrohm 862 compact titrosampler (Herisau, Switzerland). Total anthocyanin content was determined by the pH-differential method, using 2 buffer systems, namely potassium chloride (pH 1, 0.025 M) and sodium acetate (pH 4.5, 0.4 M). One millilitre of sample juice was mixed with 9 mL of buffer and the absorbance was measured at 520 and 700 nm. Total anthocyanin was calculated as cyanidin-3-glucoside according to the following equation:

\[
\text{Total anthocyanins (100 mg L}^{-1} = \frac{A \times MW \times DF \times 100}{\varepsilon \times 1}
\]

where \(A = (A_{520} - A_{700})\) pH 1 – \((A_{520} - A_{700})\) pH 4.5; MW (molecular weight) = 449.2 g mol\(^{-1}\) for cyanidin-3-glucoside; DF = dilution factor; \(1 = \) pathlength in cm; \(\varepsilon = 26,900\) molar extinction coefficient (Fawole et al., 2012). All analyses were done as 4 replicates \((n = 4)\) and values are presented as mean ± S.D.

2.7. Microbial quality

All samples were analyzed for numbers of aerobic mesophilic bacteria, yeasts and moulds by total plate count method. For aerobic mesophilic bacteria count plate count agar (PCA) was used and for the yeast and mould counts, potato dextrose agar (PDA) acidified with 10% tartaric acid. Ten grams of each sample was obtained aseptically and homogenized with 90 mL of sterile physiological solution (PS). Furthermore, 3-fold dilutions were prepared using 1.0 mL of diluents into 9.0 mL of PS. In order to enumerate microbial load, 1.0 mL of each dilution was pour-plated in triplicate onto appropriate media, PCA for aerobic mesophilic bacteria and PDA for yeast and moulds. Plates for aerobic mesophilic bacteria were incubated at 37°C for 2 days and at 25°C for 3–5 days for yeast and moulds. After incubation, plates with 15–300 colonies were counted. Microbial data were transformed into logarithms of the number colony forming units (log CFU g\(^{-1}\)).

2.8. Extraction procedure of volatile compounds and chromatographic analyses

Approximately 5 mL of aliquots of pomegranate juice were taken from samples thawed overnight at refrigerating temperature and were placed in 20 mL SPME vials. These aliquots were mixed with equal amounts of 30% NaCl to inhibit enzymatic degradation and facilitate the movement of volatiles into the headspace. The aroma volatiles were trapped and extracted from the vial headspaces an SPME method described before by Melgarejo et al. (2011) and Mayuonyi-Kirshinbaum et al. (2012). The vials were allowed to equilibrate for 10 min at 50°C in the CTC autosampler incubator and after this equilibration time, a 50/30 μm divinylbenzene/carboxen/polydimethylsiloxane coated fibre was exposed to the headspace for 20 min at 50°C. After extraction, desorption of the volatile compounds from the fibre coating was carried out in the injection port of the gas chromatography–mass spectrometry (GC–MS) during 2 min in splitless mode and then 8 min in split mode to clean fibre. The temperature of the injection was maintained at 250°C.

Separation of the volatile compounds was performed on a gas chromatograph using Agilent 6890 N (Agilent, Palo Alto, CA), coupled with an Agilent mass spectrometer detector Agilent 5975 MS (Agilent, Palo Alto, CA). The GC–MS system was equipped with an Rtx®-5Sil MS, with a 95% polydimethylsiloxane/5% polyphenylsiloxane stationary phase and the dimensions were 30 m length; 0.25 mm inner diameter; and 0.5 μm film thickness. Analyses were carried out using helium as carrier gas with a flow of 1.2 mL min\(^{-1}\). The injector temperature was maintained at 250°C. The oven temperature was as follows: 40°C for 2 min; and then ramped up to 250°C at 5°C min\(^{-1}\) and held for 5 min. The MSD was operated in full scan mode and the ion source and quadrupole were maintained at 240°C and 150°C, respectively. The transfer line temperature was maintained at 280°C. Where authentic standards were available, compounds were tentatively identified by comparison of retention times (RI); Kovats retention indices (KI); and, by comparison with mass spectral libraries (NIST, version 2.0). For quantification, the calculated relative abundances were used.

2.9. Statistical analysis

The experimental data obtained were treated with one-way analysis of variance (ANOVA) at 95% confidence interval to evaluate the effect of amount of pomegranate arils inside the MAP, storage time, temperature and their interaction on the quality attributes. Least significant difference (LSD) and Tukey post hoc tests were performed to identify specific differences in factor levels. All experimental data were analyzed using Statistical software (Statistical 10.0, Statsoft, USA).

3. Results and discussion

3.1. Package headspace gas composition

Headspace O\(_2\) content significantly decreased over time inside packages at the different storage temperatures, reaching an equilibrium concentration, although the final concentration of O\(_2\) was different for each temperature (Fig. 1a). Oxygen composition went below 2% in packages stored at 10 and 15°C after days 10 and 7, respectively, while samples at 5°C did not reach below 2% throughout the study. According to Soliva-Fortuny et al. (2004), a decrease in O\(_2\) level below a fermentative threshold limit of <2% could induce anaerobic respiration, which results in the production of off-flavours and -odours. On the other hand, CO\(_2\) levels increased significantly during storage for all packaging conditions; however, the increase was highest at 15°C (Fig. 1b). At the end of the storage
at 5 °C, O₂ and CO₂ concentrations reached approximately 4.8 and 27.8%, respectively. This observation suggests that a rapid increase in CO₂ can indicate the end of product shelf-life, and also highlights the need for a polymeric film with higher permeability for CO₂ in order to avoid accumulation inside the package.

3.2. Changes in physical attributes

Pomegranate aril weight constantly decreased with time at all storage temperatures after day 3 (Fig. 2). Weight loss did not exceed 0.53 and 0.79% for ‘Acco’ and ‘Herskawitz’ at 5 °C; 0.70 and 0.91% at 10 °C; and 2.14 and 1.94% at 15 °C. MAP samples at 5 and 10 °C showed an initial increase in weight at the early stage of storage. The observed increase in weight could be associated with the evaporation of moisture from the aril surface and initial condensation inside the package. Gil et al. (1996) reported a weight loss of 0.52, 0.56 and 0.70% for chlorine-treated pomegranate arils cv. ‘Mollar Elche’ stored at 1, 4 and 8 °C, respectively, and weight loss of 0.68, 0.84 and 0.72% for those treated with chlorine plus antioxidant at 1, 4 and 8 °C stored for 7 days. The use of polymeric films in MAP serves as a mechanical barrier to the movement of water vapour and this helps to maintain a high level of RH within the package, and reduce produce weight loss (Suparlan and Itoh, 2003).

Firmness at day 0 was 76.10±5.1 N and 85.55±8.4 N for cv. ‘Acco’ and ‘Herskawitz’, and did not exceed 77.50±7.4 N and 102.36±7.6 N, respectively, at day 14 as shown in Supplementary Table 1. Storage temperature, time, and their interaction, had no significant effect on aril firmness as storage progressed (p<0.05). This observation is similar to that in a report by Ayhan and Eştürk (2009), who observed slight or no significant change in firmness for ‘Hicaznar’ pomegranate arils stored in passive-MAP or active-MAP until day 15 at 5 °C.

With regards to colour characteristics of pomegranate arils, the average L* values measured ranged from 39.55 to 26.22 for ‘Acco’ and 41.58 to 30.03 for ‘Herskawitz’, while a* ranged from 29.29 to 19.30 for ‘Acco’ and 33.95 to 22.60 for ‘Herskawitz’, and, b* ranged from 19.66 to 12.28 for ‘Acco’ and 18.98 to 12.90 for ‘Herskawitz’ across all storage conditions. Comparison of the two cultivars showed that ‘Herskawitz’ had better colour stability than ‘Acco’, based on the overall analysis of variance (ANOVA) and Tukey Post hoc test. Storage time had no significant effect on colour parameters L* (lightness), a* (redness) and b* (yellowness) (p>0.05) which are commonly used in industry as indicators of colour stability (Supplementary Table 2). The non-significant or fluctuating effects of passive MAP and temperature on colour parameters agrees with data reported for other minimally processed pomegranate arils stored under MAP conditions (Gil et al., 1996; Sepővéda et al., 2000; Ayhan and Eştürk, 2009). Gil et al. (1996) reported a relatively small change in L* values for ‘Mollar’ arils packed in oriented polypropylene (OPP) bags stored at 8.4 and 1 °C for 7 days. Sepővéda et al. (2000) observed no colour change in minimally processed ‘Wonderful’ arils stored at 4 °C ± 0.5 in semi-permeable films for 14 day. Ayhan and Eştürk (2009) reported that MAP application or storage time had no significant effect on redness a* and yellowness of b*, but observed small fluctuations throughout the 18 days of storage at 5 °C.

3.3. Changes in pH, total soluble solids, total titratable acidity and total anthocyanin content

A comparison of both cultivars showed that ‘Herskawitz’ had a significantly higher TA and pH than ‘Acco’, while ‘Acco’ had a relatively higher TSS. The TSS/TA ratio was influenced by storage duration and temperature (Supplementary Table 3). The interaction of storage temperature and time had a significant effect on all chemical quality parameters evaluated (p<0.05). There was a significant decrease in TA on day 3 (p<0.05), although it stayed relatively unchanged for the rest of the storage period but was significantly higher than day 3. The decrease observed in acidity reported on day 3, could be related to initial response and metabolic activities of the arils during storage. Our findings corroborate other reports on the effect of packaging on the stability of chemical attributes of pomegranate arils (Artés et al., 2000; Ayhan and Eştürk, 2009). Artés et al. (2000) reported in their study, that at the end of the shelf-life, all MAP treatments maintained or had an increase in pH values, except for samples stored in perforated PP at 5 °C, which had lower pH values. Ayhan and Eştürk (2009) also observed little change in chemical quality of minimally processed pomegranate arils stored under modified atmosphere conditions. The variability of pH, TSS, and TA values found in the studies could be explained by several factors such as cultivar differences and the relative solubility effect of CO₂ in water molecules surrounding the freshly packed pomegranate arils (Caleb et al., 2012).

There was a significant effect of storage temperature and duration, as well as their interaction on the total anthocyanin content (p<0.05). A general trend of a decrease in total anthocyanin content was observed as the storage time increased for all treatments (Supplementary Table 3). Total anthocyanin content was within the range of 21.13 to 13.32 mg C3G per 100 mL−1 of pomegranate juice for ‘Acco’, and 20.42 mg C3G per 100 mL−1 to 12.32 mg C3G per 100 mL−1 for ‘Herskawitz’. Ayhan and Eştürk (2009) also reported that the total anthocyanin content of pomegranate arils of ‘Hicaznar’ were significantly influenced by packaging, storage time, and their interaction. The total content of anthocyanin reported in this study was lower than that reported by Ayhan and Eştürk (2009), for pomegranate ‘Hicaznar’ (31.13–26.53 mg C3G per 100 mL−1). This is probably due to differences in cultivar and agro-climatic regions.

3.4. Microbial quality

Total aerobic mesophilic bacterial and fungal counts remained below detection limits until days 10 and 7 of storage at 5 °C, respectively, for both cultivars. Yeast and mould counts were higher than...
bacterial counts at all storage conditions. Yeast and mould counts were in the range of 0.36–2.17 log CFU g\(^{-1}\) for ‘Herskawitz’ and 1.76–2.59 log CFU g\(^{-1}\) for ‘Acco’ after 14 days of storage at 5 °C. The aerobic mesophilic bacterial counts were in the range of 1.10 and 1.73 for ‘Herskawitz’ and 1.76–2.41 log CFU g\(^{-1}\) for ‘Acco’ after 14 days of storage at 5 °C (Fig. 3). The higher counts of yeasts and moulds with a shorter lag phase found in this study may be attributed to the fact that yeasts and moulds are capable of growing at lower pH in comparison with aerobic mesophilic bacteria (Suárez-Jacobo et al., 2010; Varela-Santos et al., 2012). These findings agree with the report by Varela-Santos et al. (2012), who observed that aerobic mesophilic bacterial counts were lower than yeast and mould counts in untreated ‘Wonderful’ pomegranate juice. Although, the highest yeast and mould counts in all passive MAP applications were fewer than 5 log CFU g\(^{-1}\), which is the maximum limit for yeasts and moulds in raw and fresh-cut fruit by the South African legislation (FCD, Act 54 1979). However, at days 10, 7, and 3, fermentative headspace gases resulting in off-odours were observed at 5, 10 and 15 °C, respectively. Furthermore, the lower microbial counts observed for ‘Herskawitz’ in comparison with ‘Acco’ may be attributed to differences in chemical characteristics of the two pomegranate cultivars. For instance, ‘Herskawitz’ has higher TA and pH than ‘Acco’, and this can influence microbial growth. Soliva-Fortuny and Martín-Belloso (2003) reported that fruit physicochemical properties such as pH and TA have an important effect on microbial shelf-life of fresh-cut fruit.

### 3.5. Volatile composition and changes with time

Using GC–MS analysis of pomegranate juice HS-SPME extract, a total of 18 and 17 volatiles were detected for ‘Herskawitz’ and ‘Acco’, respectively. In general, temperature and storage duration had significant effects on the production of volatiles \(p > 0.05\). The cultivars differed quantitatively in aroma compounds; however, they exhibited the same volatile profiles which were categorized into primary and secondary volatiles. Primary volatiles were identified in day 0 (fresh samples), while secondary volatiles changed over the storage period at different temperatures. The volatile compounds found in pomegranate juices can be grouped into 7 chemical classes: (a) monoterpenes; limonene; (b) monoterpenes alcohols; terpinen-4-ol, α-terpineol; (c) aldehydes; benzenacetaldehyde; (d) ketones: 2-octanone, 2-nonanone and 2-undecanone; (e) alcohols: trans-3-hexen-1-ol, 1-hexanol, 2-phenylethanol and 2-nonanol; (f) esters: 3-methyl-1-butanol acetate, cis-3-hexenyl acetate, hexyl acetate, 2-phenylethyl acetate, octanoic acid-, decanoic acid- and dodecanoic acid–ethyl ester; and (g) sesquiterpenes: trans-α-bergamotene (Table 1). The most abundant volatiles in both cultivars were trans-3-hexen-1-ol, 1-hexanol, 3-methyl-1-butanol acetate, hexyl acetate and 2-octanone (only in ‘Acco’). Several of the other volatiles identified in both cultivars were present in very low concentrations (%), for example limonene, benzeneacetaldehyde, α-terpineol, and 2-nonanone (Supplementary Table 4).

The concentration of ketones decreased over time during storage. Concentrations of aldehydes, alcohols and esters decreased in the following order during the storage period: aldehydes < alcohols < ester. It is well known that most types of fruit have the ability to metabolize aldehydes into alcohols, and then into their corresponding esters during ripening as well as storage (Dixon and Hewett, 2000; Pelayo et al., 2003; Vazquez-Cruz et al., 2012). For instance, Pelayo et al. (2003) reported a decrease in the level of aldehydes and alcohols at the end of postharvest life of CO\(_2\)-stored ‘Aromas’ and ‘Diamante’ strawberries with a notable increase in the concentration of ethyl esters. This was also the finding in this study, where concentration and composition of ethyl esters increased with the storage period. For example, 2-phenylethyl acetate, octanoic acid ethyl ester and decanoic acid ethyl ester were detected from day 3 and in all storage conditions and durations, with 2-phenylethyl acetate exhibiting the highest concentration among all esters. A higher level of ethyl esters was observed in ‘Acco’ in comparison to ‘Herskawitz’, as well as perception of off-flavours when packages were opened at day 10 of...
Table 1
Identification and characteristics of volatile compounds found in juices of fresh and packaged pomegranate arils.

<table>
<thead>
<tr>
<th>Classes</th>
<th>Volatile compound(s)</th>
<th>RT (min)</th>
<th>Kovats index</th>
<th>Descriptor*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monoterpenes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aldehydes</td>
<td>Limonene*</td>
<td>13.70</td>
<td>1020</td>
<td>Mild, citrus, sweet, orange, lemon</td>
</tr>
<tr>
<td></td>
<td>Benzeneacetaldehyde*</td>
<td>14.16</td>
<td>1036</td>
<td>Honey, sweet, flowery</td>
</tr>
<tr>
<td>Monoterpenoids</td>
<td>4-L-Terpine-4-ol*</td>
<td>18.41</td>
<td>1137</td>
<td>Must, turpentine, nutmeg</td>
</tr>
<tr>
<td></td>
<td>α-Terpinol*</td>
<td>18.82</td>
<td>1169</td>
<td>Lilac</td>
</tr>
<tr>
<td>Alcohols</td>
<td>trans-3-Hexen-1-ol*</td>
<td>8.26</td>
<td>814</td>
<td>Plant, fruity, aromatic</td>
</tr>
<tr>
<td></td>
<td>1-Hexanol*</td>
<td>8.63</td>
<td>831</td>
<td>Mint, grass</td>
</tr>
<tr>
<td></td>
<td>2-Phenylethanol*</td>
<td>16.33</td>
<td>1081</td>
<td>Flowery, roses</td>
</tr>
<tr>
<td></td>
<td>2-Nonanol*</td>
<td>15.93</td>
<td>1087</td>
<td>Cucumber</td>
</tr>
<tr>
<td>Esters</td>
<td>3-Methyl-1-butanol acetate*</td>
<td>8.84</td>
<td>850</td>
<td>Fruity, sweet-like, banana</td>
</tr>
<tr>
<td></td>
<td>cis-3-Hexenyl Acetate*</td>
<td>12.88</td>
<td>986</td>
<td>Fresh, leafy, green, vegetable</td>
</tr>
<tr>
<td></td>
<td>Acetic acid, hexyl ester*</td>
<td>13.10</td>
<td>997</td>
<td>Apple, cherry, floral, pear</td>
</tr>
<tr>
<td></td>
<td>Ethyl hexanoate*</td>
<td>12.68</td>
<td>984</td>
<td>Fruity, candy</td>
</tr>
<tr>
<td></td>
<td>Octanoic acid, ethyl ester*</td>
<td>18.76</td>
<td>1183</td>
<td>Fruity, fresh, sweet-like</td>
</tr>
<tr>
<td></td>
<td>2-Phenylethyl acetate*</td>
<td>20.48</td>
<td>1224</td>
<td>Flowery, fruity, cooked apple,</td>
</tr>
<tr>
<td></td>
<td>Decanoic acid, ethyl ester*</td>
<td>24.25</td>
<td>1383</td>
<td>Rancid</td>
</tr>
<tr>
<td></td>
<td>Dodecanoic acid, ethyl ester*</td>
<td>29.17</td>
<td>1581</td>
<td>Dry, metallic</td>
</tr>
<tr>
<td>Ketones</td>
<td>1-Octanone*</td>
<td>12.40</td>
<td>977</td>
<td>Cheesy, green, fruity, dairy, buttery</td>
</tr>
<tr>
<td></td>
<td>2-Nonanone*</td>
<td>15.59</td>
<td>1069</td>
<td>Cheesy, green, fruity, dairy, buttery</td>
</tr>
<tr>
<td></td>
<td>2-Undecanone*</td>
<td>21.52</td>
<td>1274</td>
<td>Cheesy, green, fruity, dairy, buttery</td>
</tr>
<tr>
<td>Sesquiterpenes</td>
<td>trans-α-Bergamotene*</td>
<td>25.33</td>
<td>1434</td>
<td>Woody, terpene-like</td>
</tr>
</tbody>
</table>

* Primary detected volatiles.
* Secondary detected volatiles.
* Only detected in ‘Herskawitz’.
* Only detected in ‘Acco’.

Storage. Enhanced synthesis of ethyl esters requires a fermentation process in order to supply high amounts of alcohol precursor.

According to Purvis (1997), fermentative metabolism can be enhanced in fruit via various stress factors such as extrinsic (temperature, hypoxic conditions), intrinsic (ripening, senescence) and biotic (microbial growth) factors. In this study we observed that increased temperature and microbial growth were correlated with the production of ethyl esters after day 3 of storage. Microorganisms have been reported to synthesize high levels of ethyl esters and alcohols on fresh or processed food (Longo and Sanromán, 2006; Deetae et al., 2007). In addition, enhanced production of ethyl esters exhibits an increased activity of alcohol acyltransferase enzyme (AAT), which promotes the last stage in biosynthesis of esters (Zhu et al., 2008). Therefore, the ability to maintain the original volatile profile during MAP storage depends on maintaining strict optimal cold chain and processing hygiene to reduce microbial load and the ability of pomegranate cultivars to maintain a reduced rate of fermentative metabolism. ‘Acco’ pomegranate arils did not maintain a low rate of this metabolic pathway compared to ‘Herskawitz’. This observation highlights the need for precise definition of stored produce flavour life (Pelayo et al., 2003), and offers the possibility to use volatile production in MA-packaged fresh/fresh-cut material as an indicator for intelligent packaging.

Furthermore, the esters had the highest percentage composition and representation among the isolated, identified and quantified groups of volatiles which evolved as secondary volatiles. The
findings in this study is in contrast with others in the literature by Calín-Sánchez et al. (2011), Melgarejo et al. (2011) and Mayoumi-Kirshinbaum et al. (2012). For instance, Calín-Sánchez et al. (2011) and Melgarejo et al. (2011) identified 18 and 21 aroma volatiles, respectively, in juices of nine different Spanish pomegranate cultivars. The most abundant of these volatiles were limonene, hexanal, cis-3-hexenol and trans-2-hexenal. Mayoumi-Kirshinbaum et al. (2012) reported that majority of aroma compounds in ‘Wonderful’ pomegranate were terpenes and aldehydes. These differences could be associated with cultivar, influence of agro-climatic regions on the fruit as well as the extraction methods used and retention time reported. However, the observation in this study is principally due to the influence of minimal processing, storage condition and duration on fresh pomegranate arils, which was not included in the studies conducted by Calín-Sánchez et al. (2011), Melgarejo et al. (2011) and Mayoumi-Kirshinbaum et al. (2012).

Flavour life can be defined as the maximum period of storage in which fruit maintain a similar flavour profile to that detected in freshly harvested fruit (Pelayo et al., 2003). However, the described changes in aroma compounds during MAP storage created new profiles that could influence flavour perception. Thus, flavour life could be described based on the compounds with the highest concentration/odour threshold that contributes to the global odour of a given food (Alonso et al., 2009). Based on the development of off-flavours and correlations found between the increasing levels of ethyl esters, changes in volatile composition and microbial growth during MAP storage (Figs. 4 and 5), both cultivars exhibited a shorter flavour life (7 days) than postharvest life (10 days). Therefore, changes in headspace volatile composition could serve as an indicator of microbial stability and postharvest shelf-life for MA-packaged pomegranate arils. However, additional sensory evaluations are needed to confirm this observation.

4. Conclusion

Temperature had a significant effect on changes on volatile profile concentration and composition. Changes in quality attributes and aroma compounds were dependent on cultivar differences, and storage temperature and duration. Under 5 °C storage conditions, MA-packaged ‘Acco’ and ‘Herskawitz’ pomegranate arils were best kept than at 10 and 15 °C. This was evident from the extension of the postharvest life based on physicochemical properties and the inhibition of microbial growth at the lowest storage temperature in the two cultivars. This shows the importance of maintaining an optimal cold chain in postharvest handling of fresh/fresh-cut produce. Flavour life was shorter than the postharvest life and was significantly influenced by storage temperature. Additional sensory evaluations are needed to confirm this report. This further highlights the need for a paradigm shift from traditional food quality attributes to flavour assessment. Although this is difficult to establish, due to cultivar differences, a more precise definition of flavour shelf-life is required for MA-packaged pomegranate arils. This could be achieved by considering recommended levels of flavour components in order to ensure acceptable flavour. Further research is warranted in this area, especially given the importance of flavour in consumer perception of quality and purchase of pomegranate arils and other fresh produce.

Acknowledgments

This work is based upon research supported by the South African Research Chairs Initiative of the Department of Science and Technology and National Research Foundation. The authors are grateful to the South African Postharvest Innovation Programme (PHI-2), CitroGold Ltd., and South African Perishable Products Export Control Board (PPECB) for financial support. Mr. Fan Olivier of Houdconstant Pack-House, Porterville for assistance with fruit procurement, processing and packaging.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.postharvbio.2013.01.006.

References


