Characterising water loss in pomegranate fruit cultivars (‘Acco’, ‘Herskawitz’ & ‘Wonderful’) under cold and shelf storage conditions

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Abstract

Fruit water loss results in a huge financial loss to the industry due to loss of aesthetic appeal and direct loss of saleable fruit weight. There is currently a limited knowledge on the mechanism of water loss in pomegranate fruits, given their complex structure. Therefore, this study aimed to characterise water loss in the most common export pomegranate cultivars (‘Acco’, ‘Herskawitz’ and ‘Wonderful’) of South Africa. Fruits were stored for 42 d at 7 °C and 90 % RH and thereafter transferred to shelf at 23 °C and 58 % RH. Another batch of fruit was immediately stored under prolonged shelf conditions for 16 d. Water loss, respiration rate, arils-peel proportions and moisture content, peel thickness and colour attributes, puncture resistance property and chemical quality attributes of fruit juice were measured. The study revealed that despite the physiological and structural differences among pomegranate cultivars, water loss was similar during the 42 d of cold storage. However, the medium-sized fruits (‘Herskawitz’ and ‘Wonderful’) had significantly higher water loss (0.32 ± 0.01 g cm\(^{-2}\)) than the small-sized fruits
(‘Acco’) (0.25 ± 0.01 g cm⁻²) during the prolonged 16 d of shelf storage. The observed maximum water loss of 24.2 % is mainly from the peel proportion. Therefore, research should primarily focus on the peel fraction in addressing the water loss problems of pomegranate fruits.

**Keywords:** Punica granatum, Weight loss, Transpiration, Water relations, Postharvest quality, Fruit physiology.

1 **Introduction**

Pomegranate fruit (Punica granatum L.) is a fruit of old, native to the region between Iran and Himalayans in northern India and has over 500 cultivars. Current global production is estimated at three million tons annually with major cultivation carried out in the Northern Hemisphere and Europe providing the largest global market. Though the pomegranate grows favourably under the Mediterranean climate, it is highly adaptable to various climates and therefore importantly cultivated in the sub-tropical and tropical regions. The wide knowledge and increasing public awareness about the health benefits associated with pomegranates have tremendously increased consumption especially in the western part of the world. Particularly, there has been a growing demand for high quality, healthy and exotic fruits both for fresh use and local processing into juices and other products. This has created an opportunity for countries in the Southern Hemisphere including South Africa to export their pomegranates to Europe during the counter off-season in the Northern Hemisphere. Therefore, there is increasing research interest focusing on maintaining pomegranate fruit quality throughout the supply chain: harvesting, packaging, transportation, storage and marketing.

Pomegranates are highly perishable fruits despite having a relatively lower respiration rate. Particularly, pomegranate fruits are prone to moisture loss due to the plentiful micro-pores and slits in the skin, despite having a thick rind. Research showed that cultivars such as
‘Bhagwa’, ‘Ruby’ and ‘Wonderful’ can lose 20-25% of the initial fruit weight within 4 weeks at temperature and relative humidity of 22 °C and 65% 13,17. During prolonged cold storage, the fruits ‘Bhagwa’, ‘Ruby’ and ‘Wonderful’ lose between 10 - 16% of their weight within 12 weeks at 5 - 7 °C and 90 - 95% RH 13,17-19. A weight loss above 5% causes shrivelling 14,20. Even in the absence of any visible shrivelling, water loss can undesirably affect the visual appearance, flavour and textural properties of the fruits 21. Excessive water loss results into browning of the peel and arils and hardening of the rind 12,22. It is important to note that pomegranates are luxurious fruits that sell well in the higher market segment 4. Therefore water loss can easily cause a huge financial loss to the industry through direct loss of marketable fresh weight and the associated diminished commercial value of affected fruits 22. Various water loss control techniques have been presented and investigated by many researchers. Storage temperature and relative humidity are important water loss control parameters 13,23. Plastic liners and modified atmosphere packaging 18,19,24–28, individual shrink wrapping 6,19,29, waxing and surface coatings 6,15,30. These techniques have been applied with great success in minimising the loss of water. However, if not properly used, shrink wrapping and surface coating/waxing can cause anaerobic respiration which leads to the production of off flavours 31,32 while plastic liners cause moisture condensation within the bags promoting fruit decay 18. Therefore, there is a need to improve the control techniques which to some extent is hampered by the limited knowledge on the characteristics of water loss of pomegranate fruits, given their complex structure. Hence, this study aimed to characterise the water loss of pomegranate fruits based on the fundamental physical and physio-chemical attributes. Secondly, the susceptibility of pomegranate fruit cultivars to water loss was assessed and the contribution of the different parts of the fruit to the water loss was examined. The study was done on the most important export
pomegranate cultivars (‘Acco’, ‘Herskawitz’ and ‘Wonderful’) of South Africa, under cold storage and shelf conditions.

2 Materials and methods

2.1 Fruit acquisition

Pomegranate fruit (*Punica granatum* L.) of cultivars ‘Acco’, ‘Herskawitz’ and ‘Wonderful’ at commercial maturity (early morning harvest) were obtained from a commercial orchard located at Porterville, Wellington (33° 38’ S, 19° 00’ E), Western Cape Province, South Africa. The fruits were packed in ventilated plastic trays cushioned with paper pads and transported using an air-conditioned refrigerated truck to the postharvest research laboratory, Stellenbosch University. Sorting was carried out to ensure size uniformity and that the fruits were free from surface defects such as cracks. Fruits were packed in dozens inside single layer display type corrugated fibreboard carton, cushioned with paper trays at the bottom.

2.2 Experimental design

A total of 84 fruits (seven cartons) for each of the three cultivars (‘Acco’, ‘Herskawitz’ and ‘Wonderful’) was used in the study. Twelve fruits (replicates) were used to assess the initial quality before storage and the remaining 72 fruits were stored in two batches each of 36. Batch 1 fruits were stored at 7 °C and 90 % RH for 42 d and thereafter transferred to shelf conditions of 23 °C and 58 % RH for eight days. This was to mimic the maximum sea freight duration of pomegranate fruits from South Africa to Europe across the Atlantic Ocean, followed by open shelf marketing before consumption. Twelve fruits (replicates) were selected for quality assessment after 42 d of cold storage and again after an additional eight days of shelf storage.
Batch 2 fruits were immediately stored under shelf conditions of 23 °C and 58 % RH. Then twelve fruits (replicates) were sampled for quality assessment at eight days and sixteen days of shelf storage. This procedure mimics fruits that are placed directly on open shelves for marketing.

2.3 Measurements

2.3.1 Size, weight and colour monitoring

Twelve fruits were randomly selected from each batch and labelled for weight, size and external peel colour monitoring. Measurements were taken before storage and at intervals of seven days throughout the 42 d of cold storage and afterwards at intervals of two days during the additional shelf period of eight days of Batch 1. For Batch 2, measurements were taken at two days’ interval.

The three linear dimensions of the fruit were measured using a digital Vernier calliper (Mitutoyo, Kawasaki, Japan, ± 0.01 mm). Fruit length (L) measures the longitudinal dimension (excluding the fruit calyx), while the width (W) and thickness (T) measures the dimensions on the equator (cheeks) of the fruit. Fruit weight was determined using an electronic scientific scale (Mettler Toledo, model ML3002E, Switzerland, 0.0001 g accuracy).

Fruit peel colour was monitored using a digital colourimeter (Minolta, model CR-400, Tokyo, Japan) at the same storage time interval as fruit weight and size. Follow up measurements were carried out at the same marked positions on two opposites sides of each fruit. The lightness (L*), redness (a*), yellowness (b*), hue angle (h °) and chroma (C*) colour properties were measured according to Commission Internationale de l’Eclairage (CIE), 1976.
A different set of randomly sampled fruits were cut open by hand with the aid of a sharp knife and the arils (edible portion) were separated from the peel. Peel thickness was measured using a pair of digital Vernier callipers (Mitutoyo, model CD-6 CX, Japan) of accuracy 0.01 mm. Opposite peel segments of the fruit were obtained using sharp blades. Measurements were then taken at the opposite mid-side positions of each segment, obtaining four readings from each of the twelve sampled fruits. The weight of the arils and peels from each fruit was measured using an electronic scientific scale (Mettler Toledo, model ML3002E, Switzerland, 0.0001 g accuracy) to determine their proportions.

2.3.2 Headspace gas composition

The headspace gas composition (O₂ and CO₂) was determined using a closed system. Two fruits were enclosed in an equilibrated hermetically sealed glass jar, in triplicates, for each of the storage conditions. Measurements were taken before and after two hours using a calibrated gas analyser (CheckPoint, PBI-Dansensor A/S, Denmark), for separate setups at low temperature (7 °C) and high temperature (23 °C).

2.3.3 Fruit firmness

Fruit firmness was determined as puncture resistance with a 5 mm diameter probe (GÜSS-FTA, South Africa). The probe was set to penetrate 8.9 mm into the fruit at 10 mm s⁻¹. The test was carried out on opposite sides of the fruit cheeks, and the peak force (N) required to puncture the fruit was reported as puncture resistance of 24 readings (2 × 12 fruits (replicates)).

2.3.4 Moisture content and chemical attributes

Moisture contents of the arils and peel fractions of the fruits were determined by a drying oven method. The samples were dried at 105 ± 0.5 °C for 24 h in a preheated oven (Model 072160,
Prolab Instruments, Sep Sci., South Africa) to achieve a constant weight. The tests were

135 carried out in five replications.

136 Fresh juice was extracted from the arils using a blender (Mellerware, South Africa) with a pre-
137 fitted screen for filtering. Total soluble solids (TSS) of the fruit juice was measured using a
digital refractometer (Atago, Tokyo, Japan). Titratable acidity (TA) was determined
140 potentiometrically, where 2 ml of pomegranate juice (PJ) in 70 ml of distilled water was titrated
141 with 0.1N NaOH to an endpoint of pH 8.2 using a compact auto titrosampler (Metrohm 862,
142 Herisau, Switzerland). Titratable acidity was expressed in milligrams of citric acid (CA) per a
143 hundred millilitres of juice.

2.4 Calculations

2.4.1 Fruit surface area

Fruit surface area (equation 1) was calculated from the fruit geometric mean diameter (equation

2) according to Dhineshkumar et al. 36.

\[
A = \pi (D_g)^2
\]  

(1)

\[
D_g = (LW T)^{1/3}
\]  

(2)

Where \(A\) (cm²) is the surface area and \(D_g\) (cm) is the geometric mean diameter of the fruit,

calculated from the length (L (cm)), width (W (cm)) and thickness (T (cm)) of the fruit.

2.4.2 Water loss

Cumulative water loss was calculated with respect to the unit fruit mass (equation 3) and with
respect to the unit surface area (equation 4) because of the variability in fruit size among

cultivars.
\[ WL = \frac{(m_i - m_t)}{m_i} \times 100 \]  
\[ WL_A = \frac{(m_i - m_t)}{A} \]  

Where; \( WL \) is water loss per unit fruit mass (%), \( WL_A \) is water loss per unit surface area of the fruit (g cm\(^{-2}\)), \( m_i \) (g) is the initial fruit mass, \( m_t \) (g) is the mass of the fruit after storage days.

### 2.4.3 Respiration rate

The respiration rate (\( RR \)) was calculated in terms of carbon dioxide production rate (\( R_{CO_2} \)) in mL kg\(^{-1}\) h\(^{-1}\) by fitting experimentally obtained data into equation (5) \(^{33}\).

\[ R_{CO_2} = 10 \times \frac{V_f}{m} \times \left( \frac{C_{CO_2t} - C_{CO_2i}}{t - t_i} \right) \]  

Where \( C_{CO_2t} \) and \( C_{CO_2i} \) are concentrations (%) of CO\(_2\) at a time \( t \) (h) and initial time \( t_i \) (h), respectively. In this study \((t - t_i)\) is constant and equals to 2 h. \( V_f \) is the free volume (mL) in the jar which is the total volume minus the volume occupied by the fruit and \( m \) (g) is the mass of the fruit inside the jar, the constant 10 is a unit conversion factor (g kg\(^{-1}\)).

### 2.4.4 Colour attributes

The colour intensity describing the length of the colour vector in the \( a^* - b^* \) plane was calculated and expressed as chroma (\( C^* \)) using equation (6) \(^{22}\) while the hue angle of the colour vectors and the total colour difference (\( TCD \)) were given by equations (7) and (8), respectively.

\[ C^* = \sqrt{a^{*2} + b^{*2}} \]  
\[ h^o = \tan^{-1}\left(\frac{b^*}{a^*}\right) \]  
\[ TCD = \left( (L^* - L^*)^2 + (a^* - a^*)^2 + (b^* - b^*)^2 \right)^{1/2} \]
Where $L^*$, $a^*$ and $b^*$ are the reference values and $L^*$, $a^*$ and $b^*$ are the respective values of lightness, redness and yellowness colour parameters at a given time.

### 2.4.5 TSS/TA and BrimA

The balance between sweetness and sourness of the pomegranate juice was estimated inform of TSS/TA and BrimA which have been reported to influence consumer acceptability. BrimA was calculated using equation (9).

$$BrimA = ^{°}Brix - k \times TA$$

where $k$ is a constant that ranges from 2 -10, depending on acid and sugar proportions. The $k$ value of 2 was used to avoid a negative BrimA index.

### 2.5 Statistical analysis

Measured and calculated data on fruit physical and physio-chemical attributes were analysed using Statistica software (Statistica 14.0, Statsoft, USA). The data was also subjected to analysis of variance (ANOVA) to assess the main effects of cultivar and storage duration. A Duncan’s Multiple Range Test was carried to test for statistical significance at $p < 0.05$. Principal component analysis (PCA) and Pearson correlation test were carried out using XLSTAT software (version 2019.1, Addinsofr, France) to assess the variability and to establish relationships among quality parameters.

### Table 1 Size differences in pomegranate fruit cultivars.

$D_g$ is the geometric mean diameter a function of the length ($L$), width ($W$) and thickness ($T$) of the fruit. Values are means ± standard deviation of $n=12$ fruits.

<table>
<thead>
<tr>
<th>Fruit cultivar</th>
<th>Mass (g)</th>
<th>$W$ (cm)</th>
<th>$T$ (cm)</th>
<th>$L$ (cm)</th>
<th>$D_g$ (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acco</td>
<td>$185.71 \pm 12.99$</td>
<td>$73.36 \pm 3.18$</td>
<td>$72.62 \pm 3.25$</td>
<td>$58.67 \pm 3.65$</td>
<td>$67.86 \pm 2.94$</td>
</tr>
<tr>
<td>Herskawitz</td>
<td>$302.17 \pm 45.36$</td>
<td>$86.54 \pm 7.34$</td>
<td>$81.33 \pm 5.55$</td>
<td>$76.82 \pm 6.51$</td>
<td>$82.54 \pm 5.11$</td>
</tr>
<tr>
<td>Wonderful</td>
<td>$336.32 \pm 31.10$</td>
<td>$90.03 \pm 4.24$</td>
<td>$89.67 \pm 4.32$</td>
<td>$80.91 \pm 4.90$</td>
<td>$86.77 \pm 4.20$</td>
</tr>
</tbody>
</table>
Figure 1 Weight loss profile of pomegranate fruit cultivars ('Acco', 'Wonderful' and 'Herskawitz') expressed per unit fruit mass (WL) and per unit surface area (WLA) during storage: (a and d) for 42 d at 7 °C / 90 % RH, (b and e) followed by additional 8 d of shelf storage at 23 °C / 58 % RH and (c and f) under immediate prolonged shelf storage of 16 d at 23 °C / 58 % RH. The data points are means (n = 12) and the vertical lines represent standard error of the mean. The lines in d, e and f are predictive trend lines fitted on the experimental data. Numerical values of A and B are p-values.
3 Results and discussion

3.1 Fruit size and water loss

The three cultivars differed in mass, specific size dimensions along their $L$, $W$, $T$ and overall geometric mean diameter $D_g$ (Table 1). Generally, ‘Acco’ was the smaller-fruit cultivar compared to ‘Herskowitz’ and ‘Wonderful’. It is important to note that fruit size influences the overall surface area to volume ratio and therefore the rate of water loss to the surrounding environment.

The water loss profiles of the three pomegranate fruit cultivars are presented in Figure 1. Generally, water loss per unit fruit mass ($WL$) was not significantly different ($P > 0.05$) among the three cultivars during cold storage (Figure 1a) and the additional days of shelf storage (Figure 1b), despite higher water loss in ‘Herskowitz’ than in ‘Acco’ and ‘Wonderful’. On average, water loss reached to 8.74 % at 42 d of cold storage. The subsequent eight days of shelf storage subjected the fruits to an extra 9.04 % water loss. Similarly, there was no significant difference among cultivars during the 10 d shelf storage at which water loss reached an average value of 16.8 %. Afterwards, water loss became significantly higher in ‘Herskowitz’ than in ‘Acco’ (Figure 1c). Weight loss was also observed to be cultivar indifferent by Al-Mughrabi et al. [38] between ‘Taeifi’, ‘Banati’ and ‘Manfaloti’ cultivars of pomegranate throughout the cold storage at different temperatures. Furthermore, Fawole and Opara [17] observed that the weight loss in ‘Ruby’ was relatively similar (20-25 %) to that in ‘Bhagwa’ cultivars of pomegranate fruit after 28 d of shelf storage at 22 °C and 65 % RH.

However, the water loss per unit surface area ($WL_A$) showed cultivar dependence during the additional shelf storage (Figure 1d-f). The $WL_A$ was significantly higher in ‘Herskowitz’ than in ‘Acco’ during the additional eight days of shelf storage (Figure 1e). Similarly, $WL_A$ was
significantly lower in fruit cultivar ‘Acco’ than in ‘Herskawitz’ and ‘Wonderful’ during the 16 d of immediate prolonged shelf storage (Figure 1f).

3.2 Respiration rate (RR)

Figure 2 summarises the results on RR across all tested conditions. RR was significantly (P < 0.05) influenced by cultivar across all storage conditions. The RR was lowest in ‘Wonderful’ than in other cultivars. At low temperatures (7 °C), a higher RR was observed in ‘Acco’ (8.268 mL Kg⁻¹ h⁻¹) and ‘Herskawitz’ (6.948 mL Kg⁻¹ h⁻¹) than in ‘Wonderful’ (3.289 mL Kg⁻¹ h⁻¹) fruit cultivars initially. The change in RR was insignificant after 42 d of cold storage at 7 °C and 90 % RH and in the subsequent shelf storage period (Figure 2a). On the other hand, the effects of cultivar, storage duration and their interaction significantly influenced the RR during the 16 d of shelf life (Figure 2b). In this case, RR at high temperatures (23 °C) decreased from 18.350, 37.936 and 30.612 mL Kg⁻¹ h⁻¹ before storage to 9.676, 20.300 and 11.740 mL Kg⁻¹ h⁻¹ in ‘Wonderful’, ‘Herskawitz’ and ‘Acco’, respectively, by the end of storage. Other studies have reported a decrease in RR of pomegranate fruits with storage duration and this could be attributed to progressive senescence of the fruits. A similar situation was observed in climacteric fruits (pear) stored at different temperature-relative humidity combinations. On the contrary, an increase of RR with storage duration has been reported in pomegranate arils, uncoated and coated fruit.
Figure 2 Changes in the respiratory carbon dioxide production rate ($R_{CO_2}$) for pomegranate fruit cultivars (‘Acco’, ‘Wonderful’ and ‘Herskawitz’) during storage: (a) for 42 d at 7 °C / 90 % RH followed by additional 8 d of shelf storage at 23 °C / 58 % RH and (b) under prolonged immediate shelf storage of 16 d at 23 °C / 58 % RH. Measurements taken at 7 °C (a) and 23 °C (b). The bars represent mean values (n=12) and the vertical lines are standard errors of the mean. Bars with different letters are significantly different (P < 0.05) and numerical values of A and B are p-values.
3.3 Peel colour of the three cultivars

Fruit peel colour influences visual appeal and acceptance of pomegranate during marketing\textsuperscript{14,31}. The three cultivars are different in peel colour. Generally, all colour parameters were significantly (P < 0.05) influenced by cultivar differences and storage duration. Lightness $L^*$ decreased with storage duration under all tested conditions and was significantly lower in ‘Herskawitz’ than in ‘Acco’ and ‘Wonderful’ at the end of fruit storage (Figure 3a-c). Similarly, peel redness $a^*$ (Figure 3d-f) and chroma $C^*$ (Figure 4a-c) decreased with storage duration and were significantly lower in ‘Wonderful’ than in other cultivars. Furthermore $a^*$ and $C^*$ were more stable with only a slight decrease during the cold storage regime as compared to a steep decline under the shelf life regime. Similar observations were made for the ‘Wonderful’ cultivar by Mukama et al.\textsuperscript{41} who observed a continuous reduction in $a^*$ and $C^*$ with storage duration for fruits under low and high relative humidity. The colour change is attributed to the degradation of colour pigments due to water stress\textsuperscript{42}. On the other hand, Arendse et al.\textsuperscript{13} observed an initial increase in $a^*$ and $C^*$ for the first 84 d followed by a decrease to the end of the 140 d of storage at 5, 7.5 and 10 °C. The difference in observations among these studies could be attributed to several pre-harvest and harvest factors such as sunlight exposure.

The three cultivars differed in hue angle $h^\circ$ (Figure 4d-f). Generally, $h^\circ$ was highest in ‘Wonderful’, followed by ‘Acco’ and least in ‘Herskawitz’. Hue angle decreased with storage time, except in ‘Wonderful’ where $h^\circ$ increased after 21 d of cold storage. Total colour difference ($TCD$) between the initial reading and at a given time during storage is presented in Figure 4g-i. $TCD$ significantly and progressively increased with storage duration and was highest in ‘Herskawitz’, followed by ‘Acco’ and least in ‘Wonderful’. This suggests that $TCD$ could be used as a good indicator for predicting storage duration for pomegranate fruits under cold and shelf conditions.
Figure 3 Variation of peel colour attributes of lightness ($L^*$) and redness ($a^*$) for pomegranate fruit cultivars (‘Acco’, ‘Wonderful’ and ‘Herskawitz’) during storage: (a and d) for 42 d at 7°C / 90% RH, (b and e) followed by additional 8 d of shelf storage at 23°C / 58% RH and (b and e) under immediate prolonged shelf storage of 16 d at 23°C / 58% RH (c and f). The data points represent mean values ($n = 12$) and the vertical lines represent standard error of the mean. Numerical values of A and B are p-values.
Figure 4 Variation in peel chroma intensity ($C^*$), hue angle ($h^\circ$) and total colour difference ($TCD$) for pomegranate fruit cultivars (‘Acco’, ‘Wonderful’ and ‘Herskawitz’) during storage: (a and d) for 42 d at 7 $^\circ$C / 90% RH, (b and e) followed by additional 8 d of shelf storage at 23 $^\circ$C / 58% RH and (c and f) under immediate prolonged shelf storage of 16 d at 23 $^\circ$C / 58% RH (c and f). The data points represent mean values ($n = 12$) and the vertical lines represent standard error of the mean. Numerical values of A and B are p-values.
3.4 Fruit firmness

Figure 5 shows the results of fruit firmness test. In general, a significant difference was observed between cultivars. Initially, firmness was significantly lowest in ‘Herskawitz’ (93.04 N) than in ‘Wonderful’ (119.32 N) and ‘Acco’ (131.21 N). The observed difference correlates negatively to the fruit size of the cultivars, with small-sized ‘Acco’ ($Dg = 7.25 \pm 0.27$ cm) having higher firmness than medium-sized ‘Herskawitz’ ($Dg = 8.45 \pm 4.92$ cm) and ‘Wonderful’ ($Dg = 9.23 \pm 0.40$ cm) fruits. These results are buttressed with findings reported by Volz et al. who observed higher firmness in apple fruits (cv. Royal Gala) from the smallest-size class than fruit from the largest-size class. This was attributed to smaller cells, less air space and greater cell packing in smaller fruits than in larger fruits. An increase in fruit firmness was observed at the end of the 42 d of cold storage followed by a decrease by the end of additional eight days of shelf life especially in ‘Acco’ and ‘Wonderful’ cultivars (Figure 5a). A quite similar situation (an increase followed by a decrease) is observed for fruits stored immediately at prolonged shelf conditions for 16 d especially for ‘Wonderful’ cultivar (Figure 5b). Arendse et al. also observed similar behaviour for pomegranate fruits stored under different temperatures ($5 – 21^\circ$ C) attributing the initial increase in puncture resistance to the decrease in the moisture content of the fruit peel resulting into toughening of the peel. The subsequent decline in puncture resistance can be attributed to the observed reduction in peel thickness and senescence of the fruits.
Figure 5 Variation in the puncture resistance force for pomegranate fruit cultivars ('Acco', 'Wonderful' and 'Herskawitz') during storage: (a) for 42 d at 7 °C / 90 % RH followed by additional 8 d of shelf storage at 23 °C / 58 % RH and (b) under prolonged immediate shelf storage of 16 d at 23 °C / 58 % RH. The bars represent mean values (n=12) and the vertical lines are standard errors of the mean. Bars with different letters are significantly different (P < 0.05) and numerical values of A and B are p-values.
3.5 Physical and chemical characteristics of fruit fractions

3.5.1 Proportions of fruit fractions

Cultivar, storage duration and their interaction significantly influenced the proportion of fruit fractions. The three cultivars differed in the proportions of arils and peel as summarized in Figure 6; 59.0 and 41.0 % in ‘Acco’, 50.1 and 49.9 % in ‘Herskawitz’ and 55.4 and 44.6 % in ‘Wonderful’, respectively before storage. However, our results are in close range with the 50.8 – 58.3 % arils and 41.7 – 49.2 % peels reported for other cultivars grown in Oman. Furthermore, the aril proportions in our study are comparable with the 55.6 % in ‘Ruby’ cultivar grown in Morocco, 58 % in ‘Ruby’, and 48.5 % in ‘Wonderful’ cultivars grown in South Africa.

Generally, there was no significant increase in the proportion of arils and a decrease in the proportion of peel fractions during the 42 d of cold storage (Figure 6a-b). However, by the end of the additional eight days of shelf life the proportion of arils fraction significantly increased with the decrease in peel proportion. A more similar scenario was observed during the 16 d of direct shelf life storage, with a higher peel proportion in ‘Herskawitz’ than in other cultivars (Figure 6c-d). Secondly, the fruit peel proportion decreased with storage time.

Despite having tough thick rind, pomegranate fruit is reported to be more susceptible to weight loss due to the numerous micro-pores on the outer peel. The findings of the current study further suggest that water loss in pomegranate fruits is primarily and majorly from the peel fraction. Fruits of the ‘Herskawitz’ cultivar had the highest percentage of peel fraction and therefore the highest water loss by the end of each storage regime within the tested conditions. These findings are in agreement with previous research and are further supported by the results on peel thickness and moisture content analysis reported in the following sections.
Figure 6 Changes in the aril and peel fractions for pomegranate fruit cultivars (‘Acco’, ‘Wonderful’ and ‘Herskawitz’) during storage: (a and b) for 42 d at 7°C / 90% RH followed by additional 8 d of shelf storage at 23°C / 58% RH and (c and d) under prolonged immediate shelf storage of 16 d at 23°C / 58% RH. The bars represent mean values (n=12) and the vertical lines are standard errors of the mean. Bars with different letters are significantly different (P < 0.05) and numerical values of A and B are p-values.

3.5.2 Peel thickness

Peel thickness measurements initially ranged from 2.66 to 4.38 mm as shown in Figure 7a-b. These values are in the range (2.68 – 4.70 mm) reported by Al-Said et al. 35 on four pomegranate cultivars grown in Oman. Peel thickness varied significantly among cultivars, greatest in ‘Herskawitz’ (4.38 mm), followed by ‘Wonderful’ (4.03 mm) and least in ‘Acco’ (2.66 mm) initially. Then it decreased with storage duration to 2.13, 1.51 and 1.51 mm, respectively, at the
end of the 42 d of cold storage and the additional eight days of shelf storage. Similarly, peel thickness reduced to 1.93, 1.74 and 1.35 mm in ‘Herskawitz’, ‘Wonderful’ and ‘Acco’, respectively after 16 d of absolute shelf storage. The thinning of the peel in time shows that water loss in pomegranate fruits comes to a great degree from the peel fraction as compared to the aril fraction.

3.5.3 Moisture content

The aril moisture content of the three cultivars was similar (81.66 ± 0.99 %) across all conditions of storage while the peel moisture content decreased with storage time (Figure 7c-f). This indicated that the peel was the main contributor to the water loss of the fruits. Interestingly, the level of water loss observed was not enough to induct any change in the aril moisture content and consequently aril weight. The total water loss of the fruits, in the range of the tested conditions, reached up to 24 %. The observed increase in the proportion of arils in the fruit is due to the reduction in the proportion of the peels. Similar to our results, Arendse et al.\textsuperscript{13} reported aril moisture content in the range of 79.74 – 85.11 % with no significant change, for pomegranate fruits (cv. Wonderful grown in South Africa) under prolonged storage for 28 d at 21 °C and 140 d at 10, 7.5 and 5 °C. However, slightly lower aril moisture content (76.01 – 79.09 %) were observed in other cultivars grown in Oman\textsuperscript{35}. These differences could be attributed to cultivar and geographical variation.

On the other hand, cultivar and storage duration and their interaction significantly influenced peel moisture content (Figure 7e-f). Initially, the peel moisture content was greatest in ‘Herskawitz’ (74.29 %), followed by ‘Wonderful’ (71.46 %) and least in ‘Acco’ (63.91 %). Furthermore, peel moisture content decreased with storage time during cold storage and shelf storage. By the end of the 42 d of cold storage plus additional eight days of shelf life, peel
moisture content had reduced to 17.18, 30.65, and 36.36 % in ‘Herskawitz’, ‘Wonderful’ and ‘Acco’, respectively.

Figure 7 Changes in the peel thickness, aril and peel moisture content for pomegranate fruit cultivars (‘Acco’, ‘Wonderful’ and ‘Herskawitz’) during storage: (a, c and e) for 42 d at 7 °C / 90 % RH followed by additional 8 d of shelf storage at 23 °C / 58 % RH and (b, d and f) under prolonged immediate shelf storage of 16 d at 23 °C / 58 % RH. The bars represent mean values.
(n=12) and the vertical lines are standard errors of the mean. Bars with different letters are significantly different (P < 0.05) and numerical values of A and B are p-values.

3.5.4 TSS, TA, TSS/TA and BrimA

Chemical attributes of TSS and TA are important in describing the sweetness and sourness of fruit juice taste, respectively \(^{35,47}\). The changes in the chemical attributes of the fruit juice with storage time are presented in Tables 2 and 3. Generally, TSS significantly varied among cultivars and was highest in ‘Wonderful’ (16.50 \(^0\) Brix) than in ‘Acco’ (14.67 \(^0\) Brix) and ‘Herskawitz’ (14.82 \(^0\) Brix). A non-significant increase of TSS was observed at the end of the 42 d of cold storage and the end of the additional eight days of shelf storage (Table 2). On the other hand, storage duration had a significant influence on the increase in TSS for batch 2 fruits stored immediately under shelf conditions (Table 3). Our results are comparable with the findings of Mukama et al. \(^{41}\) who observed a non-significant change in TSS for fruits stored under low RH (65 %) and a significant increase for fruits under high RH (95 %) at 20 °C for 30 d. The authors attributed the increase to the concentration of soluble sugars due to moisture, however, in our particular study we observed no significant change in aril moisture content. Therefore, we attribute the increase in TSS to the hydrolysis of starch and polysaccharides into soluble sugar substrates that are required for utilization in the respiration process \(^{48,49}\).

Titratable acidity (TA) was significantly influenced by cultivar effect and the interaction between cultivar and storage time. Before storage TA was lowest in ‘Acco’ (0.25 mg 100 mL\(^{-1}\)) than in ‘Heskawitz’ (1.15 mg 100 mL\(^{-1}\)) and ‘Wonderful’ (1.62 mg 100 mL\(^{-1}\)). TA increased at 42 d of cold storage followed by a decline at an additional eight days of shelf storage, except in ‘Wonderful’ where a consistent decline was observed (Table 2). It is important to note that TA was significantly lower in ‘Acco’ than in ‘Wonderful’ and ‘Herskawitz’ across all tested
conditions. Acco is generally considered as a sweet cultivar as compared to ‘Wonderful’ and ‘Herskawitz’ in the sweet-sour to the sour range. TA remained stable in ‘Herskawitz’ during the 16 d of shelf storage of Batch 2 fruits as compared to a decrease in ‘Wonderful’ and an increase in ‘Acco’ at the end of the storage period. Therefore, the different cultivars of the fruits responded differently to the storage conditions. Comparably, a decrease in TA has been reported in different cultivars of pomegranates including ‘Wonderful’, ‘Hicrannar’ and ‘Hicaznar’ under different storage conditions, attributing it to the utilization of organic acids in metabolic process. On the contrary, Mukama et al. observed an increase in TA for the ‘Wonderful’ cultivar under shelf conditions, attributing it to moisture loss from the fruits.

The effects of cultivar, storage time and their interaction significantly influenced the TSS/TA ratio which was highest in ‘Acco’ than in other cultivars across all tested conditions of storage. TSS/TA decreased at 42 d followed by an increase at additional shelf life storage for ‘Acco’ cultivar as compared to the no-significant change in ‘Herskawitz’ and a persistent increase in ‘Wonderful’. On the other hand, TSS /TA decreased in ‘Acco’ cultivar, however, remained stable in ‘Wonderful’ and ‘Herskawitz’ during the 16 d of shelf storage. Generally, fruits with a higher TSS/TA ratio are more preferred by consumers.

BrimA index is a variant of TSS/TA ratio that incorporates the tongue’s taste sensitivity and is important in assessing the effect of chemical changes on the flavour. BrimA was significantly lower in ‘Acco’ than in other cultivars, however, it was not significantly influenced by storage duration (Table 2). On the other hand, both cultivar and storage effects significantly influenced BrimA during the 16 d of shelf storage (Table 3). In this case, BrimA increased with storage time and was generally lower in ‘Herskawitz’ cultivar. Similar results are reported by Arendse et al. for fruits stored under different temperatures.
Table 2 Chemical attributes of fruit juice from pomegranate cultivars (‘Acco’, ‘Wonderful’ and ‘Herskawitz’) stored for 42 d at 7°C/90% RH followed by additional 8 d of shelf storage at 23°C/58% RH

<table>
<thead>
<tr>
<th>Storage conditions</th>
<th>Cultivar</th>
<th>TSS (°Brix)</th>
<th>TA (mg 100 mL⁻¹)</th>
<th>TSS/TA</th>
<th>BrimA</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 d</td>
<td>Acco</td>
<td>14.67 ± 0.41b</td>
<td>0.25 ± 0.02f</td>
<td>59.67 ± 4.25d</td>
<td>14.17 ± 0.41b</td>
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<tr>
<td></td>
<td>Wonderful</td>
<td>16.50 ± 0.21a</td>
<td>1.62 ± 0.04b</td>
<td>10.24 ± 0.28f</td>
<td>13.26 ± 0.22c</td>
</tr>
<tr>
<td></td>
<td>Herskawitz</td>
<td>14.82 ± 0.37b</td>
<td>1.16 ± 0.04c</td>
<td>12.89 ± 0.51d</td>
<td>12.51 ± 0.37d</td>
</tr>
<tr>
<td>42 d [7 °C]</td>
<td>Acco</td>
<td>14.99 ± 0.17b</td>
<td>0.58 ± 0.03e</td>
<td>26.60 ± 1.29c</td>
<td>13.84 ± 0.18bc</td>
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<tr>
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<td>Wonderful</td>
<td>16.75 ± 0.23a</td>
<td>1.05 ± 0.05c</td>
<td>16.16 ± 0.72de</td>
<td>14.64 ± 0.25f</td>
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<tr>
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<td>Herskawitz</td>
<td>14.84 ± 0.27b</td>
<td>1.81 ± 0.10a</td>
<td>8.32 ± 0.46f</td>
<td>11.23 ± 0.29e</td>
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<tr>
<td>42 d [7 °C] + 8 d</td>
<td>Acco</td>
<td>15.12 ± 0.20c</td>
<td>0.48 ± 0.01e</td>
<td>31.89 ± 1.08b</td>
<td>14.16 ± 0.21bc</td>
</tr>
<tr>
<td>[23 °C]</td>
<td>Wonderful</td>
<td>16.49 ± 0.15a</td>
<td>0.91 ± 0.05d</td>
<td>18.75 ± 1.13d</td>
<td>14.68 ± 0.19a</td>
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<tr>
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<td>Herskawitz</td>
<td>15.30 ± 0.15b</td>
<td>1.56 ± 0.05b</td>
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<td>12.19 ± 0.19d</td>
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P-values

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<tr>
<th>Cultivar (A)</th>
<th>P-value</th>
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<th>&lt; 0.0001</th>
<th>&lt; 0.0001</th>
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A × B 0.6294 < 0.0001 < 0.0001 < 0.0001

Table 3 Chemical attributes of fruit juice from pomegranate cultivars (‘Acco’, ‘Wonderful’ and ‘Herskawitz’) stored under prolonged immediate shelf storage of 16 d at 23°C/58% RH

<table>
<thead>
<tr>
<th>Storage conditions</th>
<th>Cultivar</th>
<th>TSS (°Brix)</th>
<th>TA (mg 100 mL⁻¹)</th>
<th>TSS/TA</th>
<th>BrimA</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 d</td>
<td>Acco</td>
<td>14.67 ± 0.41c</td>
<td>0.25 ± 0.02e</td>
<td>59.67 ± 4.25a</td>
<td>14.17 ± 0.41bcd</td>
</tr>
<tr>
<td></td>
<td>Wonderful</td>
<td>16.50 ± 0.21b</td>
<td>1.62 ± 0.04b</td>
<td>10.24 ± 0.28f</td>
<td>13.26 ± 0.22de</td>
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<tr>
<td></td>
<td>Herskawitz</td>
<td>14.82 ± 0.37c</td>
<td>1.16 ± 0.04c</td>
<td>12.89 ± 0.51d</td>
<td>12.51 ± 0.37e</td>
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<td>8 d [23 °C]</td>
<td>Acco</td>
<td>15.89 ± 0.21b</td>
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<td>Wonderful</td>
<td>17.71 ± 0.18a</td>
<td>1.91 ± 0.17a</td>
<td>9.82 ± 0.76c</td>
<td>13.89 ± 0.41d</td>
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<td>Herskawitz</td>
<td>15.90 ± 0.39b</td>
<td>1.26 ± 0.11c</td>
<td>13.16 ± 0.93c</td>
<td>13.38 ± 0.30de</td>
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<td>16 d [23 °C]</td>
<td>Acco</td>
<td>16.26 ± 0.12b</td>
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<td>14.89 ± 0.15abc</td>
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<td>Wonderful</td>
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<td>14.77 ± 1.13c</td>
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<td>Herskawitz</td>
<td>16.41 ± 0.23b</td>
<td>1.20 ± 0.08c</td>
<td>14.14 ± 1.00c</td>
<td>14.00 ± 0.22cd</td>
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P-values

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<tr>
<th>Cultivar (A)</th>
<th>P-value</th>
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<th>&lt; 0.0001</th>
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3.6  Multivariate analysis

3.6.1  Principal component analysis (PCA)

The variability of physical and physio-chemical attributes of fruits from the three cultivars was summarised in a PCA. Figure 8a-b shows that fruit cultivars exhibited both similar and distinct variability in characteristics at different storage periods. The total variability at the different storage conditions was explained by 11 principal factors (F1 to F11) and the first two factors were considered. The first two factors explained 63.3 % of the total variability, with F1 & F2 characterising 39.7 and 23.6 %, respectively. Along F1 of Figure 8a, postharvest characteristics of ‘Herskawitz’ and ‘Wonderful’ fruits at 42 d of cold storage was to a great extent similar to their characteristics at eight days of shelf storage. This could be attributed to the comparable average water loss of 8.80 % observed in each case. On the other hand, fruits at 42 d of cold storage and an additional eight days of shelf storage are comparable in characteristics with fruits kept for 16 d under immediate shelf conditions. Therefore, pomegranate fruits subjected under enhanced water loss conditions of shelf life, can to a substantial extent be used to make inferences on fruits under prolonged cold storage. Along F2, the characteristics of ‘Acco’ and ‘Herskawitz’ were more on the opposite ends while ‘Wonderful’ was intermediate with a greater leaning towards ‘Acco’ than ‘Herskawitz’.

Along F1, Figure 8b reveals that of all the tested quality characteristics, fruits at 42 d of cold storage and eight days of immediate shelf life were more characterised by peel proportion, moisture content and thickness, colour redness ($a^*$) and chroma intensity ($C^*$) and fruit puncture resistance. On the other hand, fruit water loss, water loss per unit surface area and aril proportion can be used to characterise pomegranate fruits at the end of cold storage and additional days of shelf life or fruit under prolonged shelf storage. Along F2, ‘Acco’ fruits (especially at eight days
of shelf storage) are characterised by aril moisture content, peel colour lightness \((L^*)\) and hue angle \((h^\circ)\), TSS/TA and BrimA. On the other hand, the total colour difference \((TCD)\) and titratable acidity (TA) characterise ‘Herskawitz’ fruits especially at the end of both shelf storage regimes.

3.6.2 Pearson correlation

The Pearson correlation test was performed to establish the relationships among the physical physio-chemical attributes of pomegranate fruits, across all the tested storage conditions (Table 4). Significant \((P < 0.05)\) correlations were observed among most of the analysed quality attributes of the pomegranate fruits (Table 4). Fruit water loss per unit fruit mass \(WL\) exhibited strong positive correlations with water loss per unit surface area \(WLA\) \((r = 0.981)\) and total colour difference \(TCD\) \((r = 0.774)\) as well as strong negative correlations with peel thickness \(PT\) \((r = -0.647)\), peel colour lightness \(L^*\) \((-0.751)\), redness \((r = -0.648)\) and chroma intensity \(C^*\) \((r = -0.793)\). This implies that fruits such as ‘Herskawitz’ with high water loss per unit surface area and total colour difference are more susceptible to water loss while fruits with high peel lightness, redness and chroma intensity such as ‘Acco’, are less susceptible to water loss (Figure 8b).

A significant and moderate negative correlation \((r = -0.508)\) exists between water loss \(WL\) and peel proportion across all tested conditions. However, this relationship is specifically very strong \((r = -0.823)\) for fruits under cold storage and subsequent shelf storage. Furthermore, a significant and very strong positive correlation is observed between peel proportion with peel moisture content \((r = 0.839)\) and thickness \((r = 0.892)\), as observed in ‘Herskawitz’ fruits.
Peel chroma intensity is strongly influenced by fruit water loss \((r = -0.793)\) and very strongly influenced by redness colour \((r = 0.950)\) and positively correlated with fruit puncture resistance \((0.531)\), peel thickness \((0.597)\) and moisture content \((0.566)\).

Other attributes that significantly \((P < 0.05)\) correlated with water loss in pomegranates include respiration rate, fruit puncture resistance, peel hue angle, TSS, TSS/TA and BrimA. Respiration rate was significantly and positively correlated with peel redness \((0.555)\). As observed, fruits with higher peel redness \(\text{‘Acco’ and ‘Herskawitz’}\) also had significantly higher respiration rate compared to fruits \(\text{‘Wonderful’}\) with lower peel redness value. Furthermore, the respiration rate was significantly influenced by the TSS as expected while TSS/TA was strongly and negatively influenced by TA \((r = -0.848)\) as opposed to TSS \((r = -0.217)\). The puncture resistance of the fruits was significantly influenced by the peel moisture content and thickness as expected.
Figure 8 Principal component analysis of the first two components (F1 and F2) showing observations (a) and variables (b) based on the physical, mechanical and physio-chemical attributes of pomegranate fruit cultivars (‘Acco’, ‘Wonderful’ and ‘Herskawitz’) stored for 42 d at 7°C / 90% RH followed by additional 8 d of shelf storage at 23°C / 58% RH and under prolonged immediate shelf storage of 16 d. WL and WL_A, weight loss per unit mass and unit surface area, respectively; TCD, total colour difference; MC_Arils, moisture content (wet basis) of arils; MC_peel, moisture content (wet basis) of peel; PFR, puncture force resistance; PT, peel thickness; L*, lightness; a*, redness; C*, chroma; h°, hue angle; TSS, total soluble solids; TA, titratable acidity; RR, respiration rate.
Table 4 Pearson correlation coefficient matrix between the physical and physio-chemical attributes of pomegranate fruit (‘Acco’, ‘Wonderful’ and ‘Herskowitz’) stored for 42 d at 7 °C/90 % RH followed by additional 8 d of shelf storage at 23 °C/58 % RH and under prolonged immediate shelf storage of 16 d

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<th>Variables</th>
<th>WL</th>
<th>WLₐ (%)</th>
<th>Arils (%)</th>
<th>Peel (%)</th>
<th>MC_Arils</th>
<th>MC_Peel</th>
<th>PT</th>
<th>PFR</th>
<th>L*</th>
<th>a*</th>
<th>C*</th>
<th>h⁰</th>
<th>TCD</th>
<th>RR</th>
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<th>TA</th>
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<td>Peel (%)</td>
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WL and WLₐ, weight loss per unit mass and unit surface area, respectively; TCD, total colour difference; MC_Arils, moisture content (wet basis) of arils; MC_peel, moisture content (wet basis) of peel; PFR, puncture force resistance; PT, peel thickness; L*, lightness; a*, redness; C*, chroma; h⁰, hue angle; TSS, total soluble solids; TA, titratable acidity; RR, respiration rate. Values in bold are different from 0 with a significance level alpha=0.95.
4 Conclusion

The study aimed to characterise the water loss susceptibility of pomegranate fruit cultivars (‘Acco’, ‘Wonderful’ and ‘Herskawitz’) based on the fundamental physical and physio-chemical attributes during cold shipping conditions and open shelf market conditions. Generally, the major effect factors: cultivar, storage conditions, and their interaction significantly influenced water loss, respiration rate, peel thickness, peel colour redness and chroma, total colour difference, peel moisture content, fruit firmness, and titratable acid. The study reveals that despite physiological and structural differences among pomegranate cultivars, water loss characteristics are similar during the 42 d of cold storage. However, medium-sized fruits (‘Herskawitz’ and ‘Wonderful’) are characterised by a relatively higher water loss than small-sized fruits (‘Acco’) during prolonged storage.

The study revealed that water loss in pomegranate fruits is primarily and majorly from the peel proportion and that peel related properties such as thickness, moisture content and puncture resistance were significantly influenced by the storage duration. Despite the fact that water loss resulted into deterioration of external aesthetic appeal of the fruits, the edible portion of the fruits (the arils) remained unaffected even at total water loss of 24.2%. Hence, juice yield was minimally affected by the tested storage duration. Therefore, research should primarily focus on the peel fraction in addressing the water loss problems of pomegranate fruits. This information is helpful to plant breeders in selecting against water loss susceptible cultivars.

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7  **Author contributions**

Lufu R carried out the experiment and wrote the manuscript. Opara UL and Ambaw A conceptualised and designed the research study, and edited the Manuscript.

8  **Ethics declarations**

Competing interests: the authors declare no competing interests.