Maturity Indexing and Postharvest Performance of Newly Developed ‘Lamb Hass’ Avocado Fruit

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\textbf{ABSTRACT}

This study was conducted to identify reliable science-based maturity indices for ‘Lamb Hass’ avocado fruit and evaluate the effect of harvest maturity on the fruit postharvest performance. Fruits were harvested from outside and inside canopy positions at early and mid-season for quality assessment. The results showed that the canopy position had a highly significant effect ($p < .001$) on all maturity parameters: dry matter (DM) content, moisture content (MC) and oil content (OC). The outside harvested fruit were more mature (30% DM, 70% MC, 14.9% OC) than those harvested from the inside canopy (28.9% DM, 71.1% MC, 11.75% OC) at minimum maturity. However, fruit DM and MC did not differ significantly ($p > .05$) between the early and mid-season fruit, whilst significant differences in OC were found between the two harvesting stages. In both harvesting stages, the canopy positions did not affect the rate of fruit mass loss. Nonetheless, the overall analysis showed that early season fruit had a significantly ($p < .001$) higher fruit mass loss rate than the mid-season fruit. Notably, fruit maturity did not have a significant effect ($p > .05$) on the fruit softening rate as insignificant differences between the two harvesting stages were observed. In both the harvesting stages, inside canopy fruit were characterized by a higher concentration of mannosepentulose and perseitol than the outside canopy fruit. The early and mid-season fruit also differed significantly ($p < .05$) in phenolic content; with the mid-season fruit having a high phenolic content (2.7 µm/g) than the early season fruit (1.8 µm/g), suggesting the possibility of increased mesocarp discoloration with the advancement in maturity. These findings will be a valuable tool to assist growers in assessing fruit readiness for harvest and understanding the influence of maturity on the fruit postharvest behavior.

\textbf{KEYWORDS}

Canopy position; dry matter; oil content; uneven ripening; fruit quality

\textbf{Introduction}

The South African avocado industry is ranked as the fifth biggest exporter in Africa with more than 45% of its production exported annually (Mogala, 2015). During the 2009/10 season, about 64% of South African avocados were exported; however, the amount declined drastically after this season. The decline was attributed mainly to the inferior quality of fruit observed during the 2009/10 export season (Nelson, 2010). Uneven ripening is among the important quality defects resulting in marketing difficulties of South Africa’s avocado fruit (Nelson, 2010).
Most countries in the European Union (EU) have adopted the ‘ready-to-eat’ market (Hernández et al., 2016); this is because of consumers’ demand for homogeneous and high consistency in fruit quality (Hofman et al., 2013). The ‘ready-to-eat’ market is also 30% more profitable than the selling unripened fruit (Hernández et al., 2016). Thus, the uneven ripening results in difficulty to manage the marketing of fruit and inconsistent quality delivery to consumers (Blakey, 2016). Considering that the international market is highly competitive, and consumers have a broader choice of suppliers, it is critical that the South African avocado industry urgently resolve this issue in order to maintain its reputation as a reliable supplier.

Varying maturity at harvest has been the main factor resulting in uneven ripening (Ferrer et al., 2005). This is because of the high correlation between fruit ripening behavior and harvest maturity (Barmore, 1977; Mkathini et al., 2017; Zauberman et al., 1977). Thus, maturity indexing is essential for optimum fruit quality and uniform ripening (Crane et al., 2013; Lee et al., 1983; Ncama et al., 2018). Currently, the maturity indices used for the newly developed ‘Lamb Hass’ avocado cultivar are based on models previously developed for well-known commercial cultivars such as ‘Hass’ and ‘Fuerte.’ Considering the fact that cultivars perform differently from each other mainly due to the distinct genetic constitution (Carter et al., 1988), individual cultivar maturity indexing is essential (Crane et al., 2013; Lee et al., 1983; Ncama et al., 2018).

Identification of avocado physiological maturity is challenging because external changes during maturation do not fully characterize the stage of maturity (Lee, 1981; Magwaza and Tesfay, 2015). However, the use of DM, MC and OC has been reported to be strongly correlated with optimum postharvest quality and best eating quality of the avocado fruit (Clark et al., 2007; Hofman et al., 2013; Parodi et al., 2007). These parameters change significantly during maturation of avocado. The fruit OC increases with maturity, while the mesocarp MC decreases with maturity, with a reciprocal increase in DM.

Furthermore, Carvalho et al. (2014) reported that as mesocarp oil content increases, the moisture content decreases by the same amount, which implies that the ratio of oil and moisture contents remains constant throughout the life of the avocado fruit (Ncama et al., 2018). The strong relationship between these maturity parameters and their high correlation with the fruit postharvest performance and final eating quality make them to be commercially adopted as reliable indicators of avocado maturity (Lee, 1981). Thus, the aim of this study was to identify maturity indices for newly developed ‘Lamb Hass’ avocado based on the currently used maturity parameters. The study also evaluated the effect of harvest maturity on fruit quality and postharvest behavior.

**Materials and Methods**

**Description of the Study Area**

The experiment was conducted during the 2018/2019 growing season in Everdon Estate, a commercial avocado farm of Westfalia fruit (Pty) LTD. The farm is located in Howick, a cool subtropical area of KwaZulu-Natal province, South Africa (29° 26’37”S, 30°16’22”E, 1080 m altitude). The area receives mostly summer rainfall with an average of 826.57 mm per year and experiences an average maximum and minimum air temperature of 26.1 and 15.0°C in summer and 19.4 and 6.7°C in winter. Seven year old ‘Lamb Hass’ avocado trees were used in the study. The trees were spaced 4 m x 7 m apart from each other. No training system/shoot pruning techniques were performed during the season. Irrigation was carried out using micro-jet sprinklers for soil moisture to be maintained at 80–100% of field capacity.

**Fruit Growth Evaluation**

**Plant Material, Sampling and Measurements**

A total of nine 7-year old ‘Lamb Hass’ avocado trees were selected in a completely randomized design (CRD) with three replicates and each replicate consisting of three trees. The experiment consisted of two canopy positions, namely, inside canopy and outside canopy. At 67 days after full bloom (DAFB),
72 fruit (36 per canopy) from the 9 selected trees were tagged for growth measurements. Fruit were not tagged immediately after anthesis because of excessive fruit abscission during the early stages of development. The abscission is due to high resource competition between fruits and shoots of which fruit with better nutrient reserves are able to grow (Alcaraz and Hormaza, 2009). Growth parameters (length and diameter) were measured at 2–3-week intervals starting from 67 DAFB until 219 DAFB where the growth parameters remained constant. Digital Vernier calliper (Digital Vernier Dexter) was used to conduct the measurements.

**Fruit Maturity Evaluation**

Maturity was evaluated at sixteen distinct developmental stages starting from 81 DAFB and continuing at 2–3 weeks intervals (Table 1). For each of the maturity stages, 60 fruit per canopy position were randomly harvested from the nine tagged trees and immediately transported in a ventilated vehicle to the postharvest laboratory of the University of KwaZulu-Natal. Upon arrival at the laboratory, 30 fruit for each canopy were analyzed for maturity (DM, MC and OC). The other 30 fruit were left to ripen in the laboratory at room temperature (21°C) and minimum maturity was evaluated on the ability of these fruit to ripen normally without shriveling and developing a watery texture.

**Oil Content**

Mesocarp oil content was measured from a freeze-dried sample material and quantified using a method described by Meyer and Terry (2008), with slight modifications. Briefly, 9 mL of Hexane was added to 300 mg of the freeze-dried sample in the test tube and placed into a sonic bath at 50°C for 10 min. The sample was then filtered using a vacuum pump through a 90 mm Whatman filter paper into a scintillation vial of known mass and another 6 mL hexane was added to the test tube to re-extract the remaining residue. This was left for 5 min and the tube emptied into the funnel. The 15 mL extract was then dried using a GeneVac® concentrator (SP Scientific, Genevac Limited, IPSWICH ENG.). The oil percentage of the avocado mesocarp was calculated with the following equation (Carvalho et al., 2014) and expressed as %.

$$OC\% = \frac{DM\% \times \text{oilweight}(g)}{\text{dryweightofthecoredsample}(g)}$$

(1)

| Table 1. Description of different harvesting times for ‘Lamb Hass’ avocado fruit during the 2019 growing season. |
| --- | --- | --- |
| Harvest | Date | DAFB |
| H1 | 04-Mar-19 | 81 |
| H2 | 19-Mar-19 | 96 |
| H3 | 06-Apr-19 | 114 |
| H4 | 20-Apr-19 | 127 |
| H5 | 04-May-19 | 142 |
| H6 | 20-May-19 | 158 |
| H7 | 04-Jun-19 | 173 |
| H8 | 18-Jun-19 | 187 |
| H9 | 05-Jul-19 | 204 |
| H10 | 02-Jul-19 | 219 |
| H11 | 05-Aug-19 | 235 |
| H12 | 20-Aug-19 | 250 |
| H13 | 06-Sept-19 | 270 |
| H14 | 20-Sept-19 | 284 |
| H15 | 04-Oct-19 | 298 (Early season/Minimum maturity) |
| H16 | 25-Oct-19 | 319 (Mid-season) |
Moisture Content and Dry Matter Content
Fruit MC was determined by measuring the fresh mass of a core sample using a digital weighing balance (RADWAG, WTB 2000 Precision Balance) and removing the free water of the cored portion by drying the fruit sample using a freeze-dryer (VirTis BenchTop Pro with Omnitronics freeze dryer) (Sugiyama and Tsuta, 2010). The MC and DM were calculated using 2 and 3, respectively;

\[
MC\% = \left( \frac{M_f - M_d}{M_f} \right) \times 100
\]

\[
DM\% = \left( \frac{M_f - M_d}{M_d} \right) \times 100
\]

Where MC is mesocarp moisture content, DM is dry matter content, Mf is fresh mass of the cored sample and Md is dry mass of the cored sample.

The Effect of Harvest Maturity on Fruit Quality and Postharvest Performance
For this experiment, a total of 200 fruit (100 per canopy position) were harvested randomly from the tagged trees at 298 and 319 DAFB, respectively, corresponding to early season/minimum maturity and mid-season. The earliest/minimum acceptable maturity stage was judged by the ability of the fruit to ripen without shriveling and developing a watery texture. Due to extremely windy conditions that prevailed during the season, the fruit size was generally small and there was a high incidence of wind damage. An attempt was made to selectively pick fruit that are bigger in size. However, this was not possible in many cases due to the lack of fruit. Fruit were then packed in boxes and immediately transported in a well-ventilated vehicle to the postharvest laboratory in the University of KwaZulu-Natal.

The fruit were stored in a cold room for 28 days at a temperature of 5.5°C and 95% RH; simulating shipment conditions, followed by eight days at room temperature (21°C). The fruit were sampled at 7 days intervals in cold storage and 2 days intervals at shelf-life. Five fruit were used for physical assessment, while another set of five fruit were destructed for biochemical analysis. The samples were put in a freezer at −10°C and thereafter dried using a freeze-dryer (VirTis BenchTop Pro freeze dryer) until the samples were completely dry. The dried samples were grinded into powder using a blender (Bennett Read Blender, KBD202) and stored in a freezer at −10°C for further analysis.

Mass Loss
The mass loss was determined by weighing the fruit on a digital weighing scale (RADWAG Wagi Electronic Inc., Poland), and was expressed as mass loss percentage based on the initial fruit mass at the day of harvest.

Fruit Firmness
Fruit firmness was determined using a hand-held firmness tester (Bareiss, Germany). Two firmness readings were taken in the equatorial regions by rotating the fruit 180°. The firmness tester that was used measures firmness on a scale from 100 (very hard, unripe fruit) to < 64 (soft, ripe fruit) (Standard ISO 7619, International Organization for Standardization).

Fruit Color
Fruit skin color was measured objectively using a colourimeter (Konica Minolta Chroma Meter CR-400) by averaging three measurements taken around three fruit positions. Calibration of the device was done with white standard tile. The parameters determined were Lightness \([L^* = 0\) (black) and \(L^* = 100\) (white)], Chroma, \((C^*)\) and hue angle \((h^*)\).
Phenolic Compounds Extraction and Quantification

Total phenolic compound extraction and quantification was carried out using a Folin-Ciocalteau method previously described by Lamien-Meda et al. (2008) with slight modifications. Briefly, a freeze-dried mesocarp sample powder (0.5 g) was extracted with 70% acetone (5 mL). The mixture covered with aluminum foil was kept for 10 min at room temperature to facilitate the extraction of phenolics. The mixture was then centrifuged for 10 min using GenVac® centrifuge (SP Scientific, Genevac LTD., Suffolk, UK) to obtain a clear extract.

Each extract (100 µL) was mixed with 2 N Folin-Ciocalteau reagent (125 µL) and allowed to stand for 3 min. Thereafter, 7.5% sodium carbonate (1250 µL) was added and samples were incubated for 1 h in dark room temperature. The absorbance was measured at 760 nm using a UV-1800 Spectrophotometer (Shimadzu Scientific Instruments INC., Columbia, USA) against acetone as blank.

Non-structural Carbohydrates Identification and Quantification

The soluble sugars were extracted as described by Tesfay and Magwaza (2017). Briefly, 10 mL 80% v/v ethanol/H2O was added into 0.1 g of freeze-dried sample and the mixture was homogenized using Ultraturrax for 1 min. The sample was then placed for 60 min in a shaking water bath at 80°C. Thereafter, the sample was removed from the water bath and stored in a refrigerator at 4°C for 24 hrs to facilitate the release of soluble sugars. The mixture was then centrifuged at 10,000 rpm (11953 g) for 15 minutes in a refrigerated centrifuge at 4°C. The supernatant was filtered through glass wool and the filtrate was dried under vacuum in a GenVac1 concentrator (SP Scientific, Genevac LTD., Suffolk, UK) for overnight. Dry extracts were reconstituted by adding 2 mL ultra-pure water and centrifuged at 10,000 rpm (5 min.). The supernatants were then filtered through a 0.45 mm nylon syringe filter into an HPLC vial. Sugars were analyzed using an isocratic HPLC system equipped with a refractive index detector, according to Liu et al. (1999), using a Ca+ (8%) column of 7.8 mm diameter x 300 mm (Phomenex, Torrance, CA, USA) with a Carbo-Ca2+ guard column of 3 mm x 4 mm x (Phomenex). The column temperature was kept at 80°C using a thermoregulated column compartment. The mobile phase was ultra-pure water at a flow rate of 0.6 mL/min. The concentration and the presence of individual sugars (sucrose, glucose, fructose, mannoheptulose, and perseitol) were determined by matching peak areas of samples with the peak areas and concentration of standards (0.05–1.25 mg/L; R² = 0.99).

Statistical Data Analysis

The collected data was subjected to the analysis of variance (ANOVA) using GenStat statistical software (GenStat®, 18.2 edition, VSN International, UK). Mean separation was performed using Fisher’s least significant difference (LSD) at 5% level of significance. Standard error values were calculated where a significant standard deviation was found at p ≤ .05 between individual values.

Results and Discussion

Fruit Growth

Results of change in fruit size during growth and development are presented in Figure 1 and Figure 2. Fruit length increased rapidly from the first sampling date until 204 DAFB which thereafter remained fairly constant (Figure 1). The fruit diameter followed the same pattern, but the changes were smaller to those of fruit length (Figure 2); fruit length had a maximum value of (97 mm) while; maximum of (75 mm) was recorded for fruit diameter. The trend of fruit length and diameter followed a single sigmoid pattern (Figure 1 and 2), confirming previous findings by Lewis (1978) that avocado fruit growth follows a single sigmoid pattern characterized by a very rapid cell division during the early stages of fruit growth. The author, furthermore, explained that the reduced growth rate in the later
stages of development is due to reduced cell division, which occurs over the whole season and accounts for the continued fruit growth. Similarly, Moore-Gordon (1997) showed that the rate of cell division is at a maximum during the exponential growth phase.

While canopy position did not have a significant effect ($p > .05$) on fruit diameter, highly significant differences ($p < .001$) between inside and outside canopy on fruit length were observed. The fruit from the two canopy positions showed a similar trend, however, differed significantly in size with the outside canopy having the highest fruit length with a minimum of 65.6 mm and maximum of 97.2 mm compared to the inside canopy fruit with the respective values of 57.2 mm and 93.4 mm (Figure 1). Findings by Shezi et al. (2019) revealed that the outside canopy fruit are characterized by a high rate of metabolic processes due to high light and temperature exposure. This explains the observed high fruit length of the outside canopy which was because of the enhanced rate of cell division. A significant

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Figure 1. Seasonal pattern of ‘Lamb-Hass’ avocado fruit length measured from 67 DAFB until 219 DAFB on fruit harvested from the inside and outside canopy positions. Data presented as mean ± standard error; CP, canopy position; DABF, days after full bloom; IC, inside canopy; OC, outside canopy.

Figure 2. Seasonal pattern of ‘Lamb-Hass’ avocado fruit diameter measured from 60 DAFB until 224 DAFB on fruit harvested from the inside and outside canopy positions. Data presented as mean ± standard error; CP, canopy position; DABF, days after full bloom; IC, inside canopy; OC, outside canopy.
variation in the rate of growth in fruit from the different canopy positions suggests that the fruit will reach harvest maturity at different times and ripe unevenly due to an imbalance in the accumulated carbohydrates during growth and development.

**Fruit Maturity**

The fruit dry matter content increased until the last maturity stage, and there were highly significant \( p < .001 \) differences between the harvesting stages except for the early harvesting stage and mid-harvesting stage where the difference was not significant (Figure 3A). The fruit DM accumulation was also significantly affected \( p < .001 \) by the canopy position with the outside canopy having the highest DM content throughout growth and development. However, the interaction between the harvesting stage and canopy position \( (H*CP) \) was not significant \( p > .05 \).

The minimum maturity was reached at 30% and 28.8% DM for outside and inside canopy fruit, respectively. The differences in DM content for the outside and inside canopy fruit can be hypothesized to be due to different environmental stress experienced by the tree canopies. The outside canopy is exposed to more direct sun radiation and experience higher temperatures as compared to the inside canopy which is shaded and receives less direct sunlight (Woolf et al., 2000). The different exposure to environmental stress triggers a diverse physiological response and results in a different rate of metabolic processes between the fruit of the two canopy positions. Shezi et al. (2019) reported that avocado fruit from the outside canopy were characterized by a higher assimilation rate and enhanced sink strength than the inside canopy fruit, resulting in higher DM accumulation.

On average, the ‘Lamb-Hass’ avocado fruit reached the minimum maturity stage at 29% DM content. This is in agreement with the previous work by Chen et al. (2009) who found 29% DM content to be the minimum standard where best-eating quality can be assured for South Konan ‘Hass’ avocados. Kremer-Köhne (2000) reported the DM of 27% to be the minimum harvest standard for Lamb-Hass in the South Africa-Mooketsi area (hot, dry and tropical region). The harvest time was from August. The current study was conducted in a cool subtropical region which the harvest time was from October at 29% DM content. This suggests that (i) fruit maturity is delayed in cooler environments when compared with hotter regions, and (ii) longer fruit hanging time enhances dry matter accumulation, indicating that avocado maturity is also region-specific.

Moisture and oil contents were also significantly affected by the canopy position \( p < .001 \) and also showed highly significant differences \( p < .001 \) between the different harvesting stages (Figure 3B and 3C). The interaction between the harvesting stage and canopy position \( (H*CP) \) for the two maturity parameters was insignificant \( p > .05 \). Generally, fruit moisture content decreased as from the first sampling stage and throughout the season (Figure 3B). The minimum MC where the fruit was able to ripen normally was established at 70% and 71.2% for outside and inside canopy fruit, respectively.

The oil content of 11.8% and 14.9% for inside and outside canopy fruit, respectively, was defined to be the minimum maturity standards for ‘Lamb Hass’ avocado fruit (Figure 3C). Our results agree with those of Lee (1981) and Carvalho et al. (2014) that the widely accepted minimum maturity standard of 8% OC is too low for many cultivars as fruit have been observed to be immature at this oil content level. This is due to the varying ability of avocado cultivars to synthesize oil as a result of cultivar hybridization (Barmore, 1977). Fruit of the Mexican type have the highest oil content followed by the Guatemalan and West Indian types (Barmore, 1977). Considering that ‘Lamb Hass’ is predominantly Guatemalan; this explains the observed high oil content at maturity. One of the distinguishable characteristics of Guatemalan cultivars is that they take a longer time to maturity compared to other races. Therefore, long hanging time might have enhanced the ability of the fruit to synthesize more oil (Kremer-Kohne and Kohne, 2001). The oil content increased from 1.4% to 12.8% and from 1.6% to 15.8% for inside and outside canopy fruit, respectively, from the early growth stages to the mid-harvest stage later in the season. Our results are consistent with those of Villa-Rodriguez et al. (2011) who reported that the late season fruit have a higher oil content.
Figure 3. Seasonal changes in mesocarp (A); dry matter content, (B); moisture content and (C); oil content of ‘Lamb-Hass’ avocado fruit harvested from the inside and outside canopy positions. Data presented as mean ± standard error; CP, canopy position; H, harvesting stage; IC, inside canopy; OC, outside canopy. The red arrow indicates the minimum maturity/Early harvesting stage, whereas the blue arrow indicates the mid-harvesting stage.
The synthesis of avocado oil requires high energy input which is derived from the stored fruit carbohydrates derived from photosynthesis. The main avocado fruit carbohydrates are the C7 sugars (mannohexulose and perseitol), which are the predominantly stored sugars in avocado (Liu et al., 1999). Previous work by Davenport and Ellis (1959) also showed that a decrease in sugar concentration of 'Fuerte' avocado during growth and development was accompanied by an increase in oil concentration of the fruit. Thus, a significant variation in the oil content between inside and outside canopy means that the fruit from the two canopy positions will differ significantly in the content of the C7 sugars. Considering that C7 sugars are the major respiratory substrates during ripening of the avocado fruit (Tesfay et al., 2010), the uneven distribution of C7 sugars between inside and outside canopy fruit would result in uneven ripening.

**Fruit Postharvest Performance**

**Mass Loss**

Fruit mass loss was investigated for early and mid-harvesting stages (Figure 4A-C). The mass loss rate increased with an increase in storage time for both harvesting stages; irrespective of the canopy position (Figure 4A-B), the increase was temperature-dependent. This was observed when a sharp increase in the rate of fruit mass loss was observed when fruit were transferred from a cold room to shelf-life/room temperature (day 28 to day 36). Similar results were reported by Ahmed and Ahmed (2005) on ‘Hass’ and ‘Fuerte’ avocado fruit, whereby, transfer of fruit from 5°C to 20°C increased the rate of mass loss. Blakey (2011) reported that the loss of fruit mass is due to respiration and transpiration. The transpiration process of fruit is driven by the vapor pressure deficit difference between the fruit which is almost saturated and the environment which is less saturated, thus lowering the storage temperatures and increased relative humidity reduces respiration rate and the vapor pressure which reduce the transpiration rate, thereby delaying mass loss (Tesfay and Magwaza, 2017). Hence, the observed low mass-loss rate during cold storage.

![Figure 4](image_url)

**Figure 4.** Mass loss percentage of 'Lamb-Hass' avocado fruit harvested at early harvest (A) and mid-harvest (B) maturity stages from inside and outside canopy positions and overall (C) during storage at 5.5°C; 95% RH for 28 days followed by 8 days at 21°C. Data presented as mean ± standard error; CP, canopy position; T, time (storage); IC, inside canopy; OC, outside canopy.
In both harvesting stages, the canopy position did not have a significant effect \((p > .05)\) on the rate of fruit mass loss. Even though no significant effect of the canopy position was observed, the inside canopy fruit were characterized by a higher mass loss rate than fruit from the outside canopy in both early and mid-season harvest stages. The interaction between the harvesting stage and storage period was significant \((p < .05)\). This was characterized by highly significant differences \((p < .001)\) between the two harvesting stages with the early harvesting stage having a higher fruit mass loss rate compared with the mid-harvesting stage (Figure 4C). In agreement with these findings, Cutting and Wolstenholme (1992a) reported that increasing maturity reduces the amount of water lost by the fruit during postharvest storage.

**Firmness**

Fruit firmness decreased significantly \((p < .05)\) with the storage period for both the harvesting stages and tree canopies (Figure 5A–C). The cold storage retained the fruit firmness as no decline was evident during this period (day 0 to day 28) for both the harvest stages. Thus, the overall mesocarp softening was observed during the shelf-life period, irrespective of harvest stage or canopy position. One factor that leads to a gradual decline in fruit firmness is pectin depolymerization during ripening, resulting in the weakening of the cell structure, loss of cell membrane integrity, and hydrolysis of cellulose and hemicellulose (Huber et al., 2001).

The breakdown of pectin polysaccharides results from different enzymes activities catalyzing various metabolic reactions; these enzymes include β-galactosidase, pectate lyase, polygalacturonase and pectin methylesterase (Pesis et al., 1978). The activity of these enzymes is enhanced by increased temperatures (Bower and Magwaza, 2004). Hence, the observed high softening rate during shelf-life.

The canopy position significantly affected \((p < .05)\) the fruit softening rate for the early season fruit, whereas, the mid-season fruit were not significantly affected by the canopy position (Figure 5A–B). However, in both harvesting stages, the outside canopy fruit were characterized by a lower softening rate than the inside canopy. Woolf et al. (1999) reported similar results where outside canopy ‘Fuerte’ avocado fruit had a low ripening rate than inside canopy fruit. The high accumulation of heat shock proteins (HSPs) in the outside canopy fruit could explain the observed ripening heterogeneity of

![Figure 5. Firmness changes of ‘Lamb-Hass’ avocado fruit harvested at early harvest (A) and mid-harvest (B) maturity stages from inside and outside canopy positions and overall (C) during storage at 5.5°C; 95% RH for 28 days followed by 8 days at 21°C. Data presented as mean ± standard error; CP, canopy position; T, time (storage); IC, inside canopy; OC, outside canopy.](image-url)
avocado fruit (Woolf et al., 2000). Due to higher temperatures stress experienced by the outside canopy fruit, the synthesis of normal protein production is reduced, and the synthesis of HSPs is induced. Due to the protective role of HSPs on proteins, metabolic processes occur at a very low rate until the stress is relieved (Ul Haq et al., 2019). This causes the metabolic processes to occur in an unsynchronized pattern between the outside and inside canopy fruit. Thus, results in different physiological status at harvest for the two canopies; hence, the different postharvest behavior was observed.

No significant changes ($p > .05$) were observed in fruit softening rate between the early and mid-harvesting stage (Figure 5C). In contrast, Cutting and Wolstenholme (1992b) reported a higher softening rate of more mature fruit. The interaction of harvest stage and storage time ($H^*T$) also had a significant effect on firmness decline rate. Although there were non-significant differences brought by the harvesting stage, the early harvest stage had higher firmness retention compared with the mid-harvest stage. The firmness declined from 94.9 N (day 28) to 66 N (day 36) for the early harvest stage and from 99.85 N (day 28) to 62 N (day 36) for the mid-harvest stage. This indicates that early-season fruit took longer to ripen than their mid-season counterparts. These findings are in agreement with those reported by Cutting and Wolstenholme (1992b), that time to ripening is a function of fruit maturity, with less time to ripen being observed with increasing maturity.

**Color**

Ripening was characterized by a color change from green to black. Hue angle ($h^\circ$), chroma ($C^*$) and lightness ($L^*$) all decreased with ripening regardless of the harvesting stage and the canopy position (Figure 6A-F). Slight color changes were observed during cold storage, with significant changes in color observed during shelf-life (day 32) where $L^*$, $C^*$ and $h^\circ$ all declined rapidly. This suggests that the color of avocado fruit is temperature-dependent with higher temperatures enhancing color change. This is in agreement with the study by Cox et al. (2004), which reported that higher ripening temperatures accelerated anthocyanin accumulation, resulting in rapid avocado fruit color change. No significant differences were found in color from day 32 to the last day of shelf-life (day 36). This means that after 4 days at shelf-life, all the fruit had changed color to black. Considering that the ‘ready-to-eat’ fruit stage was reached after only 8 days of shelf-life (Figure 7A-C), color is not an

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**Figure 6.** Color attributes of ‘Lamb-Hass’ avocado fruit harvested at early harvest (A, C and E) and mid-harvest (B, D and F) maturity stages from inside and outside canopy positions and overall (C) during storage at 5.5°C; 95% RH for 28 days followed by 8 days at 21°C. Data presented as mean ± standard error; CP, canopy position; T, time (storage); IC, inside canopy; OC, outside canopy.
appropriate indicator of fruit softness but can be rather used as ripening indicator. These observations contrast with the study by Cox et al. (2004), which reported that skin color change is an indication of fruit softness. However, the findings of the present study are comparable to those communicated by Donetti and Terry (2012), who reported a poor correlation between anthocyanin accumulation and mesocarp softening.

![Figure 7](Image)

**Figure 7.** Overall postharvest changes in color parameters (A): Lightness, (B): Chroma and (C): hue angle of ‘Lamb-Hass’ avocado fruit harvested at early harvest (EH) and mid-harvest (MH) maturity stages during storage at 5.5°C; 95% RH for 28 days followed by 8 days at 21°C. Data presented as mean ± standard error; H, Harvest (maturity stage); T, Time (storage).
Lightness differed significantly \((p < .05)\) between the canopy position for the mid-harvesting stage, whereas no significant differences \((p > .05)\) were observed between the canopy position of the early harvesting stage (Figure 6A-B). The early and mid-harvesting stage differed significantly \((p < .05)\) in L*. Notably, L* decreased from 33.2 (day 0) to 25.91 (day 36) and from 31.57 (day 28) to 25.25 (day 36) for the early and mid-harvest stage, respectively. Furthermore, the interaction of the harvest stage and storage time \((H^*T)\) had a significant effect on L*. The above results suggest that mid-season fruit were darker than the early season fruit. Donetti and Terry (2012) also found higher chlorophyll content in early season fruit compared to the mid and late season fruit at harvest.

Non-significant differences \((p > .05)\) were observed in Chroma \((C^*)\) and Hue angle \((h^o)\) between the canopies for both the harvesting stages (Figure 6C-F). These findings agree with the report by Mathaba et al. (2015) where canopy position did not have an effect on ‘Hass’ avocado fruit skin color change during ripening. The mid-harvest fruit were characterized by a more rapid initial decrease in \(h^o\) when compared with early season fruit.

**Non-structural Carbohydrates**

At harvest (Day 0), the early season fruit had a higher concentration of mannoheptulose and perseitol than the mid-season fruit. Both the harvesting stages had a predominant concentration of mannoheptulose (Figure 9 A-B). This suggests that the content of the C7 sugars is reduced with the advancement of maturity (Tesfay et al., 2010). The initial concentration of the sugars at harvest (Day 0) was highly variable as indicated by high error bars. Significant differences \((p < .05)\) in mannoheptulose were found between the canopy positions of the early season fruit while no significant differences \((p > .05)\) were found between the canopy positions of mid-season fruit.

The concentration of perseitol did not differ significantly between the canopy positions in both the harvesting stages (Figure 8 A-D). Nevertheless, in both the harvesting stages, inside canopy fruit were characterized by a higher concentration of mannoheptulose and perseitol than the outside canopy fruit, bearing in mind that the outside canopy fruit were more mature than the outside inside canopy fruit. This observation is in agreement with a previous report by Tesfay et al. (2010) that the content of the C7 sugars declines with the advancement of maturity. Shezi et al. (2019) furthermore reported that

![Figure 8](image-url)

**Figure 8.** Postharvest carbohydrates changes of ‘Lamb-Hass’ avocado fruit harvested at early season (A and C) and mid-harvest (B and D) maturity stages from inside canopy (IC) and outside canopy (OC) during storage at 5.5°C; 95% RH for 28 days followed by 8 days at 21°C. Data presented as mean ± standard error; CP, canopy position; T, time (storage).
the cooler temperature inside the canopy results in a lower rate of physiological processes during growth and development, considering that the C7 sugars as the major substrates for the processes, they will be slowly used up and thus, conserved for longer.

A study by Blakey et al. (2015) reported a correlation between the ripening rate and the content of C7 sugars of ‘Hass’ avocado fruit, the content of the C7 sugars was found to be high in slow ripening fruit than the fast-ripening fruit. However, in the current study, inside canopy fruit with higher mannoheptulose and perseitol concentration had faster-softening rate than outside canopy fruit. Similarly, Woolf et al. (1999) observed that the ethylene production peak of ‘Hass’ avocado was delayed by 2–5 days in outside canopy fruit compared to inside canopy fruit. In agreement with the findings, the authors noted that the outside canopy fruit were more mature than the inside canopy fruit.

The interaction between the harvest stage and storage had a significant effect ($p < .05$) on perseitol with the early season fruit having a high amount of the sugar than the mid-season fruit. The concentration of both mannoheptulose and perseitol decreased with storage. Notably, low concentration of the sugars was found on the last day of shelf-life (Day 36) compared to the day of harvest (Day 0). Liu et al. (2002) also reported a decline in the content of these sugars with ripening. The authors concluded that avocado ripening process is associated with the catabolism of the C7 sugars. Similarly, Tesfay et al. (2010) reported that C7 sugars are the major respiratory substrates during

Figure 9. Overall carbohydrates changes (A; Mannoheptulose, B; Perseitol) of ‘Lamb-Hass’ avocado fruit harvested at early harvest (EH) and mid-harvest (MH) maturity stages during storage at 5.5°C; 95% RH for 28 days followed by 8 days at 21°C. Data presented as mean ± standard error; H, Harvest (maturity stage); T, Time (storage).
ripening of avocado. Therefore, the deterioration in the postharvest quality of avocado is due to a decline in the concentration of mannheptulose and perseitol with ripening. The decline in the content of the C7 sugars with the advancement of avocado maturity explains the high fruit susceptibility to various physiological disorders such as mesocarp browning and a shorter postharvest life at higher maturity (Bertling et al., 2007).

**Phenolic Compounds**

The total phenolic concentration (TPC) increased with storage, irrespective of the canopy position or the harvesting stage (Figure 10A-C). Significant differences (p < .001) existed between the canopy positions of the early harvest with the outside canopy fruit having lower TPC compared with inside canopy fruit while insignificant differences (p > .05) were observed between the canopy positions of the mid-season fruit. Even though the mechanism of the canopy effect on phenolic accumulation is not completely understood, Woolf and Ferguson (2000) suggested that the high content of heat shock proteins in the outside canopy fruit results of the inhibition of the synthesis of phenolic compounds or other secondary metabolites.

Mid-season fruit showed an initial decline in TPC at the first week of cold storage (Day 7) which thereafter increased rapidly until the last day of cold storage (Day 28) and slight changes were observed after, thus, a uniform pattern was observed during shelf-life. In the early harvested fruit, the content decreased until the last day of cold storage which then increased until day 4 of shelf-life and thereafter, a uniform pattern was observed.

There were highly significant differences (p < .001) between the harvesting stages, with mid-season fruit having higher TPC compared with early season fruit (0.92 and 1.36 µg/g for early and mid-season fruit, respectively (Figure 10C). These findings corroborate with those of Van Rooyen and Bower (2003) who also reported that the total phenolic content of avocado fruit increase with the advancement of maturity. Furthermore, the authors reported that the delayed harvest fruit with higher phenolic content were characterized by poor membrane stability. This agrees with the current study results where the mid-season fruit had poor firmness retention ability compared with the early season fruit (Figure 5C).

![Figure 10](image-url)  
**Figure 10.** Changes in total phenolic content of ‘Lamb-Hass’ avocado fruit harvested at early harvest (A) and mid-harvest (B) maturity stages from inside and outside canopy positions and overall (C) during storage at 5.5°C; 95% RH for 28 days followed by 8 days at 21°C. Data presented as mean ± standard error; CP, canopy position; T, time (storage); IC, inside canopy; OC, outside canopy.
Whilst no significant differences were observed in DM between the harvesting stages (Figure 3A), highly significant differences in phenolic content between the harvesting stages suggest that DM alone is not an accurate measure of avocado fruit maturity. Van Rooyen and Bower (2006) found significant differences in TPC between different harvest dates whereas, fruit MC was not significantly different. In addition to the currently used maturity parameters, work identifying other maturity markers where mesocarp browning can be predicted is still essential for optimum fruit quality.

Conclusion
The findings of the study showed that the canopy position had a highly significant effect on fruit maturation. The outside canopy fruit were more mature than those harvested from the inside canopy at minimum maturity and this trend was observed throughout growth and development. Notably, fruit DM and MC did not significantly differ between the early and mid-season fruit, whilst significant differences in OC were found between the two harvesting stages. This suggests that the use of DM and MC alone as commercially adopted maturity indices of avocado does not reliably measure fruit maturation. However, this requires further investigation to confirm our argument. This was further supported by the highly significant differences in phenolic content between fruit of the two harvesting stages during postharvest storage. In both harvesting stages, the canopy positions did not have a significant effect on the rate of fruit mass loss. However, the overall analysis showed that early season fruit had a higher fruit mass loss rate than the mid-season fruit. Notably, fruit maturity did not have a significant effect on the fruit softening rate as insignificant differences between the two harvesting stages were observed. However, the mid-season fruit were characterized by a higher softening rate throughout the storage. The insignificance between the fruit softening rate of the two harvesting stages can be due to the less precision of the handheld texture analyzer used in the study.

‘Lamb-Hass’ avocado fruit is temperature-dependent with higher temperatures inducing rapid color change. All the fruit had changed color to black after 4 days at shelf-life. However, considering that the ‘ready-to-eat’ fruit stage was reached after only 6 and 8 days of shelf-life; color is not an appropriate indicator of fruit softness. In both the harvesting stages, inside canopy fruit were characterized by a higher concentration of mannoheptulose and perseitol than the outside canopy fruit. The early and mid-season fruit also notably differed in phenolic content; with the mid-season fruit having a high phenolic content (2.7 µm/g) than the early season fruit (1.8 µm/g), suggesting the possibility of increased mesocarp discoloration with the advancement in maturity. These findings will be a valuable tool to assist growers in assessing fruit readiness for harvest and understanding the influence of maturity on the fruit postharvest behavior. Further, canopy position has a significant effect toward avocado uneven ripening behavior, thus, in solving the problem of uneven ripening, preharvest and postharvest factors contributing to this phenomenon must be carefully considered.

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