

Post-harvest avocado physiology

RJ Blakey, JP Bower and I Bertling

Horticultural Science, School of Agricultural Sciences and Agribusiness
University of KwaZulu-Natal, Pietermaritzburg
Private Bag X01, Scottsville 3209, South Africa
E-mail: robblakey@gmail.com; bowerj@ukzn.ac.za

ABSTRACT

A three year study considered the effects of production location, maturity, storage temperature, modified atmosphere (MA) storage and a cold chain break on avocado ripening physiology. The aim of this study was to better understand post-harvest avocado physiology to enable improved post-harvest management to maintain fruit quality longer. Focus was placed on sugars, ripening enzymes, respiration, ethylene production and water relations. The seven carbon sugars, mannoheptulose and perseitol, were confirmed as the predominant sugars and possible energy source of avocado fruit. The six carbon sugars – glucose, fructose and sucrose – were not present in sufficient quantities nor do their trends suggest that they are used as an energy source. The seven carbon sugars are present in low concentrations in the mesocarp and need to be preserved during cold storage to enable proper ripening. The activity of three enzymes (cellulase, polygalacturonase and pectin methylesterase) was measured during ripening. The activity of these ripening enzymes is essential for softening and palatability. Polygalacturonase (PG) was not affected by different storage treatments although increased because of storage but cellulase activity was highly dependent. Fruit that were not stored in MA storage had a higher cellulase activity post-storage and fruit stored at 1°C had a slightly higher activity. The activity of pectin methylesterase (PME) was initially higher in fruit that were not stored in MA bags, but PME showed only slight variations post-storage. The 1°C and MA storage both reduced the respiration rate, ethylene production rate, mass loss, water loss and heptose consumption. The MA storage also reduced protein synthesis and external chilling injury. These two treatments together were found to be an effective means of maintaining fruit quality. These treatments negated the symptoms of long-term cold storage: the reduced storage temperature reduced the respiration rate and the MA storage reduced desiccation, but had a minimal effect of the ripening rate. A cold chain break, at any time during cold storage, increased mannoheptulose usage and water loss, and caused a spike in respiration and ethylene production. A cold chain break also increased shrivelling, greatly reducing fruit quality. Water content of fruit was measured using near infrared spectroscopy (NIRS). The model was validated in 2009 after two years of model development. It was found that the model was more accurate for fruit from KwaZulu-Natal, probably due to less

time loss between harvesting and measurement than fruit from other areas.

INTRODUCTION

The avocado (*Persea americana* Mill.) is a fruit crop with physiology which does not always appear to follow that of other fruit. The popularity of the avocado fruit has grown during the last fifty years and the fruit is produced extensively in a broad range of climatic zones, including semi-desert, temperate, subtropical and tropical. Avocados are also an important export crop in most of the countries of production. The cold storage of avocados has brought about many challenges for horticultural scientists. Unfortunately for the consumer, there are still many unsolved problems. These problems can only be solved once the avocado physiology is properly understood. Progress in understanding avocado physiology has been slow because of its highly variable nature and high concentrations of oil and phenols, requiring large datasets and compli-

cated assays. Research resources are also divided into pathology, agronomy, cultivar development and post-harvest technology, further slowing advances in post-harvest management.

The South African avocado industry is export driven. However, export also results in high costs of shipping and handling. Profits can be increased substantially with optimised management of the fruit. Management decisions need to have a physiological basis, because fruit quality is imperative for maintaining – and growing – market share. Two low-cost post-harvest techniques were implemented in this study: 1°C storage and modified atmosphere (MA) storage. The MA was achieved by enclosing fruit in micro-perforated polypropylene bags (polybags).

Avocados are climacteric fruit, generating large amounts of carbon dioxide and ethylene during ripening. The texture of fruit changes substantially during ripening. These changes are brought about by the



action of cell wall degrading enzymes on the cellulose, hemicellulose and pectin of the cell wall. These metabolic changes require an energy source and this area of avocado physiology is not sufficiently understood. The importance of water relations on fruit quality has been known for decades. Near-infrared spectroscopy now allows for improved studies on fruit water relations because the water concentration of fruit can be measured non-destructively. These factors were all studied to provide a better understanding of ripening physiology.

The aim of this study was to better understand post-harvest avocado physiology to enable better post-harvest management and enhance post shipment shelf life.

MATERIALS AND METHODS

Fruit, treatments, sampling and statistical analysis

Count 16 (236–265 g) fruit (cv Hass) were sourced from Tzaneen and Howick. Fruit from Tzaneen reached the laboratory within two days of picking, after refrigerated transport. Fruit from Howick were picked and immediately transported to the laboratory in Pietermaritzburg. Fruit were stored for 28 days at either 1.0°C or 5.5°C ($\pm 0.5^\circ\text{C}$). Four fruit per treatment were heat-sealed in a 30 μm thick polypropylene bag with 9 μm perforations and an anti-mist coating (Polylam Packaging, Johannesburg) before being placed in cold storage. Fruit tissue sampling was done by removing a core with a 15 mm diameter cork borer and immediately freezing in liquid nitrogen, the tissue lyophilised, and stored at -20°C until later analysis. The volume of fruit removed by this method was sealed with petroleum jelly, thus allowing the fruit to continue being used for ripening evaluation. A factorial design was used, with ten fruit as replications per treatment combination. ANOVA was performed using Genstat v12 (VSN International, Hemel Hempstead, UK).

Respiration rate

Carbon dioxide production was measured using an EGM-1 environmental gas monitor (PP Systems, Hitchin, Hertfordshire, UK). Fruit were incubated in a 1 L

container for 15 min. The headspace CO_2 concentration (ppm) was converted to respiration rate, taking into account fruit mass and volume, free space in the jar and the ambient CO_2 concentration (Van Rooyen, 2006).

Ethylene production rate

The ethylene production rate was measured using a DANI 1000 gas chromatograph (DANI Instruments, Monzese, Italy). A 20 mL glass autosampler vial and individual fruit were placed in a sealed 1 L jar for 30 min, thereafter the autosampler vial was sealed and transferred to the autosampler (HT250D, HTA s.r.l., Brescia, Italy). The GC was fitted with a flame ionisation detector, stainless steel column packed with an alumina-F1 stationary phase, and instrument-grade nitrogen as the mobile phase. The injector, column and detector temperatures were 160, 80, and 180°C , respectively. Results were corrected for fruit mass and volume.

Firmness

Fruit were deemed ripe when the average reading on a hand-held 5 mm round tip densimeter (Bareiss, Oberdischingen, Germany) was less than 60 (Köhne *et al.*, 1998).

Sugar concentration

Sugar content and concentrations were determined by HPLC, based on the method of Liu *et al.* (1999). The elution was isocratic, using ultrapure water as the mobile phase. Individual sugars were identified by comparing retention times of standards. Sugar concentration is expressed as mg sugar per g dry mass (mg g^{-1}).

Enzyme activity

Protein extraction was based on the method of Kanellis and Kalaitzis (1992). Protein quantification was done using the Bradford micro-assay (Bradford, 1976) and a calibration curve using bovine serum albumin applied.

Oil concentration

Oil concentration was measured using the method of Meyer and Terry (2008).

Near-infrared spectroscopy

Fruit were scanned before and after storage using a Foss NIRS 6500 spectrometer (Foss NIR Systems, Silver Spring, USA). To calculate water loss during storage, the water content of individual fruit was quantified using the method of Blakey *et al.* (2009).

RESULTS AND DISCUSSION

Figure 1 shows the trends of the individual sugars and total protein in the mesocarp of unstored fruit during ripening. Mannoheptulose and protein declined rapidly during the first week and then remained fairly constant at 1–2 mg g^{-1} DM. Sucrose declined slightly during the first three days and thereafter remained at about 3 mg g^{-1} DM. Glucose and fructose were present in very low concentrations at harvest but increased slightly during ripening to reach approximately 2 and 1 mg g^{-1} DM, respectively. Total protein increased from about 15 mg

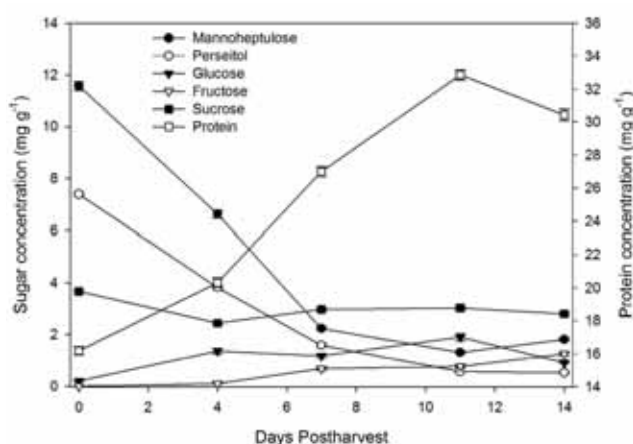


Figure 1. Sugars and protein concentration in the mesocarp of unstored fruit during ripening.



g^{-1} DM, reaching a peak of 33 mg g^{-1} DM at 11 days post-harvest and then declined to 30 mg g^{-1} DM. Glucose is unlikely to be the major stored energy source of avocados because the concentration increases during ripening. If glucose was the stored energy source it would decline, considerably. This trend is observed in the heptose sugars mannoheptulose and perseitol, implying that these sugars are the predominant energy sources in avocado (Liu *et al.*, 1999). These fruit have previously been linked to fruit quality (Bertling & Bower, 2005). Mannoheptulose also functions as the major anti-oxidant in the mesocarp (Tesfay, 2009), thus maintaining the concentration of these sugars is critical for fruit quality.

The effects of the storage treatments are illustrated in **Figure 2**. The 1°C and MA storage both reduced the respiration rate, ethylene production rate, mass loss, water loss and heptose consumption. The MA storage also reduced protein synthesis and external chilling injury. These two treatments together appear to be an effective means of maintaining fruit quality. The combination of these treatments negated the symptoms of long term cold storage: the reduced storage temperature reduced the respiration rate and the MA storage reduced desiccation (and thereby external chilling injury), but had a minimal effect on the ripening rate, only prolonging ripening by one to three days. Fructose and glucose both increased during storage, this being most evident in the conventionally stored fruit (5.5°C and unwrapped). Sucrose declined during storage, but there was no significant difference between treatments. The combination of 1°C and MA storage consistently resulted in the best quality fruit. This treatment needs to be compared to that of using 1-MCP.

While this treatment does not address the effects of post-harvest stress, it does block ethylene reception which could be expected to alter the ripening physiology. Previous work by Pesis *et al.* (2002) indicated that the presence of ethylene during storage does adversely affect fruit quality.

Enzyme activity generally increased rapidly after cold storage, implying some activity during storage and explaining why stored fruit ripen faster than unstored fruit. To prevent softening during storage, the activity of cellulase and PG needs to be minimised, because these enzymes break down the cell wall – PME prepares pectin for hydrolysis by PG. This is best achieved by the 1°C , MA storage treatment combination. Fruit stored at this treatment regime had no significant increase in cellulase activity (**Figure 3A**) after removal from storage. There was no significant difference between the treatments, with regards to polygalacturonase activity, although the maximum activity was higher in stored fruit. PME activity was generally constant within the MA stored fruit but fluctuated greatly in the unwrapped fruit. The activity in fruit stored at 5.5°C increased, while in those stored at the lower temperature the activity declined considerably.

There was no significant difference between the two storage temperatures with regards to cellulase activity, but there was between unwrapped and wrapped fruit (**Figure 4**). Unwrapped fruit had a higher activity immediately after storage, which was sustained throughout ripening, until ripeness. The wrapped fruit had no significant difference to the unstored fruit immediately after cold storage, but had a significantly higher activity during ripening compared to the unstored fruit.

The trends for oil concentration from each harvest

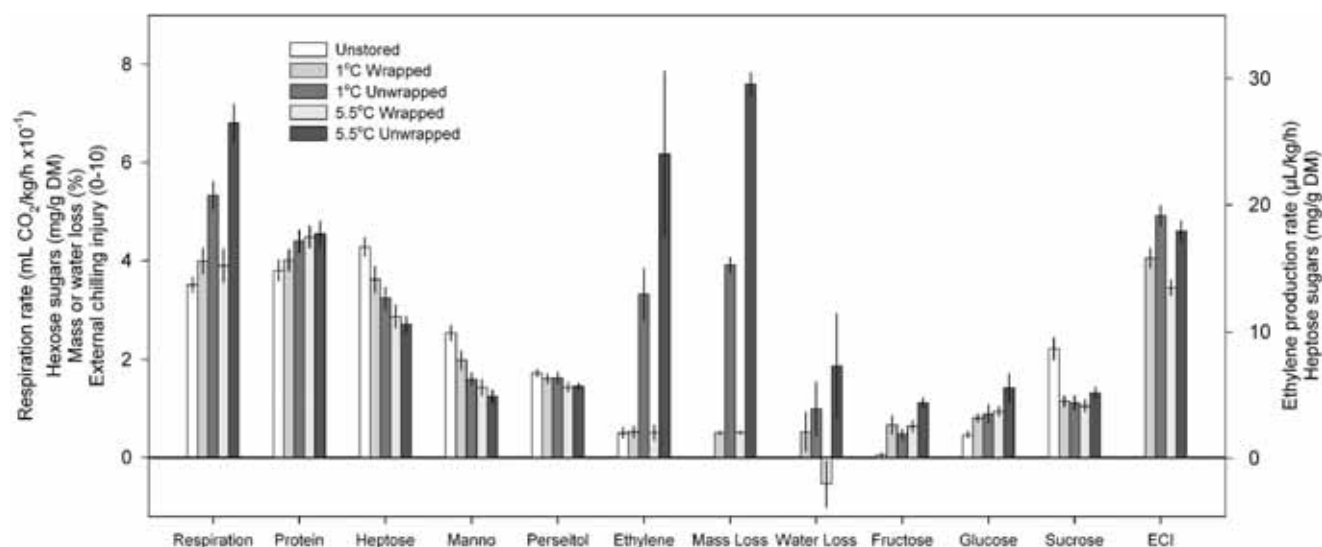


Figure 2. A range of parameters measured after harvest and after storage at 1.0 or 5.5°C and wrapped in polybags or not.

*Manno: mannoheptulose

ECI: External chilling injury

Table 1. Difference (percentage) between predicted and actual water content for 'Hass' and green skin cultivars, and local and transported fruit.

	'Hass'	Green skin	Local	Transported
Predicted-actual	1.79	1.47	0.87	2.72



are similar and thus the results in **Figure 5** are combined from six harvests. The oil concentration of un-stored fruit increased exponentially from 32% DM to 41% DM, but only increased slightly during the first week (**Figure 5**). This may be because of improved extraction efficiency or a relative increase as the cell wall is hydrolysed. The storage treatments resulted in aberrant trends but the MA storage had similar trends to the unwrapped fruit having a higher terminal concentration compared to the wrapped fruit.

The near-infrared spectroscopy model developed in 2007 and 2008 to measure water content, was validated in 2009. It was found that fruit that was measured on the day of harvest had the most accurate results. This has logistical implications if this technology is to be implemented commercially. There did not seem to be a difference between 'Hass' and green skin culti-

vars, although 90% of the fruit used in the model were 'Hass' (**Table 1**). The instrument operated in reflection mode, so the penetration depth of the NIR was only a few millimetres and affected by the exocarp. A transmission or intercanthance data collection method would be more suitable for fruit (Blakey *et al.*, 2008). This will be attempted in the 2010 season on a semi-commercial basis.

In conclusion, cold storage conditions need to: i) minimise the consumption of heptose sugars, ii) minimise protein synthesis, especially the enzyme cellulase, and iii) minimise water loss which will lead to stress and increased ethylene synthesis. Utilising cost effective cold storage regimes to achieve these objectives will increase fruit quality and profitability of the avocado industry. For 'Hass' fruit, storage at 1°C would appear to achieve these objectives. However, a direct comparison between the 1°C protocol in conjunction with MA storage and the presently commercially used 1-MCP would be useful. Practical options of using polypropylene wrapping need to be found before this can

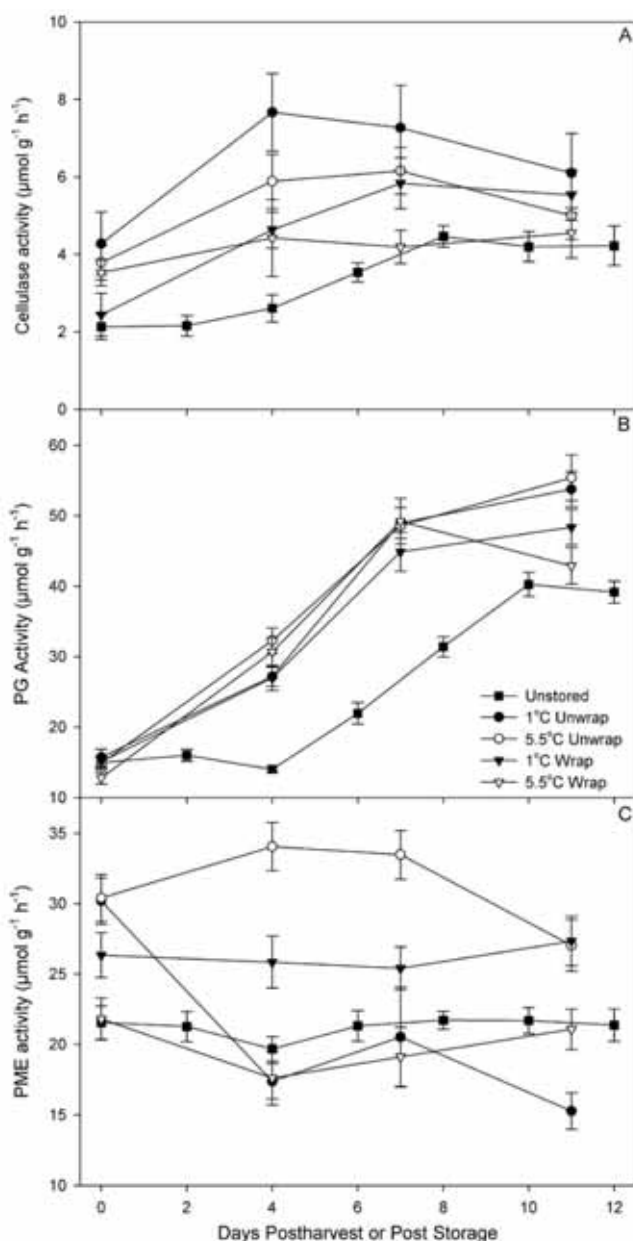


Figure 3. The activity of cellulase (A), polygalacturonase (B) and pectin methylesterase (C) for fruit stored for 28 days at 1.0 or 5.5°C and wrapped in polybags or not, during ripening.

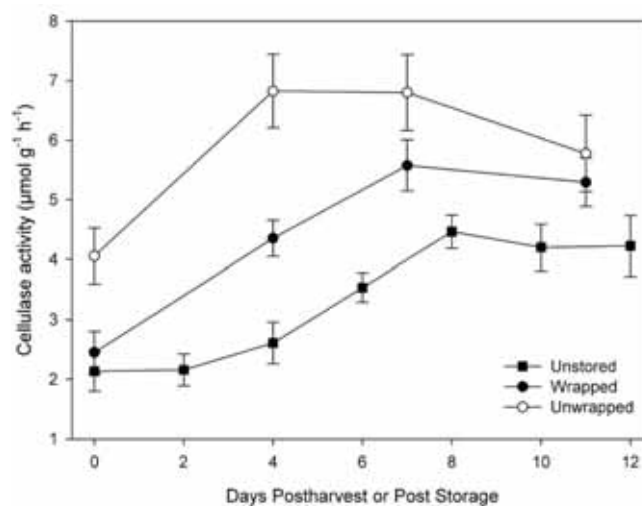


Figure 4. Cellulase activity of unstored fruit, fruit wrapped in polybags and fruit not wrapped in polybags.

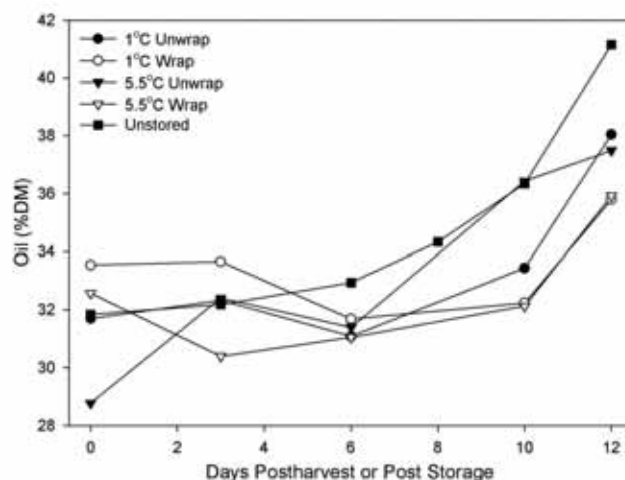


Figure 5. Oil concentration of unstored fruit, fruit wrapped in polybags and fruit not wrapped in polybags.



be used commercially. This could be carton wraps – similar to table grapes – or a pallet wrap.

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