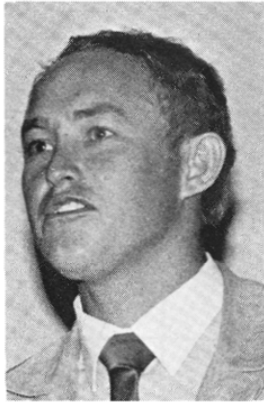


PROGRESS REPORT THE EFFECT OF STORAGE ON RESPIRATION, MITOCHONDRIAL AND LIPOXYGENASE ACTIVITY OF FUERTE AVOCADO FRUITS



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OPSOMMING

Die avokados wasgestoor by aërobiese en amperanaërobiese toestande vir 5, 10 en 15 dae by 5° en 25 °C. Na hierdie periodes was die vrugte vir 12 h by 25 °C geplaas voordat waarnemings gemaak is. 'n Klimakteriese respiratoriese styging kon waargeneem word slegs by vrugte wat aërobies by 25 °C gehou was. Beson-dere hoë RK-waardes (>0,8) was gevind by vrugte wat anaerobias by 25 °C gestoor is.

Mitochondriale aktiwiteit in terme van ADP: O-verhouding RCR-waardes en stadium 3-respirasie was laag in alie gevalle. In die algemeen was suksinaat 'n beter substraat vir mitochondriese respirasie as malaat of NADH. Respirasie met alie substrate was sianiedsensitief.

SHAM-sensitiwiteit net slegs voorgekom met suksinaat as respiratoriese substraat. Hierdie sensitiwiteit het ontwikkel tydens die stoortyd.

Lipo-oksigenase-aktiwiteit was hoër by pH 9 as pH 6. Hoër waardes was waargeneem by vrugte gestoor onder anaërobiese toestande waar 'n piek waargeneem kon word na 6 dae stoortyd. Onder aërobiese toestande het die pH 6-lipo-oksigenase-aktiwiteit langsaam toegeneem maar die pH 9-aktiwiteit effens afgeneem of nie verander nie. Koueskade kon nie waargeneem word nie en kon dus nie in verband gebring word met die metings wat gemaak is nie.

SUMMARY

In the main study fruits were stored at near anaerobic and aerobic conditions at 5 °C for 5, 10 and 15 days. After each period fruits were placed at 25 °C for 12 h before making measurements. A climacteric respiratory rise was only observed in fruits stored aerobically at 25 °C. Extremely high RQ-values (>8,0) were found in fruits stored anaerobically at 25 °C.

Mitochondrial activity in terms of ADP: An O ratio, RCR-values and State 3 respiration

was poor in all cases. In general succinate was a better substrate for mitochondrial respiration than malate or NADH. Respiration with all substrates was CN-sensitive, SHAM-sensitivity was only observed with succinate as respiratory substrate. This sensitivity developed with increased storage time.

Lipoxygenase activity at pH 9 exceeded pH 6 activity. Higher values were recorded under anaerobic condition where peaking was observed after 10 days storage. Under aerobic conditions pH 6 activities increased steadily whereas pH 9 activity decreased slightly or showed no change.

Chilling injury was not observed and could therefore not be related to the activities measured.

INTRODUCTION

Fruit discolouration is a major problem in the avocado Industry. Pulp spot, lead discolouration, vascular browning and cold damage have been listed as post harvest disorders (Bezuidenhout, 1983). We are not convinced that a clear cut distinction between these disorders can be made. The fundamental reason and mechanism of fruit discolouration is unknown. Various studies have implicated mitochondrial changes during the climacteric cycle of avocados and possible correlation with chilling injury (Koslyachinda & Young 1977). In apples Lipoxygenase activity has been correlated with physiological disorders developing during storage (Feys era/1980).

It was originally envisaged to study the respiratory metabolism of avocado fruits during pulp spot development. The incidence of this disorder was, however, very low in the 1983 crop. For this reason emphasis was placed on changes occurring in respiration, mitochondrial and Lipoxygenase activity and Fuerte avocados during aerobic and anaerobic storage at 5 and 25 °C.

MATERIALS AND METHODS

Material

Initial studies were performed on Fuerte avocados produced on the experimental research farm of the University of Pretoria. Storage experiments were conducted on fruits obtained from a commercial orchard in the Hartebeespoort dam area.

Storage

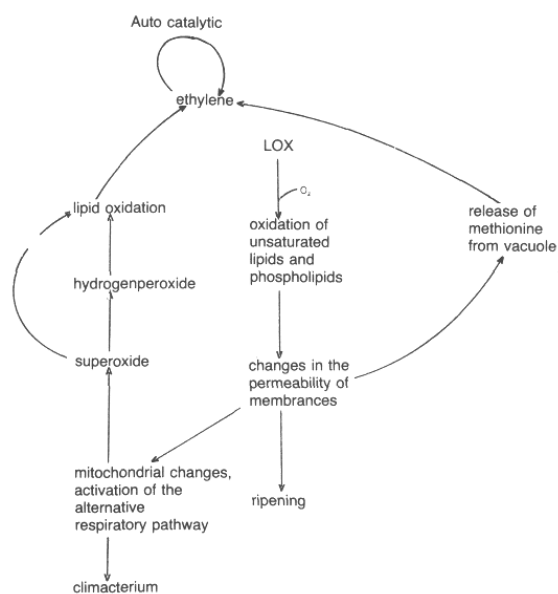
Storage experiments commenced approximately 1 h after picking. Pedicels were removed and the wound sealed with petroleum jelly. Fruits were stored in the dark at 5 ° and 25 ° ± 0,5 °C. For anaerobic storage, fruits were placed singly into 1 dm³ Consol preserving jars and sealed with gas tight lids. Anaerobic conditions were thus created by the respiratory activity of fruits. For aerobic storage, jars were left open. Details of storage experiments will be given in the results section.

TABLE 1. Effect of storage conditions on mitochondrial activity.

		RESPIRATORY SUBSTRATES												
		MALATE					SUCCINATE				NADH			
	Storage temp	Storage time (days)	State 3 resp nmo10 ₂ ₂₁ mgprot ⁻¹ min ⁻¹	RCR	Sensitivity to KCN	SHAM	State 3 resp nmo10 ₂ ₂₁ mgprot ⁻¹ min ⁻¹	RCR	Sensitivity to KCN	SHAM	State 3 resp nmo10 ₂ ₂₁ mgprot ⁻¹ min ⁻¹	RCR	Sensitivity to KCN	SHAM
Anaerobic	5 °C	5	7,9	1,06	+	-	15,3	1,5	+	-	7,4	1,0	+	-
		10	38,2	1,6	+	-	28,7	1,3	+	-	0	0	0	0
		15	9,0	1,4	+	-	10,7	1,6	+	+	7,3	1,0	+	-
	25 °C	5	0	0	0	0	9,8	1,5	+	-	0	0	0	0
		10	0	0	0	0	8,0	1,7	+	-	0	0	0	0
		15	5,5	2,03	+	-	6,7	1,0	+	+	6,9	1,0	+	-
Aerobic	5 °C	5	4,5	1,4	+	-	23,0	1,6	+	-	0,9	1,0	+	-
		10	17,1	1,4	+	-	11,17	1,7	+	-	14,3	1,0	+	-
		15	10,5	1,6	+	-	6,9	1,3	+	-	2,4	1,0	+	-
	25 °C	5	0	0	0	0	7,8	1,2	+	-	0	0	0	0
		10	17,2	1,5	+	-	30,6	5,3	+	-	9,7	1,0	+	-
		15	0	0	0	0	12,32	1,3	+	+	10,9	1,0	+	-

TABLE 2. Effect of storage conditions on lipoxygenase activity.

		Storage temp	Storage time (Days)	Lipoxygenase activity, (nmo10 ₂ mg prot ⁻¹ min ⁻¹)		
				pH 6	pH 9	Total
Anaerobic	5 °C	5	4,3	7,1	11,4	
		10	6,1	13,7	19,8	
		15	5,0	9,0	14,0	
	25 °C	5	2,4	10,6	13,0	
		10	11,1	16,2	26,3	
		15	5,8	10,6	16,4	
Aerobic	5 °C	5	2,8	12,9	15,7	
		10	3,9	9,1	13	
		15	6,2	12,3	18,5	
	25 °C	5	0,9	12,8	13,7	
		10	2,3	2,5	4,8	
		15	3,8	8,4	12,19	



Changes and pathways during climacterium and ripening.

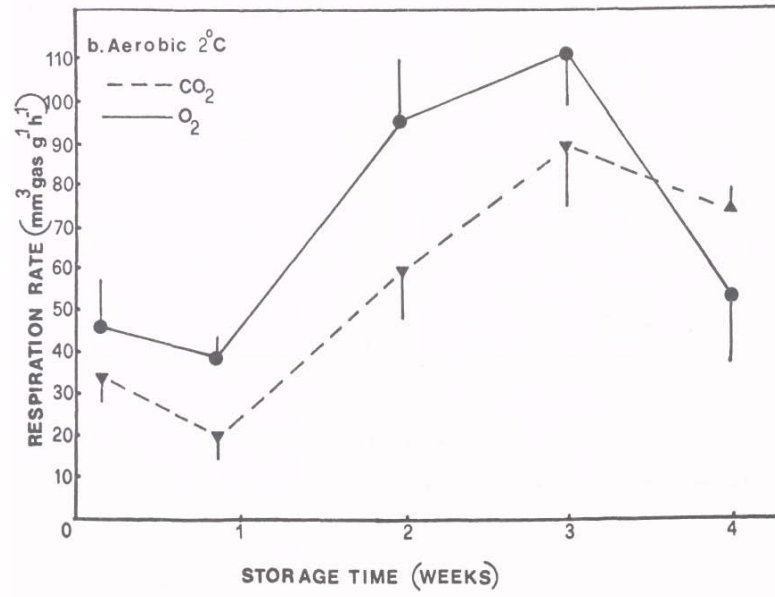
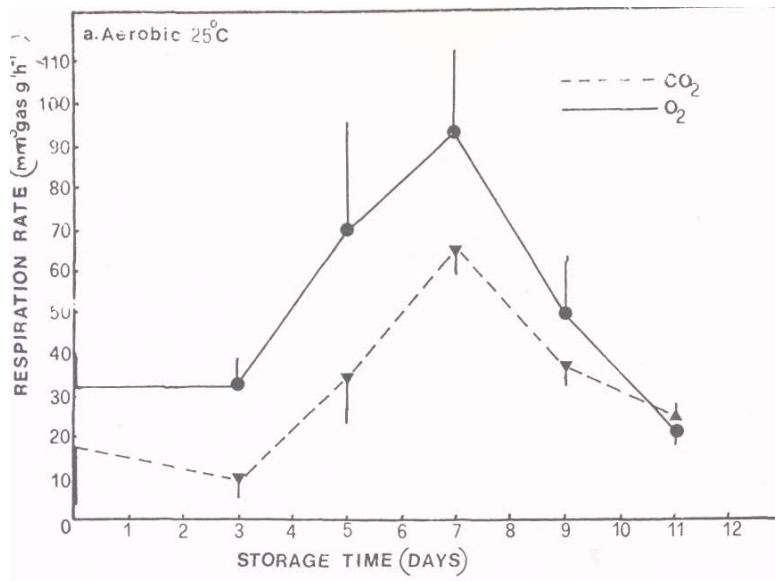


Fig. 1 Effect of storage temperature on respiratory rate

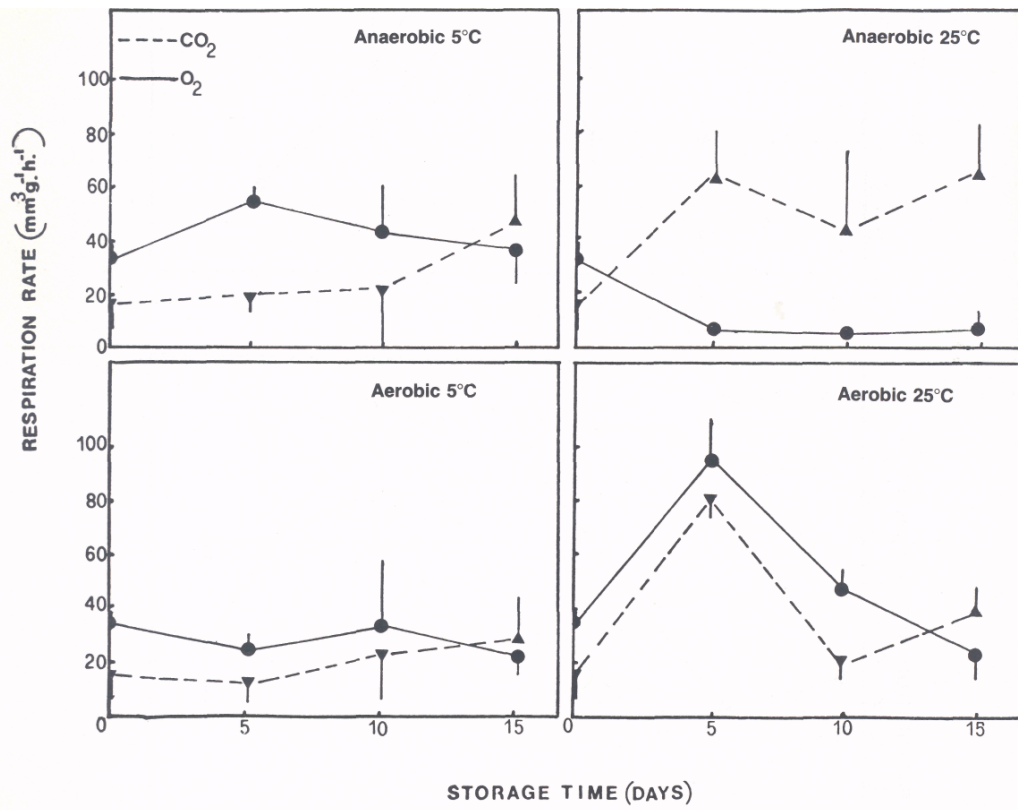


Fig. 2 Effect of storage conditions on respiration rate

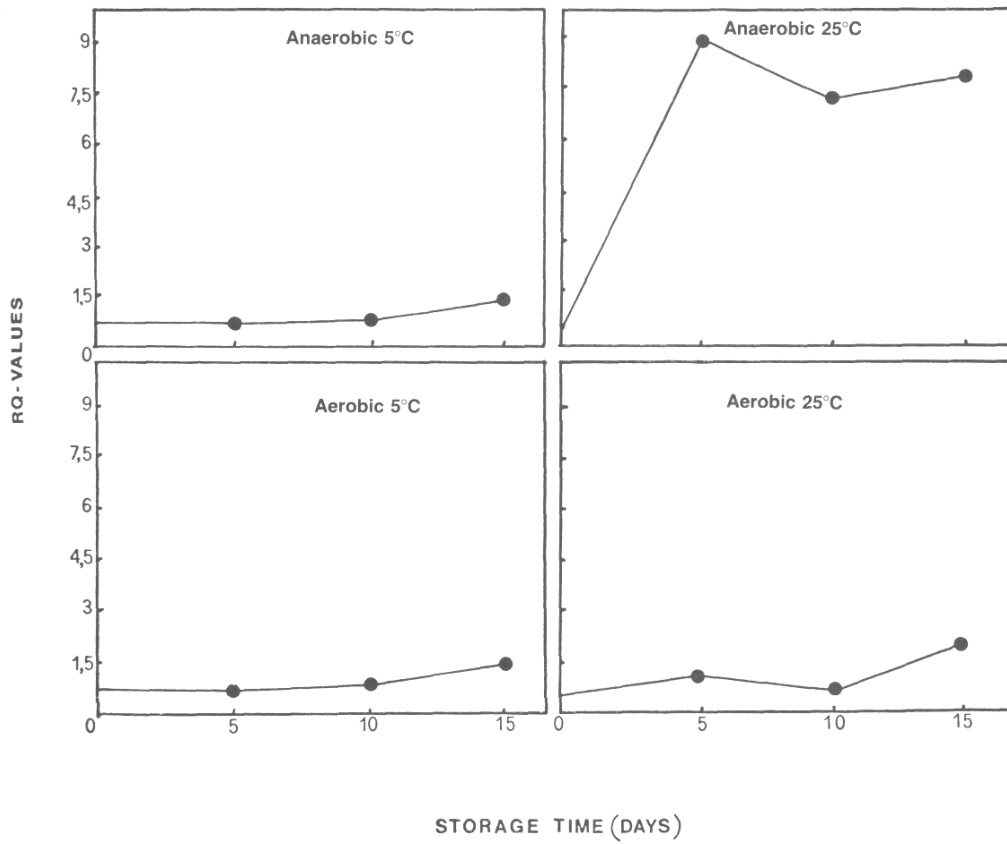


Fig. 3 Effect of storage conditions on respiratory quotient (RQ)

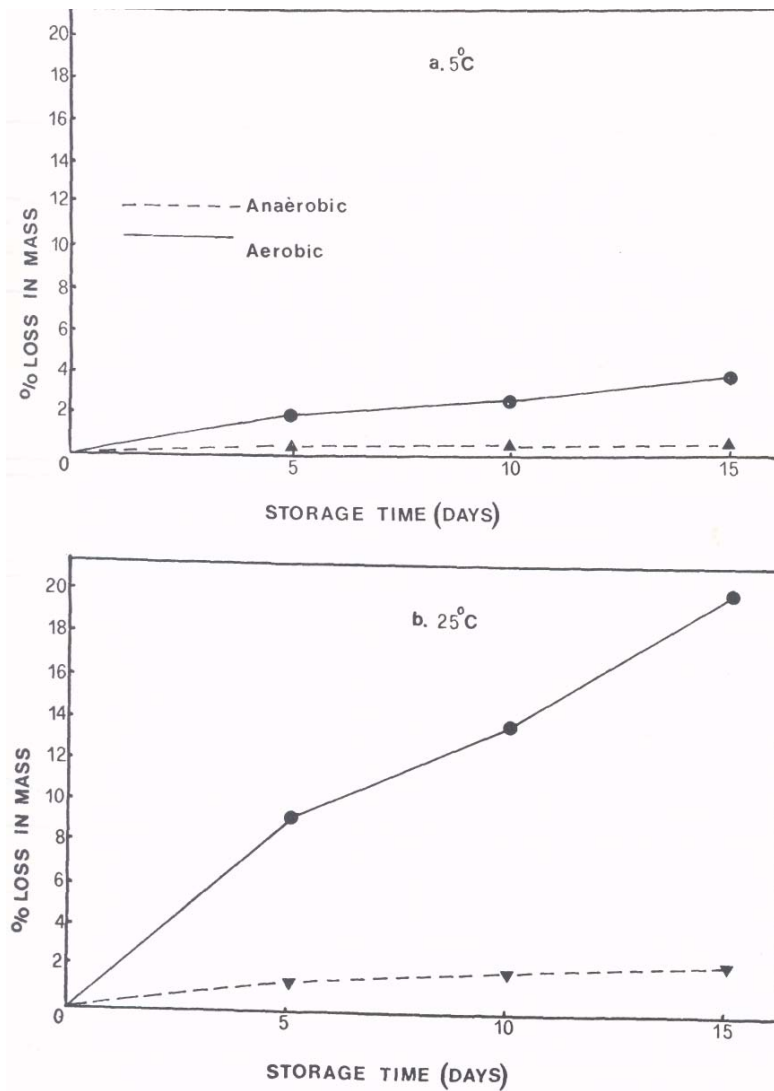


Fig. 4 Effect of storage conditions on loss of fruit mass

Respiration

Respiration of whole fruits was determined manometrically at 25 °C using a self constructed differential respirometer.

Preparation of mitochondria

Avocado tissue (100 g) was homogenized in 200 cm³ extraction buffer in a Waring Blendor and mitochondria prepared according to the method of Day & Wiskich (1974). Their extraction buffer was modified to contain 0,4 mol dm⁻³ sucrose.

Oxygen uptake of mitochondria was measured polar graphically using a Clark electrode and reaction buffer as formulated by Miller & Obendorf (1981).

Solutions of malate, succinate and NADH and ADP were prepared in reaction buffer. KCN was applied as an aqueous solution while salicyl hydrox amic acid (SHAM) was dissolved in dimethylformamide.

Lipoxygenase (LOX)

Supernatants after extraction of mitochondria were used to determine LOX activity polarographically according to the method of Grossman & Zakut (1977). Activities at both pH 6 and pH 9 were determined.

Protein

Mitochondria and supernatant protein was determined as described by Bradford (1976).

RESULTS AND DISCUSSION

Respiration

In preliminary studies the respiratory pattern of fruits (ex University of Pretoria) stored aerobically at 25 °C and 2 °C was compared. Gas exchange was measured at 25 °C and the same fruits were used during the whole study.

Fruits stored at 2 °C were allowed to equilibrate at 25 °C for 2 h before gas exchange determination were made, where after they were again stored at 2°C.

Fruits held continuously at 25 °C developed a climacteric peak for gas exchange after 7 days of storage (Fig 1a). The climacteric peak was delayed considerably (3 weeks) in cold stored fruits (Fig 1 b). It should be remembered that these fruits were exposed for short periods (3 h) to a temperature of 25 °C each week when measurements were made. This could have contributed to the ultimate attainment of a climacterium in these cold stored fruits.

In a second experiment fruits were stored in sealed (anaerobic) and unsealed jars at 5 ° and 25 °C. Twelve hours before measurements, fruits were placed at 25 °C under aerobic conditions. This was done to mimic the practical situation in which cold stored fruits (during long distance transport) are placed on the shelf at room temperature before sale.

Over a 15-day period a climacteric peak was only observed in fruits stored aerobically at 25 °C (Fig 2). A fairly constant rate of gas exchange was observed in fruits stored an aerobically and aerobically at 5 °C. The lack of a climacteric rise in gas exchange in these fruits is consistent with the results of other workers (Baile & Young 1971).

Despite the fact that fruits experienced aerobic conditions at 25 °C for 12 h prior to measurements, fruits stored an aerobically at 25 °C showed an apparent markedly higher CO₂ release rate than the rate of O₂ uptake. This led to very high apparent respiratory quotient (RQ) values in these fruits. The high RQ-values could be indicative of ethanol fermentation (Fig 3).

However, avocados appear unable to switch to fermentative metabolism when deprived of oxygen (Baile & Young 1971). This phenomenon therefore requires further study.

Change in mass

In the above study the mass of fruits was determined at the start of the storage experiment and again when respiration was measured.

From Fig 4 it is evident that little mass change occurred when fruits were stored aerobically and an aerobically at 5 °C and an aerobically at 25 °C. Fruits stored in open containers at 25 °C, however, showed a decrease of more than 20% in mass over a 15

day period. This was probably mainly due to moisture loss.

Mitochondrial activity

In order to test the efficiency of the extraction and method of activity determination avocados were obtained from the University of Pretoria experimental farm and softened at 25 °C aerobically. Mitochondria were isolated as fruits were entering the climacteric rise. Only NADH and succinate were used as respiratory substrates. Both of these were utilized well and strong coupling was observed. ADP: O ratios of 2,18 with NADH and 2,17 with succinate were obtained. From this it was concluded that the technique for studying mitochondrial activity was satisfactory.

Subsequently mitochondria! and LOX activity were studied in fruits stored aerobically and an aerobically as in the previous experiment.

As seen from State 3 respiratory rates and RCR-values NADH appeared a poor respiratory substrate and in no case could coupling be shown with NADH (Table 1). These results were in total contrast to the results obtained with University of Pretoria fruits.

In general succinate was utilized better than malate and NADH. Judged by mitochondrial activity fruits stored at 25 °C an aerobically appeared in a worse condition than those from other treatments.

The most interesting finding was that SHAM-sensitivity was detected only when succinate was used as respiratory substrate. This sensitivity developed with increasing storage time. Respiration supported by malate and NADH appeared to be sensitive to KCN only.

It is generally assumed that the pathway for electron flow from ubiquinone onwards is the same for the substrates malate, NADH and succinate (Goodwin & Mercer, 1983). The present results, however, indicate that this need not be the case.

Lipoxygenase activity

Both pH 6 and pH 9 activity was detected (Table 2). In contrast to what is generally found, pH 9 activity exceeded pH 6 activity. The pH 9 isoenzymes appear to be mainly present in the vacuole (Wardale & Lambert, 1980) while the pH 6 isoenzymes are most probably membrane bound (Feys *et al* 1982).

Under anaerobic conditions both activities showed a peak after 10 days storage with the pH 6 isoenzymes showing the largest increase in activity.

Under aerobic storage pH 6 activity showed a steady rise while pH 9 activity decreased or showed little change. These results could indicate a rise in membrane-bound Lipoxygenase activity which could result in an enhanced oxidation of unsaturated membrane lipids.

Fruit condition

Fruits stored an aerobically remained fairly hard. At 25 °C these fruits lost their green colour, becoming brown and "glassy".

It is interesting that highest LOX activities were recorded in these fruits. According to Feys *et al* (1980) Lipoxygenase may be a key enzyme in oxidation reactions of

structural membrane lipids, determining the onset of senescence in apples, and this might well be the case in avocados.

Apart from a loss in green colour fruits stored anaerobically at 5 °C appeared fairly normal. Fruits stored anaerobically at 5 °C were softer than their aerobic counterparts but appeared sound. Fruits stored anaerobically at 25 °C appeared to ripen normally being totally overripe by the 15th storage day.

Typical cold injury symptoms were not observed. It was therefore not possible to relate disorders pertaining to storage to the various activities measured in this study.

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