

CAN DYNAMIC CONTROLLED ATMOSPHERE STORAGE BE USED FOR 'HASS' AVOCADOS?*

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ABSTRACT

There is renewed interest in dynamic controlled atmosphere (CA) storage of fruit, where the O₂ level changes according to fruit response, as an alternative to static CA, where the O₂ level remains fixed during storage. The objective of this study was to assess whether dynamic CA can be used for New Zealand 'Hass' avocados by determining the changes in ethanol accumulation and chlorophyll fluorescence (CF) in response to low O₂ or high CO₂ atmospheres at storage or chilling temperatures.

The accumulation of ethanol in mesocarp tissue was measured for fruit exposed to O₂ atmospheres of 0.1, 0.5, 1, 2, 5, 10 or 21% O₂ (balance N₂), or 0, 1, 2, 5, 10 or 20% CO₂ (balance air) at 5°C. The effect of the level of O₂ on ethanol in fruit removed from stress inducing atmospheres was determined by exposing fruit to 0.1% O₂ for 24, 48, or 96 hours, then transferring back to atmospheres of 2, 5 or 21% O₂. Ethanol was not detected in fruit held in O₂ between 0.5 and 21%, but a marked accumulation of ethanol occurred in fruit held in less than 0.5% O₂. Ethanol accumulated markedly in fruit held in 0.1% O₂ at 5°C to approx. 2, 14 and 20 nmol/g FW after 24, 48 and 96 hours, respectively. Levels of ethanol in these fruit generally decreased to trace levels after 24, 48 or 96 hours respectively, when transferred to non-stress atmospheres regardless of the O₂ level in the non-stress atmosphere. Ethanol did not accumulate in fruit exposed to atmospheres of 0, 1, 2, 5, 10 or 20% CO₂.

The CF yield ($(F_m - F_0) / F_m$) was measured for fruit from 3 orchards exposed to atmospheres of 0.3, 0.5, 0.8, 1, 2, 5 or 21% O₂, (balance N₂), or 0, 2, 5, 10, 15 or 20% CO₂ (balance air) at 0° or 6°C. CF yield remained at approx. 0.8 for fruit at 6°C held in O₂ between 21 to 1%, but yield decreased sharply to 0.68 within 1 day of exposure to <1% O₂. When fruit held in <1% O₂ for 6 days were returned to air at 6°C, CF yield recovered from approx. 0.67 back to 0.8. CF yield decreased slightly when levels of CO₂ were greater than 5%, but recovered to approx. 0.8 on return to air after 6 days at 6°C. However, at 0°C the decreased CF yield became more marked with increasing levels of CO₂. Fruit from the three orchards had similar CF responses within all treatments.

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It is concluded that the physiological behaviour of New Zealand 'Hass' avocado, and in particular the kinetics of ethanol accumulation and CF yield responses, makes dynamic CA commercially realistic.

Keywords: dynamic controlled atmosphere storage, ethanol, chlorophyll fluorescence, non-destructive sensor, physiological stress

INTRODUCTION

New Zealand exports 'Hass' avocados to overseas markets by sea primarily in refrigerated holds but also in integral shipping containers. Depending on existing markets the ability to store fruit for 14 to 28 days is necessary, and if New Zealand is to supply fruit to more distant markets, even greater storage life will be needed. Some New Zealand avocados are transported in controlled atmosphere (CA) shipping containers and this form of shipping may increase when supplying fruit to the more distant markets. Although CA conditions vary with the type of container or vessel used, atmospheres typically range from 2 to 5% O₂ and 3 to 10% CO₂. Usually the O₂ and CO₂ levels are fixed, i.e. they are static, throughout the voyage.

A trend in CA storage is to consider the use of dynamic rather than static atmospheres (Van Schaik and Verschoor, 2003). For dynamic CA the atmosphere is changing (dynamic) rather than being static throughout storage. Specifically, the O₂ level is set according to a fruit response, which usually changes during storage, hence O₂ levels also change during storage. For dynamic CA to operate a non-destructive sensor is required to detect real-time changes in the physiological response of the fruit to the set atmosphere. The sensor needs to be sensitive enough to detect physiological change before irreversible low O₂ damage occurs to the fruit. Ultimately, the sensor could be linked to a control system capable of automatically adjusting the O₂ atmosphere in a CA room during storage to maximise the effect of low O₂ on fruit quality.

The detection of ethanol (EtOH; Veltman *et al.*, 2003), and more recently chlorophyll fluorescence (CF) (Prange *et al.*, 2002, 2003) has been evaluated as useful sensors for dynamic CA. To assess whether dynamic CA could be used for New Zealand 'Hass' avocados, two studies were undertaken in which fruit were held in a range of static O₂ or CO₂ levels for different durations and then the stress atmosphere returned back to 2% O₂, 5% O₂, or air.

In the first study, changes in EtOH in the fruit were determined during induction and recovery from stress-inducing atmospheres at 5°C. In the second study, changes in CF yield of fruit were determined during induction and recovery from stress-inducing atmospheres at 6°C and 0°C. The two studies were used to compare the potential for EtOH and CF to be used as sensors for dynamic CA.

MATERIALS AND METHODS

For the EtOH study, freshly harvested 'Hass' avocados from one orchard were cooled to 5°C and held in humidified atmospheres of 0.1, 0.5, 1, 2, 5, 10 and 21% O₂ (balance N₂), or 0, 1, 2, 5, 10 and 20% CO₂ (balance air) for up to 504 h to quantify EtOH induction. EtOH in the flesh was determined at intervals by removing subsamples of 5 fruit per treatment and immediately excising two plugs (approx. 2 g) of tissue from endocarp tissue on opposite sides of each fruit. The plugs were placed in a 60 ml plastic syringe, the void volume adjusted to 10 ml, capped with a rubber septum, and a vacuum established by increasing the void volume to 60 ml. After 1 min the vacuum was released and a 1 ml sample of the headspace analysed for EtOH using a FID gas chromatograph (Pye Unicam PU4500, UK) fitted with a 1.5 m Haysep N column (Alltech Associates, USA). In a separate experiment, fruit were held in 0.2% O₂ for 24, 48, or 96 h, then the O₂ backed off to 2, 5 or 21% O₂ to quantify EtOH recovery.

For the CF study, freshly harvested 'Hass' avocados from 3 orchards were cooled to 0° or 6°C, enclosed in clear plastic film bags which were vented (approx. 150 cm³/min) with humidified atmospheres of 0.3, 0.5, 0.8, 1, 2, 5, 21% O₂ (balance N₂), or 0, 2, 5, 10, 15, 20% CO₂ (balance air) for 6 days. To quantify change in CF during this induction phase, a pulse amplitude modulated fluorimeter (WALZ Mini-PAM, Germany) was used to measure CF yield ((F_m-F₀)/F_m) daily. A preliminary experiment had determined that the F₀ and F_m measurements were slightly lower when measured through the plastic film, but the CF yield was unaffected. Measurements were repeated at 24 h intervals at the same location on 5 dark-adapted fruit per orchard. To quantify change in CF of fruit on removal from stress-inducing atmospheres, the atmosphere in the bags was changed to air for a further 8 days at 0° or 6°C, and finally to air for 2 days at 20°C. The fruit were assessed for external chilling injury and low O₂ injury at the end of the experiment.

RESULTS

The level of EtOH increased markedly in fruit held in less than 0.5% O₂ for 48 h, whereas only basal levels of EtOH (0.02 nmol / g fresh weight (FW)) were measured in fruit held in 0.5 to 21% O₂ (Figure 1A). Levels of up to 20% CO₂ had no marked affect on EtOH accumulation (Figure 1B). The pattern of accumulation of EtOH over time was typical of growth or S-type curves (Figure 1C and D). The maximum EtOH level produced increased with decreasing O₂ level below 0.5%. When fruit were transferred to air after a 24, 48 and 96 h induction period accumulated EtOH decreased markedly to basal levels within a further 24, 48 or 96 h, respectively. When the O₂ atmosphere was backed off to 2 or 5% O₂ there was an initially delay in the decrease in EtOH that had accumulated over the 96 h followed by a rapid decrease in EtOH to similar or slightly higher levels than fruit backed off to air (Figure 1D).

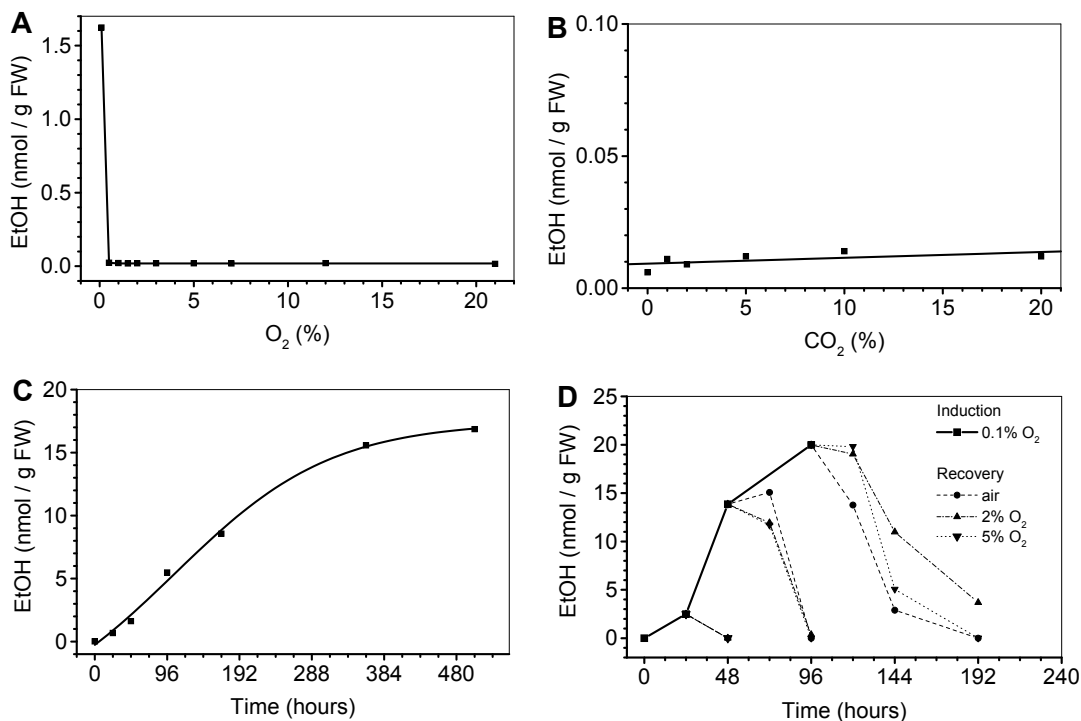


Figure 1. Ethanol (EtOH) levels in ‘Hass’ avocados held at 5°C for 48 h in 0.1 to 21% O₂ (A), or in 0 to 20% CO₂ in air (B), or in avocados during 0 to 504 h in 0.3 % O₂ at 5°C (C), or recovery in EtOH levels in avocados transferred to air, 2 or 5% O₂ after exposure to 0.1% O₂ for 24, 48 or 96 h at 5°C (D); n = 5 fruit.

The CF yield (0.8) decreased slightly over 6 days for fruit held in air (21% O₂, 0% CO₂) at 6°C over time (Figure 2A and 2B). The CF yield decreased markedly at atmospheres of less than approx. 0.8% O₂. Transfer of fruit from the various O₂ levels to air at 6°C resulted in a recovery in the yield values from approx. 0.67 to approx. 0.78 for fruit that had been held in less than 0.8% O₂, and to similar or slightly lower CF yield (approx. 0.76) when these fruit were finally transferred to air at 20°C. The level of CO₂ did not markedly affect CF yield for fruit held at 6°C, though there was a trend for slightly lower CF yield with increasing CO₂ level, and CF yield was not affected when fruit were transferred to air at 6° or 20°C (Figure 2B).

CF was affected by chilling temperature with the CF yield decreasing over time for fruit held at 0°, and particularly for fruit in 0.3% O₂ (Figure 2C). For fruit held at 0°C, increasing the level of CO₂ and duration resulted in even lower CF yields (e.g. approx. 0.61 after 6 days at 15 or 20% CO₂). The difference in CF yield between the 3 orchards was approx. 0.044 for fruit held for 20 h in air at 6°C

(Figure 2D). However, the difference in CF yield between orchards was less (approx. 0.004 to 0.020) for fruit held in atmospheres less than 2% O₂ at 6°C.

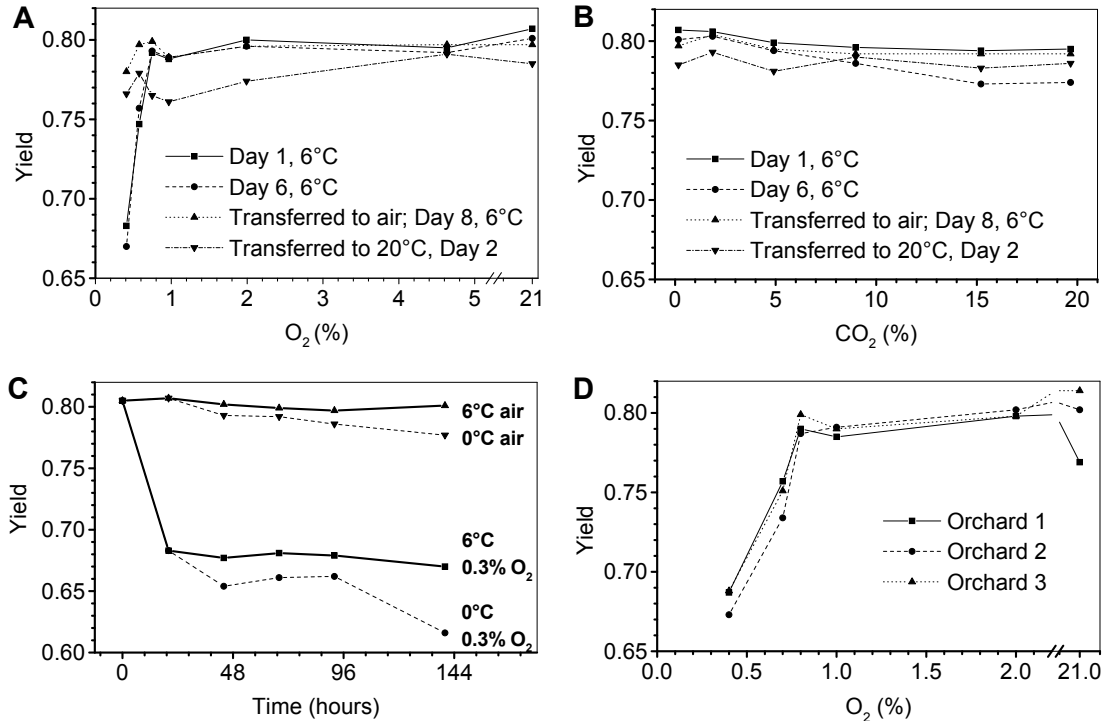


Figure 2. Chlorophyll fluorescence yield ($(F_m - F_0) / F_m$) of dark-adapted ‘Hass’ avocados during storage at 6° or 20°C in 0.3 to 21% O₂ (A), or in 0 to 20% CO₂ in air (B), or of avocados held at 0° or 6°C in 0.3% O₂ or air for up to 140 h (C), or of avocados from 3 orchards held at 6°C in 0.3 to 21% O₂ for 1 day (D); n = 15 (5 fruit for each of 3 orchards) for A, B, and C, and n = 5 fruit per orchard for D.

Fruit that had been held in 0.3% O₂ for 6 days developed low O₂ injury when returned to air, with approx. 83% or 46% of fruit affected at 0°C or 6°C, respectively. Low O₂ injury was characterised by firm irregular shaped brown or light-brown discolouration of the skin predominantly at the stem-end of the fruit. External chilling injury was observed only in fruit held at 0°C, and was characterised by small irregular charcoal-black discoloured areas with sharp margins and occurred anywhere over the fruit surface.

DISCUSSION

The kinetics of EtOH accumulation and changes in chlorophyll fluorescence yield were both capable of indicating a shift in the physiological state of avocados

exposed to low O₂, and may be appropriate physiological indicators for use in dynamic CA of avocados. An ideal sensor for dynamic CA will be sensitive enough to maximise the benefit of low O₂ throughout the storage period while minimising the risk of low O₂ injury. A significant increase in EtOH occurred at a slightly lower O₂ level than the change in CF yield (approx. <0.5% and 0.8% O₂, respectively; Figures 1A and 2A). This lower O₂ level, coupled with the initial lag in EtOH accumulation for fruit held in 0.1% O₂ suggests EtOH increase may be a less responsive, or higher risk indicator of physiological changes at low O₂ than CF.

Although EtOH that accumulated in fruit exposed to 0.1% O₂ for up to 96 h dissipated rapidly when transferred to 2, 5 or 21% O₂ (study 1), low O₂ injury was detected after 6 days in fruit held at approx. 0.3% O₂ (study 2). Therefore, the O₂ level at which there was an increase in EtOH appears to be very close to O₂ levels that resulted in low O₂ injury. Additionally, studies in our laboratory indicate a change of as little as 0.1% O₂ from the stress-inducing O₂ level can rapidly and markedly affect the CF signal in 'Hass' avocado, and therefore CF may be a more responsive sensor for dynamic CA than EtOH accumulation. Furthermore, exposure of 'Hass' avocados to approx. 0.8% O₂ for 8 weeks at 5°C did not result in low O₂ injury (Yearsley, unpublished data). The variation in response between orchards to low O₂ was only tested in the CF study, and the variation was minimal at O₂ levels near stress inducing levels. Individual lines of fruit within a dynamic CA store could simply be monitored using separate CF sensors to account for any orchard-to-orchard variation.

While a correlation between CF changes and EtOH accumulation was not determined in this study a link between them has been reported for some crops (Toivonen and DeEll, 2001). However, EtOH accumulation may not always be closely linked to O₂ level, for example in apples, EtOH accumulation may result from senescence-based fermentation in fruit held above their optimum low O₂ level (Prange *et al.*, 2003). Methods for detecting EtOH using gas chromatography or solid-state sensors may be affected by fruit or store volatiles and these may diminish the selectivity of these methods for sensing low O₂ effects on fruit. CF may also be affected by stresses other than low O₂ (DeEll *et al.*, 1999). In the present study, storage temperature and CO₂ affected the CF yield of avocados in addition to low O₂. Thus, CF may be a useful tool in determining chilling stress in avocados. However, for dynamic CA of avocados at normal storage temperatures (5° to 6°C) and typical CO₂ levels of less than 10% CO₂, the change in CF signal is likely to be primarily a response to low O₂.

CONCLUSIONS

It is concluded that, the physiological behaviour of New Zealand 'Hass' avocado, and in particular the kinetics of EtOH accumulation and CF yield response to low O₂, makes dynamic CA commercially realistic. The O₂ level at which an increase in EtOH was detected was approx. 0.5% O₂, whereas a change in CF yield was

detected at approx. 0.8% O₂. EtOH that accumulated resulting from exposure of fruit to low O₂ stress may dissipate rapidly after raising the stress-inducing O₂ level. CF is likely to change more rapidly and indicate low O₂ stress at a higher O₂ level than EtOH accumulation, and therefore reduce the risk of low O₂ injury for avocados held in a dynamic CA.

ACKNOWLEDGEMENTS

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