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Physico-chemical changes of Avocado (*Persea americana* Mill cv Hass) at some stage in Controlled Atmosphere Storage at 5°C

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ABSTRACT

Physico-chemical changes of 'Hass' Avocado at some stage in Controlled Atmosphere (CA) Storage were carried out. Experimental unit of the fruit stored in CA containing 2.5, 5 % oxygen with 5 and 7.5 % carbon dioxide in all combinations (4 mixtures) and 78.5 % N_2 (control). The occurrence of CI (Chilling Injury), textural change, the rates of respiration, ethylene production and polyamines concentration was determined. Low oxygen concentration (2.5 %) at 5°C storage induced higher levels of polyamines and significantly inhibited the softening of fruit compared to fruit stored in air. Following 3 weeks storage at 5°C no indication of CI in all treatments after ripening for 6 days, but was light discoloration after 6 and 9 weeks storage and very severe in air storage

Key word:, Controlled Atmosphere, Chilling Injury, Ethylene, Polyamine.
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INTRODUCTION

Successful export marketing must depend on declining the rate of ripening satisfactorily to permit for the shipping time and arranged marketing in the importing country [4]. Storage life of avocados stored at the recommended storage temperature 4.5 - 7°C in air [36] is frequently not long enough to permit shipping by sea to intercontinental markets. Previous research with a number of methods and/or combinations of controlled atmosphere in the absence of ethylene at low temperatures can enhance the length storage life [5] Low temperature storage of avocados is someway limited by the occurrence of chilling injury (CI) [35]. The CI symptoms of avocado contain unusual respiration and ethylene production patterns and failure to soften properly upon warming after storage [32].

Polyamines appear to be potent inhibitor of senescence related processed in a plant tissue [1,8;23]. Application of polyamine has been shown to inhibit the production of ethylene in apples tissues [34] proposed that the naturally occurring polyamines may acts as modulators of some cellular and physiological processes for the period of development and ripening of avocado fruit. Low oxygen concentration (1 %) at 3 and 3.5°C storage induced higher levels of all three polyamines and significantly inhibited the softening of apples at both temperatures compared to fruit stored in air [18,9,10,11]. This opportunity has not been fully explored in avocado fruit. Such studies require to be evaluated for each cultivars to determine their specific requirement [12]. This study was to intent the effectiveness of gas mixtures containing low oxygen and high carbon dioxide concentrations (CA) for extending storage life and reducing the occurrence of CI. The occurrence of CI, textural change, and polyamines concentration was determined. A further aim was to identify possible correlations between ethylene and rate of respiration to the polyamines concentration during ripening following CA storage at low temperatures.

MATERIAL AND METHODS

Mature avocado fruit (*Persea americana* Mill cv Hass) were obtained from New Zealand, air transported to Sydney Central Fruit Market, then transport about 30 km by road to the Horticulture Laboratory, UWS-H Richmond, Australia. Fruit were then sorted for weight uniformity (200 – 250 grams), dipped in 0.2 % ‘Sportak’ fungicide solution, dried for about 30 minutes at 20^oC and then stored in 30 L polyethylene containers at 5^oC for 9 weeks.

The containers were ventilated with 78.5% N_2 (control) or CA at a flow rate of or about 12 $L.h^{-1}$. The atmospheres were generated by mixing regulated flows of air, carbon dioxide and a nitrogen enriched stream [30]. The mixture of CA were monitored with a Fruit Store Analyser type 770 L (David Bishop Instrument, Heatfield, UK) and the composition were recorded automatically at 4 hourly intervals. Experimental unit of the fruit stored in CA containing 2.5, 5 % oxygen with 5 and 7.5 % carbon dioxide in all combinations (4 mixtures) and Air (control). Samples from each atmosphere was transferred to 20^oC at 3 weeks intervals. The harvested and sampled fruit were stored singly in polyethylene container (1 L) at ambient temperatures were then ventilated at a air-flow rate of about 8 $L.h^{-1}$ and monitored for ethylene, respiration rate and polyamine until the fruit ripened. Fruit from this experiment were assessed for their ability to ripen and the incidence of CI and textural change. The rate of respiration and ethylene production of freshly harvested and CA storage of avocado were measured daily, whereas Polyamine concentration were analysed at days 0, 2, 4 and 6 at 20^oC following CA storage.

Measurement of CI was visually performed by cutting the fruit longitudinally into halves and scoring the appearances of the pulp using a scale, where 0 = no discolouration; 1= very light discolouration; 2= light discolouration; 3= medium discolouration and 4 severe discolouration (Meir *et al* ., l995: Pesis *et al*., l996). Flesh firmness was measured on two locations on each fruit with an Effegi penetrometer mounted on a drill press (12 mm tip), following removal of small pieces of skin. Firmness was expressed as newtons (Kgf x 9,807 = Newtons (N) [16].

The rate of respiration and Ethylene production were analysed by using gas chromatograph (Gow Mac Model 500, USA) with similar method to those described by Jobling [3,15]. The rate of respiration was reported as $mLCO_2$ kg/h and ethylene production of fruit tissue as $\mu LC_2 H_4$.kg/h.

Polyamine concentrations were determined at each sampling interval in pulp sections of three individual fruit used for flesh firmness according to the procedure of Kramer *et al.,* [19]

RESULTS AND DISCUSSION

Chilling Injury

The severity of CI in the flesh was examined at day 6 after transfer of the fruit to 78.5% N_2 following CA storage for 3,6 and 9 weeks. CI was not detected in fruit stored in air and/or CA fruit after 3 weeks storage. Very light discolouration was observed in fruit containing 5 % O_2 + 5% CO_2 and 5 % CO_2 + 7.5 % CO_2. After 6 and 9 weeks the fruit stored in 2.5 % O_2 combined with 5 and 7.5 % CO2, very light discoloration was observed whereas control fruit developed severe CI symptoms (Fig. 1).

These fruit reached normal colour compared to other treatment that only achieved colour score 3. Overall, these treatment (2.5 and 5 % O_2 combined with 5 and 7.5 % CO_2) gave the best result and the fruit ripened normally. Avocado cv Ettinger fruit treated with Ethrel prior to packing and air-storage developed severe CI symptoms, expressed as mesocarp discoloration after 3 weeks at 5°C [27]. The CI symptom in air storage were black lesions in the skin and grey black discoloration of the flesh. Parallel result were observed in fruit stored at 0 and 2 [13,29].

The rates of softening of the avocado fruit after transfer to 78.5% N_2 were strikingly affected by storage temperature. Fruit were fully soft following storage at CA and this fruit developed normal brown black skin when ripe. The differences between the CA treatments and 78.5% N_2 storage were not significant. Atmospheres of five treatments (2.5 % O_2 + 5 % CO_2, 2.5 % O_2 + 7.5 % CO_2 , 5 % O_2 + 5 % CO_2 ,and 5 % O_2 + 7.5 % $CO_{2,}$78,5 % N_2, retarded softening significantly. All fruit stored for 6 and 9 weeks at CA softened normally during ripening at ambient temperature (Fig.2).

A correlation has also been noted between firmness and polyamines levels in avocado pulp. Low oxygen concentration (2.5 %) at 0^oC storage induced higher levels of polyamines and significantly inhibited the softening of fruit compared to fruit stored in 78.5% N_2. Basuki *et al* [2] found that fruit following CA storage for 6 and 8 weeks in high CO_2 concentration and 78.5% N_2 attained very severe CI after ripening in ambient temperature.

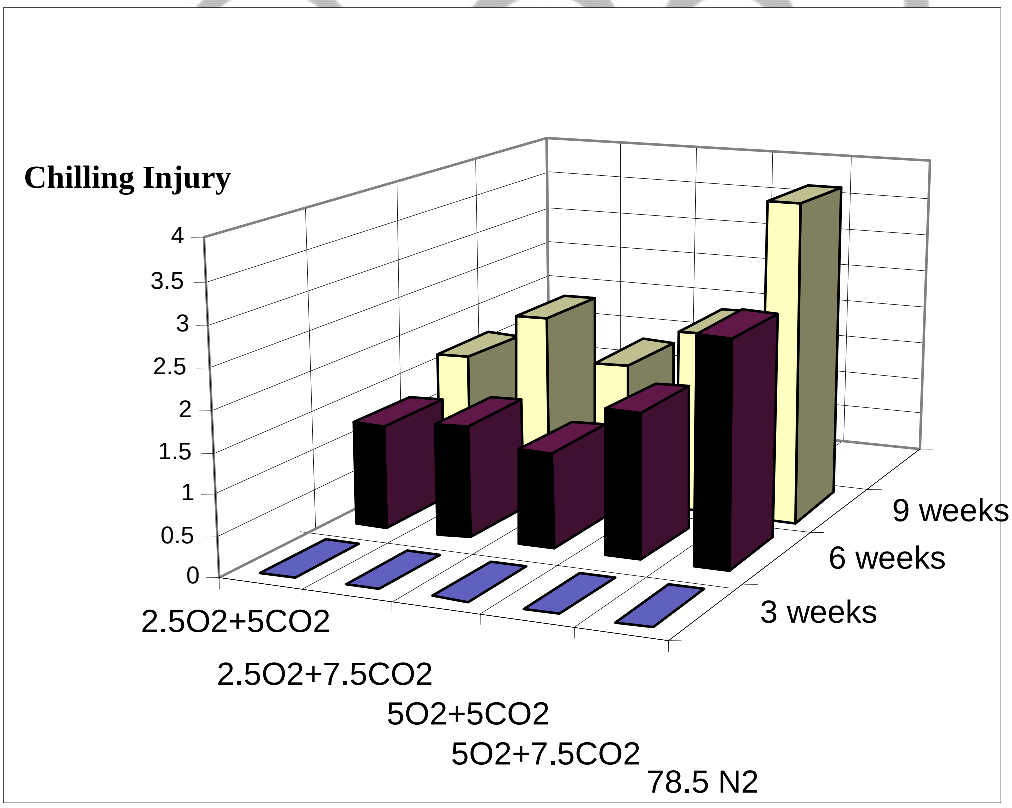

Fig. 1. Severity of chilling injury of avocado flesh after 6 days at 20^oC following transfer from CA storage at 5^oC for 3,6, and 9 weeks.

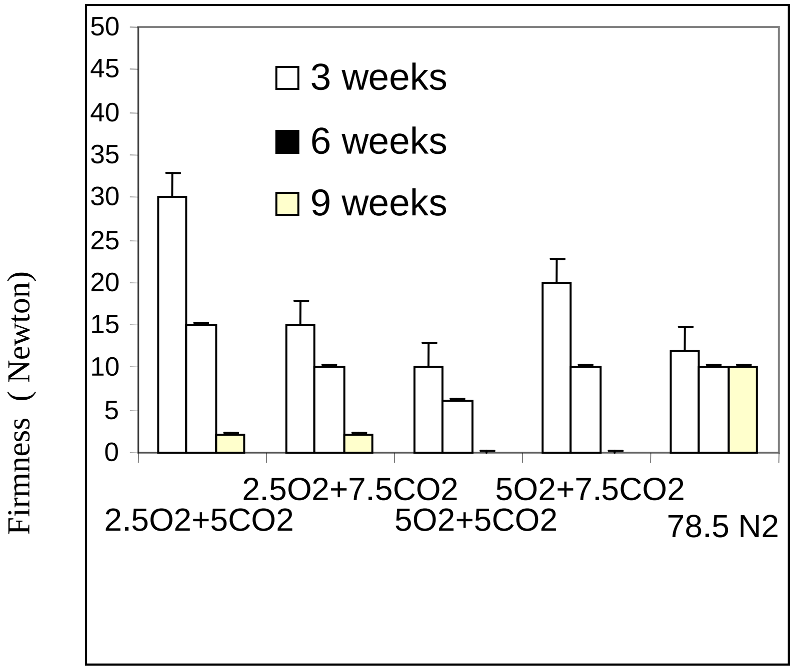

Fig. 2. Textural changes of avocado after transfer to 78.5% N_2 for 6 days following CA storage for 3,6 and 9 weeks at 5°C. The vertical bars represent standard error of the mean n=3).

Rates of respiration and ethylene production

The pattern of changes in respiration rates and ethylene production during ripening of avocado fruit transferred to 78.5% N_2 at 20°C were measured daily for 6 days, following CA storage for 3 weeks at 5°C (Fig. 3). Freshly harvested fruit indicated climacteric patterns of CO_2 and ethylene production, with peaks recorded on the 14th day . The rates of respiration and ethylene production of fruit stored in 78.5% N_2 were higher than those of fruit stored in CA treatments. Respiration rates and ethylene production show climacteric-like peaks by days 2 - 4 for 78.5% N_2 and CA compared to harvest control that reached a peak at 14 days. The lowest rates of ethylene production were recorded in fruit stored in CA mixtures of 5 % O_2 combined with 7.5 or 10 % CO_2. Generally, the CA treatments reduced the respiratory peak and ethylene production as compared to 78.5% N_2. In comparison to harvested fruit that reached a peak at day 14 these data show that ethylene production and respiration were stimulated by chilling at 0°C, peaking 2 - 3 days after transfer to 20°C and decreasing thereafter. CA treatments at low temperature (5°C) generally reduced the rates of respiration and ethylene production.

Similar patterns of changes in respiration rates and ethylene production during ripening of avocado at 20°C were observed after CA storage for 6 and 9 weeks at 5°C (data not shown). The increase in CO_2 production by avocado stored at 0°C was possibly due to the increased ethylene production stimulated by chilling. However, the rates of respiration of CA fruit were remained lower than the fruit stored in 78.5% N_2. A similar persistent repression of CO_2 production was reported for Fuerte pre treated in a low O_2 atmospheres (3 % O_2 and 97 % N_2) during storage at 2°C and 17°C [27]. An increase in respiration following chilling appears to be a common response in non-climacteric lemons, beans and potatoes [33]. The observed increase in respiration appeared to be related to development of symptoms of CI (Fig. 2). The data reported here confirm the work of Lange and Kader [21,22] who reported that Hass avocado stored in air had higher respiration rates than fruit stored with a high CO_2 concentration.

Polyamines

Changes in polyamines concentrations of fruit after transfer to air at 20°C after CA storage for 3, 6 and 9 week at 0°C. Polyamines concentrations were not calculated in freshly harvested fruit during ripening at 20°C. Unripe avocado fruit have been reported to have relatively higher concentrations of polyamines than ripe fruit [34]. In the present study, the concentrations were high in all samples on the day of transfer from 5°C to 20°C, after storage for 3, 6 and 9 weeks and subsequently decreased during storage in 78.5% N_2 at 20°C (Fig. 4.).

Comparable results were observed in Hass avocado from New Zealand [2]. This suggested that the initial concentrations in the fruit were high and CA mixtures had no consistent effects on polyamine concentrations or the rates of change during ripening at 20°C. Polyamine concentrations decrease during avocado fruit development [34,20] and between the immature and mature stages of development prior to the onset of climacteric ethylene production in tomato fruits [17].

Polyamine concentrations in CA stored avocado were higher than those in air stored fruit. This result agree with Kramer *et al.* (1989) who reported that the concentrations of polyamines were higher in CA-stored apples than in air-stored fruit and the maximum concentrations coincided with the ethylene climacteric. A close and inverse relationship has been observed between ethylene and firmness. Putrescine and spermidine concentrations evolved in a similar way during peaches storage at 1 and 5 °C and decreased in the fruits kept for 48 hours at 20 °C [31]. Polyamines and ethylene are known to have opposite effects in avocado fruit ripening. This paper present that ethylene production begins only after the concentration of polyamines decline (Fig. 3 B). Kakkar and Rai [17] reported that ethylene production reached a maximum concentration whereas the level of certain endogenous polyamine decline. During this phase accumulation of polyamines declines while extensive production of ethylene results in promotion of senescence of the plant organ [28,7]. However, Kakkar and Rai [17] stated that polyamines and ethylene biosynthesis pathways do not actively compete for the same substrates at any stage of avocado fruit development and ripening.

No such competition was observed in avocado during fruit development and ripening [20], because polyamines peak earlier than ethylene [8]. A correlation has also been reported between early cell division and putrescine and spermidine levels in avocado pulp [34].

CA storage connecting low oxygen and high CO_2 concentrations is widely used to prolong the storage life of apples. Low oxygen concentration (1 %) at 3 and 3.5°C storage induced higher levels of all three polyamines and significantly repressed the softening of apples at both temperatures compared to fruit stored in 78.5% N_2 [19,10,11].

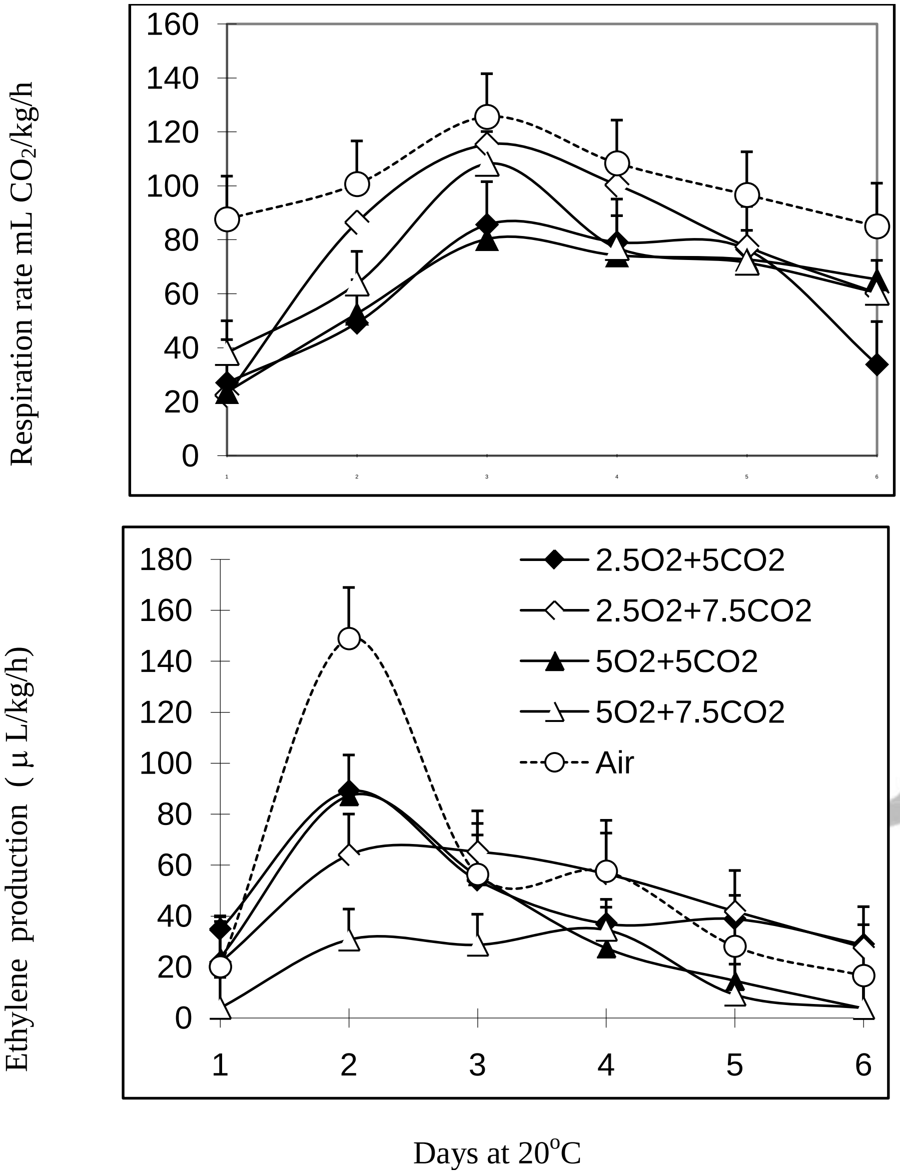

Fig. 3. The rates of respiration (above) and ethylene production (below) of Hass avocado following after transfer to air (78.5% N_2) at 20°C following CA storage for 3 weeks at 5°C. Vertical bars represent Standard Error of the means (n=3).

CONCLUSIONS

Controlled Atmosphere Storage (2.5 % to 5 %) at 5^{o}C storage induced higher levels of polyamines and significantly inhibited the softening of fruit compared to fruit stored in 78.5% N_2. Following 3 weeks storage at 5^{o}C no indication of CI in all treatments after ripening for 6 days, but was light discoloration after 6 and 9 weeks storage and very severe in 78.5% N_2 storage.Generally, the CA treatments reduced the respiratory peak and ethylene production as compared to 78.5% N_2

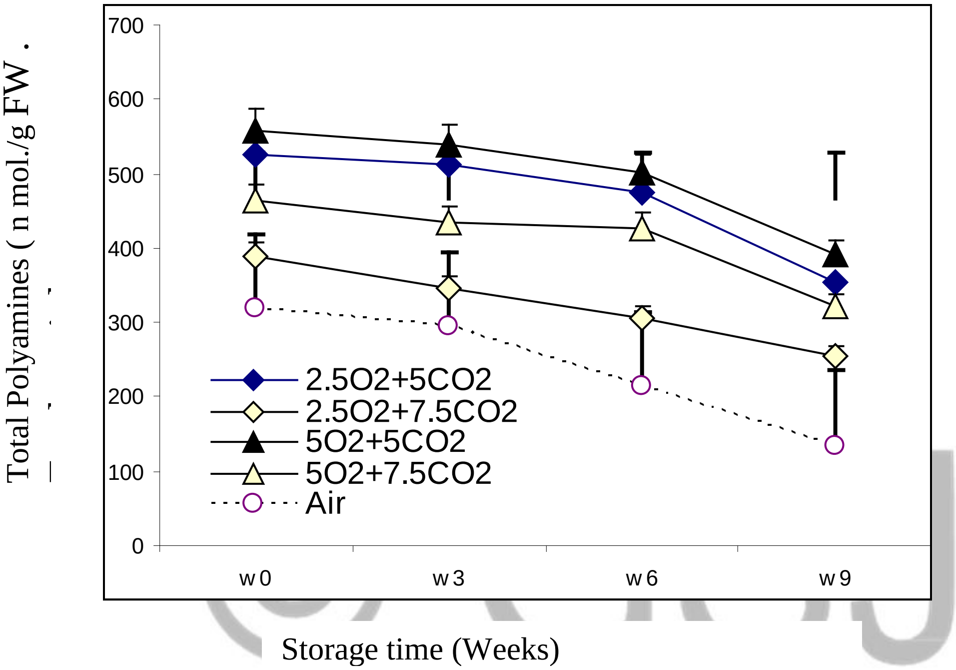

Fig. 4. Total Polyamines of avocado fruit following transfer to air (78.5% N_2) at 20^{o}C for 6 days after CA treatments for 9 weeks at 5^{o}C. The vertical bars indicate SE of the mean n = 3)

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