Polyamines Association in Textural Changes of Avocado during Controlled Atmosphere Storage

Eko Basuki¹, W. B. McGlasson² & G. Skurray³
¹Faculty of Food Technology & Agroindustry University of Mataram, Indonesia.
²University of Western Sydney Hawkesbury, Richmond, N.S.W. Australia.
³University of Western Sydney Hawkesbury, Richmond, N.S.W. Australia.

Abstract: Polyamines association in textural changes of Avocado during Controlled Atmosphere 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 Air (control). The incidence of CI, textural change, and polyamines concentration, rates of respiration, ethylene production was determined. The polyamines concentrations were high on the day fruit were transferred from 0°C to 20°C after storage 3, 6 and 9 weeks and subsequently decreased during ripening stage at 20°C. Polyamine concentrations in CA stored avocado were higher than those in air stored fruit. Low oxygen concentration (2.5 %) at 0°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 0°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: Polyamine, Controlled Atmosphere (CA), Ethylene and Texture.

INTRODUCTION

Successful export marketing must depend on decreasing the rate of ripening sufficiently to permit for the shipping time and arranged marketing in the importing country [4]. Storage life of avocados stored at the suggested storage temperature 4.5 - 7°C in air [35] is frequently not long enough to allow shipping by sea to intercontinental markets. Preceding research with a number of methods and/or combinations of controlled atmosphere (CA) in the absence of ethylene at low temperatures can increase in length storage life [5]. Low temperature storage of avocados is somew way limited by the occurrence of chilling injury (CI) [34]. The CI symptoms of avocado include 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 [8, 23]. Application of polyamine has been shown to inhibit the production of ethylene in apples tissues [1]. Naturally occurring polyamines may acts as modulators of some cellular and physiological processes during development and ripening of avocado fruit [1]. 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, and 10]. This possibility has not been fully explored in avocado fruit. Such studies need to be evaluated for each cultivar 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 incidence of CI. 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. The incidence of CI, textural change, and polyamines concentration was determined.

MATERIAL AND METHODS

Mature ‘Hass’ avocado fruit were harvested from New Zealand, air transported to Sydney Central Fruit Market, then transfer about 30 km by road to the Science Laboratory, UWS-H Richmond, Australia. Fruit were then sorted for weight uniformity, dipped in 0.2 % ‘Prochloraz’ fungicide solution, dried for about 30 minutes at 20°C and then stored in 30 L polyethylene containers. The containers were ventilated with air (control) or CA at a flow rate of about 12 L.h⁻¹. The atmospheres were generated by mixing regulated flows of air, carbon dioxide and a nitrogen enriched stream [30]. The mixtures of CA were monitored with a Fruit Store Analyser type 770 L (David Bishop Instrument, Hatfield, 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 were transferred to 20°C at 3 weeks intervals. The harvested and sampled fruit were stored singly in

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polyethylene container (1 L) at ambient temperatures were then ventilated at a air-flow rate of about 8 L.h⁻¹ and monitored for polyamine, ethylene and respiration rate 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°C following CA storage.

Assessment 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 discoulouration [24, 26]. 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 [3, 15]. The rate of respiration was reported as mLCO₂/kg/h and ethylene production of fruit tissue as µLC₂H₄/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 [18] with the following modifications. Pulp tissues were taken in the form of discs from the equatorial region with a knife to yield about 2 g fresh weight samples. Pulp samples were stored at -80°C for later extraction. Extracts for polyamine analysis were prepared by homogenising 2.0 g of tissue in 15 mL of 5 % perchloric acid using a Waring blender. Before homogenisation, 1,8-octanediamine (150 nmol.g⁻¹ fresh weight) was added as an internal standard. The homogenate was then centrifuged at 8000 x g for 20 minutes (Beckman GS-6R Centrifuge). The supernatant was saved for polyamine analysis. Dansylation was performed by mixing 400 µL of 10 mg dansyl chloride.mL⁻¹ (in acetone) and 150 µL of saturated sodium bicarbonate with 200 µL of tissue extract. After incubation overnight at room temperature, 250 µL proline.mL⁻¹ was added and the incubation was continued for one hour. After centrifugation in a Beckman GS-6R Centrifuge at 8000 x g for 10 min, the pH of the supernatant was adjusted to 6.8. Samples of 100 µL of the supernatant were used for HPLC analysis [14]. HPLC was performed with a system consisting of two pumps (Waters 501 and Waters 510). Samples were injected using a Waters U6K injector onto a reverse-phase 25 cm C-18 column (Supelco). Samples were eluted from the column at a flow rate of 1.5 mL.min⁻¹ with a programmed solvent gradient of 0, 100, 0; 15, 0, 100; 18, 0,100; where the first number was the time (minutes), the second number was the percent of buffer A (60 Methanol: 40 water), and the third number was the buffer B (10 ethanol). Elution was completed in 18 min. Products were detected with a Tunable Absorbance Detector (Waters 484) using an excitation wavelength of 365 nm. The pumps were controlled and data collected and analysed using a Computer system equipped with a Baseline 810 Chromatography Work Station (Dynamic Solutions). Total polyamines were quantified by the comparison of sample peak areas with those of the known standard [18].

RESULTS AND DISCUSSION
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 measured 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 [1]. In the present study, the concentrations were high in all samples on the day of transfer from 0°C to 20°C, after storage for 3, 6 and 9 weeks and subsequently decreased during storage in air at 20°C (Fig. 1.). Similar 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 [1, 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 [19] 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. 4 B). Ethylene production reached a maximum concentration whereas the level of certain...
endogenous polyamine decline [17]. During this phase accumulation of polyamines declines while extensive production of ethylene results in promotion of senescence of the plant organ [28,7]. However, Polyamines and ethylene biosynthesis pathways do not actively compete for the same substrates at any stage of avocado fruit development and ripening [17].

No such competition was observed in avocado during fruit development and ripening [20], because polyamines peak earlier than ethylene [6]. A correlation has also been reported between early cell division and putrescine and spermidine levels in avocado pulp [1,6].

CA storage involving low oxygen and high CO₂ 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 inhibited the softening of apples at both temperatures compared to fruit stored in air [18,9,10,11].

![Fig. 1. Total Polyamines of avocado fruit following transfer to air at 20°C for 6 days after CA treatments for 9 weeks at 0°C. The vertical bars indicate SE of the mean n = 3)](image)

**Chilling Injury**

The severity of CI in the flesh was examined at day 6 after transfer of the fruit to air 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 discoloration was observed in fruit containing 5% O₂ ± 5% CO₂ and 5% CO₂ ± 7.5% CO₂. After 6 and 9 weeks the fruit stored in 2.5% O₂ combined with 5 and 7.5% CO₂, very light discoloration was observed whereas control fruit developed severe CI symptoms (Fig. 2). These fruit reached normal colour compared to other treatment that only achieved colour score 3. Overall, these treatment (2.5 and 5% O₂ combined with 5 and 7.5% CO₂) 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. Similar result were observed in fruit stored at 0 and 2°C [13, 28]. The rates of softening of the avocado fruit after transfer to air 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 air storage were not significant. Atmospheres of four treatments (2.5% O₂ ± 5% CO₂, 2.5% O₂ ± 7.5% CO₂, 5% O₂ ± 5% CO₂, and 5% O₂ ± 7.5% CO₂), retarded softening significantly. All fruit stored for 6 and 9 weeks at CA softened normally during ripening at ambient temperature (Fig. 3).
A correlation has also been noted between firmness and polyamines levels in avocado pulp. Low oxygen concentration (2.5%) at 0°C storage induced higher levels of polyamines and significantly inhibited the softening of fruit compared to fruit stored in air. Fruit following CA storage for 6 and 8 weeks in high CO₂ concentration and air attained very severe CI after ripening in ambient temperature [2].

Rates of respiration and ethylene production

The pattern of changes in respiration rates and ethylene production during ripening of avocado fruit transferred to air at 20°C were measured daily for 6 days, following CA storage for 3 weeks at 0°C (Fig. 4). Freshly harvested fruit showed climacteric patterns of CO₂ and ethylene production, with peaks recorded on the 14th day. The rates of respiration and ethylene production of fruit stored in air were higher than those of fruit stored in CA treatments.
Respiration rates and ethylene production show climacteric-like peaks by days 2 - 4 for air 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 % O₂ combined with 7.5 or 10 % CO₂. Generally, the CA treatments reduced the respiratory peak and ethylene production as compared to air. 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 (0°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 0°C. The increase in CO₂ 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 air. A similar persistent suppression of CO₂ production was reported for Fuerte pre treated in a low O₂ atmospheres (3 % O₂ and 97 % N₂) during storage at 2°C and 17°C [26]. 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 [21,22] who reported that Hass avocado stored in air had higher respiration rates than fruit stored with a high CO₂ concentration.

Fig. 4. The rates of respiration (A) and ethylene production (B) of Hass avocado following after transfer to air at 20°C following CA storage for 3 weeks. Vertical bars represent Standard Error of the means (n=3).

CONCLUSIONS

1. The polyamines concentrations were high on the day fruit were transferred from 0°C to 20°C after storage 3, 6 and 9 weeks and subsequently decreased during ripening stage at 20°C. Polyaamine concentrations in CA stored avocado were higher than those in air stored fruit.

2. Low oxygen concentration (2.5 % and 5 %) at 0°C storage induced higher levels of polyamines.
and significantly inhibited the softening of fruit compared to fruit stored in air.

3. Following 3 weeks storage at 0°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.

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REFERENCE


