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RESEARCH ARTICLE

CORRELATION OF SOFTENING AND POLYAMINES LEVELS DURING CONTROLLED ATMOSPHERE STORAGE OF AVOCADO

[1*]Basuki, E., [1]Alamsyah, A., [1]Yasa, IWS, [2]Skurray, G and [2]McGlasson, W.B.

[1]Faculty of Food Technology and Agroindustry, University of Mataram, Indonesia 83125
[2]The University of Western Sydney, Richmond NSW 2753 Australia

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ABSTRACT

Correlation of Softening and Polyamines levels during the Controlled Atmospheres Storage of Avocado were examined. The fruit was harvested then stored for 9 weeks at 0°C in Controlled Atmospheres (CA) containing 2.5, 7.5 % O_2 with 5 and 10 % CO_2 in all combinations (4 mixtures) and air (20 % O_2). The incidence of CI, textural change and polyamines concentration, rates of respiration, ethylene production was determined. Fruit stored for 9 weeks in 2.5 % O_2 and 10 % CO_2 softened after transfer to air while fruit from other mixtures containing 10 % CO_2 did not soften. The polyamines concentrations were high on the day fruit were transferred from 0°C to 28°C after storage 3, 6 and 9 weeks and subsequently decreased during the ripening stage at 28°C. Polyamine concentrations in CA stored avocado were higher than those in air stored fruit. After 9 weeks of storage in CA at 0°C then transferred to air resulted in a slight increase in putrescine (PUT), a slight increase in spermidine (SPD) and a major decrease in spermine (SPN). 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 (20 % O_2). 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. The rate of respiration and ethylene production of fruit stored in air were higher than those of fruit stored in CA treatments.

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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 (Bower and Cutting, 1988). Storage life of avocados stored at the suggested storage temperature 4.5 - 7°C in air (Zauberman and Jobin-Decor, 1995) is frequently not long enough to allow shipping by sea to intercontinental markets. Utilize of controlled atmosphere (CA) storage appears promising for the extension of shelf life of avocado and has numerous commercial possibilities; however the specific requirements of each cultivar should be evaluated (Hatton and Spalding, 1990). Preceding research with the number of methods and/or combinations of CA in the absence of ethylene at low temperatures can increase in length storage life (Chaplin et al., 1983). The mechanism of CA also inhibits the increase in the activity of softening related enzymes such as polygalacturonase (Hatton and Spalding, 1990).

Corresponding author: Basuki, E.,
[1]Faculty of Food Technology and Agroindustry, University of Mataram, Indonesia 83125.

Low temperature storage of avocados is someway limited by the occurrence of chilling injury (CI) (Zamorano and Merodio, 1993). The CI symptoms of avocado include unusual respiration and ethylene production patterns and failure to soften properly upon warming after storage (Wang, 1990a). Polyamines appear to be potent inhibitor of senescence related processed in a plant tissue (Galston and Kaur-Sawhney, 1995; Malmberg et al., 1998). Application of polyamine has been shown to inhibit the production of ethylene in apple tissues . Naturally occurring polyamines may acts as modulators of some cellular and physiological processes during development and ripening of avocado fruit (Apelbaum, 1986). Also, the resistance of zucchini squash to CI has been correlated with elevated polyamines levels (Kramer and Wang. 1989). While there is some evidence for an inverse relationship between the concentration of polyamines and ethylene production during avocado fruit development, their roles in avocado ripening remain unknown (Kushad et al.,1988). 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 (Kramer and Wang, 1989; Gorny and Kader, 1996; Nambi et al., 2016).

This possibility has not been fully explored in avocado fruit. Such studies need to be evaluated for each cultivar to determine their specific requirements (Hatton and Spalding, 1990). In this study, we evaluate 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 levels of Hass avocado ventilated with gas mixtures containing low O_2 and high CO_2 during storage at 0 ^oC were examined. The incidence of CI, textural change, and polyamines levels were determined.

MATERIAL AND METHODS

Mature 'Hass' avocado fruit was harvested from The Centre of Lombok District, then transfer about 30 km by road to the Food Technology Laboratory, University of Mataram, Indonesia. Fruit was then sorted for weight uniformity, dipped in 0.2 % 'Prochloraz' fungicide solution, dried for about 30 minutes at 20^oC and then stored in 30 L polyethylene containers. Samples of 36 fruits were enclosed in each of thirty-polyethylene container and were stored at 0 ^oC. Groups of three containers were ventilated with the ventilated with air (20 % O_2) or CA at a flow rate of about 12 $L.h^{-1}$ (Table 1). The atmospheres were generated by mixing regulated flows of air, carbon dioxide and a nitrogen-enriched stream (Smith et al.,1997). The mixtures of CA were monitored with a Fruit Store Analyser type 770 L (David Bishop Instrument, Heatfield, UK) and the composition was recorded automatically at 4 hourly intervals. Experimental unit of the fruit stored in CA containing 2.5, 7.5 % O_2 with 10 and 5 % CO_2 in all combinations (4 mixtures) and Air (20 % O_2). Samples from each atmosphere were transferred to 28^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 an air-flow rate of about 8 $L.h^{-1}$. These fruits were used for measurement of rate of respiration and ethylene production. Another three fruit from each treatment were taken for analyses of polyamine levels at two-day intervals (Table 2). Fruit from this experiment was 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 was analysed at days 0, 2, 4 and 6 at 28^oC 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 discolouration (Meir et al., 1995; Pesis et al.,1994). 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) (Kader, 1982). 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 (Basuki, 2000; Jobling,1993). The rate of respiration was reported as $mLCO_2/kg/h$ and ethylene production of fruit tissue as $\mu LC_2H_4.kg/h$. 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 and Wang, 1989) 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^oC for later extraction. Extracts for polyamine analysis were prepared by homogenizing 2.0 g of tissue in 15 mL of 5 % perchloric acid using a Waring blender. Before homogenization, 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 (Hugo et al.,1987). 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 analyzed 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. Each mean was the average of three independent samples from each treatment. Putrescine (PUT), spermidine (SPN) and spermine (SPN) are collectively referred to as polyamines (Kramer and Wang, 1989).

RESULTS AND DISCUSSION

Polyamines: Changes in polyamines levels of fruit after transfer to air at 28^oC following CA storage for 3, 6 and 9 weeks at 0^oC. Polyamines levels were not measured in freshly harvested fruit during ripening at 28^oC. Unripe avocado fruit have been reported to have relatively higher concentrations of polyamines than ripe fruit (Apelbaum, 1986). In the present study, the concentrations were high in all samples on the day of transfer from 0^oC to 28^oC, after storage for 3, 6 and 9 weeks and subsequently decreased during storage in air at 28^oC (Fig. 1). Similar results were observed in Hass avocado from New Zealand (Basuki et al.,2016). This suggested that the initial concentrations in the fruit were high and CA mixtures had no consistent effects on polyamine levels or the rates of change during ripening at 28^oC. Polyamine levels decrease during avocado fruit development (Apelbaum, 1986;Kushad et al.,1988) and between the immature and mature stages of development prior to the onset of climacteric ethylene production in tomato fruits (Kakkar and Rai, 1993). Polyamine levels in CA stored avocado were higher than those in air stored fruit. The effect of CA on the polyamine levels in avocado are shown in Figure 2. After 9 weeks of storage in CA at then transferred to air (20 % O_2) resulted in a slight increase in PUT, and SPD except for the major decrease in SPN.

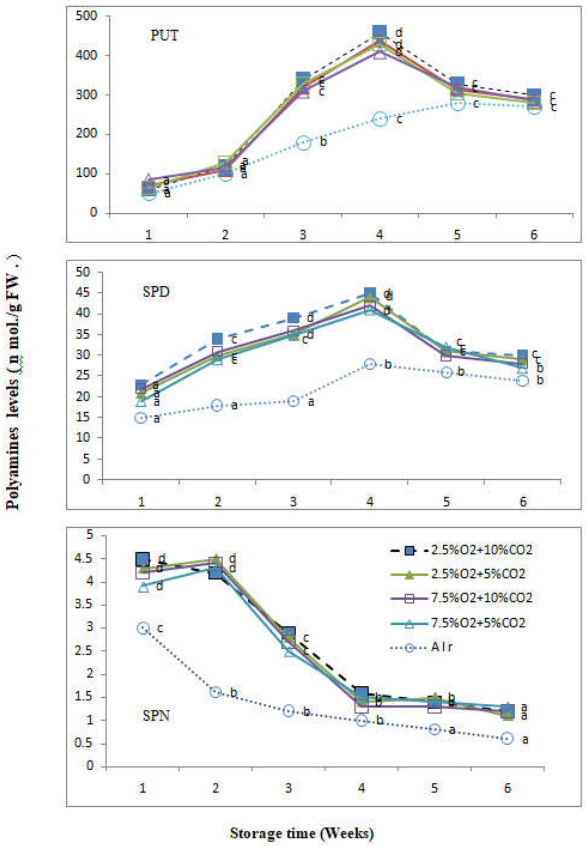

Fig. 1. Total Polyamines of avocado fruit folowing transfer to air at 28°C for 6 days after CAtreatments for 9 weeks at 0°C. Means for each treatment with different letter were significantly different at p< 0.05 (Duncan's Range Multiple Comparisons)

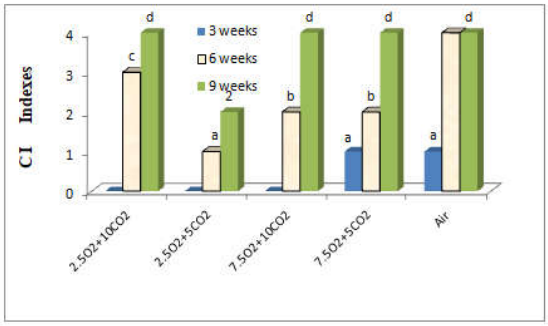

Fig. 2. Severity of chilling injury of avocado flesh after 6 days at 28°C following transfer from CA storage at 0°C for 3,6, and 9 weeks. Means for each treatment with different letter were significantly different at p< 0.05 (Duncan's Range Multiple Comparisons).

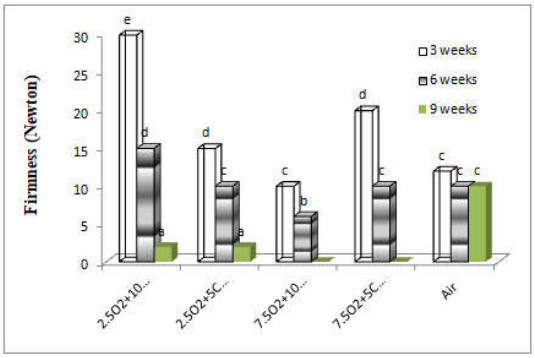

Fig. 3. Textural changes of avocado after transfer to air for 6 days following CA storage for 3,6 and 9 weeks at 0°C. Means for each treatment with different letter were significantly different at p< 0.05 (Duncan's Range Multiple Comparisons)

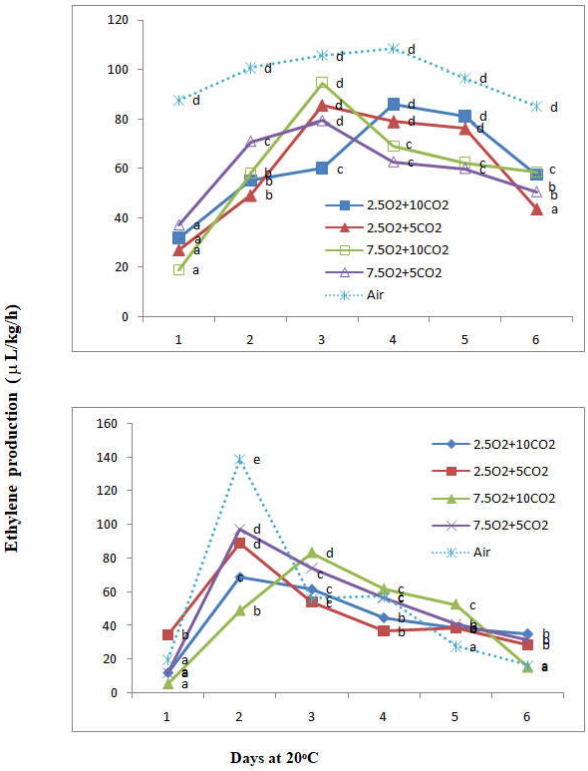

Fig. 4. The rates of respiration (Above) and ethylene production (Below) of Hass avocado after transfer to air following CA storage for 3 weeks. Means for each treatment with different letter were significantly different at p< 0.05 (Duncan's Range Multiple Comparisons)

Table 1. Experiment CA storage plan at 0 °C.

Atmospheres	Storage time (weeks)			
	0	3	6	9
2.5 % O_2 + 10 % CO_2	-	+	+	+
2.5 % O_2 + 5 % CO_2	-	+	+	+
7.5 % O_2 + 10 % CO_2	-	+	+	+
7.5 % O_2 + 5 % CO_2	-	+	+	+
Air/20% O_2	+	+	+	+

+ indicates when samples were transferred to Air

Table 2. Fruit sampling plan following storage at 0 °C

Time (days)	Respiration rateand ethylene production	Polyamines concentrations
0	+ (3)	+ (3)
1	+ (3)	-
2	+ (3)	+ (3)
3	+ (3)	-
4	+ (3)	+ (3)
5	+ (3)	-
6	+ (3)	+ (3)

PUT and SPD increased over time while SPN decreased, but all remained higher than those in air-stored fruit. Some differences are due to differences in concentration of oxygen in CA storage. These results agree with Kramer et al., (1989) who reported that the concentrations of polyamines were higher in CA-stored apples than in air-stored fruits and the maximum concentrations coincided with the ethylene climacteric. A close and inverse relationship has been observed between ethylene and firmness. PUT and SPD 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 (Valero et al., 1997).

In most cases, PUT might accumulate due to stress (Galston and Kaur-Sawhney, 1995). 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). Ethylene production reached a maximum concentration whereas the level of certain endogenous polyamine decline (Kakkar and Rai, 1993). During this phase accumulation of polyamines declines while extensive production of ethylene results in promotion of senescence of the plant organ (Saftner and Baldi, 1990;Fluhr and Matto, 1996). However, Polyamines and ethylene biosynthesis pathways do not actively compete for the same substrates at any stage of avocado fruit development and ripening (Kakkar and Rai, 1993).

No such competition was observed in avocado during fruit development and ripening (Kushad et al., 1988) because polyamines peak earlier than ethylene (Evan and Malmberg, 1989). A correlation has also been reported between early cell division and PUT and SPD levels in avocado pulp (Apelbaum, 1986; Evan and Malmberg, 1989). CA storage involving low oxygen and high CO_2 concentrations is widely used to prolong the storage life of apples. Low oxygen concentration (1 %) at 1 and 3.5^{o}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 (Kramer and Wang, 1989;Gorny and Kader, 1996;Nambi et al.,2016;Gorny and Kader, 1997). Polyamines have been shown to posses anti senescence agent, including the inhibition of degradation enzymes, fruit development and stabilization of membrane development (Galston and Kaur-Sawhney, 1995).

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 of storage. Very light discolouration was observed in fruit containing 2.5 % O_2 + 10 % CO_2 and 2.5 % O_2 + 5 % CO_2. After 6 and 9 weeks the fruit stored in 2.5 % O_2 combined with 5 and 10 % CO2, very light discoloration was observed whereas control fruit developed severe CI symptoms (Fig. 2). These fruits reached normal colour compared to other treatment that only achieved colour score 3. Overall, these treatments (2.5 and 7.5 % O_2 combined with 10 and 5 % CO_2) gave the best result and the fruit ripened normally. Avocado (*P. americana* 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 (Pesis et al.,2002). The CI symptom in air storage were black lesions in the skin and grey black discoloration of the flesh. Similar result was observed in fruit stored at 0 and 2°C (Hopkirk et al.,1994;Saftner and Baldi, 1990). 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 % O_2 + 10 % CO_2, 2.5 % O_2 +5 % CO_2 , 7.5 % O_2 + 10 % CO_2 ,and 7.5 % O_2 + 5 % CO_2), retarded softening significantly. All fruit stored for 6 and 9 weeks at CA softened normally during ripening at ambient temperature (Fig. 3).

Fruit firmness: A correlation has also been noted between firmness and polyamines levels in avocado pulp. Low oxygen concentration (2.5 %) at 0^{o}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 9 weeks in high CO_2 concentration and air attained very severe CI after ripening in ambient temperature (Basuki et al., 2016).

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 20oC were measured daily for 6 days, following CA storage for 3 weeks at 0oC (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 2.5 % O_2 combined with 5 or 10 % CO_2. 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^{o}C, peaking 2 - 3 days after transfer to 20^{o}C and decreasing thereafter. CA treatments at low temperatures (0^{o}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 28^{o}C were observed after CA storage for 6 and 9 weeks at 0^{o}C. The increase in CO_2 production by avocado stored at 0^{o}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. Similar persistent suppression 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^{o}C and 17^{o}C (Pesis et al.,1994). An increase in respiration following chilling appears to be a common response in non-climacteric lemons, beans and potatoes (Wang, 1990b). 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 CO_2 concentration.

Conclusions

Polyamine concentrations in CA stored avocado were higher than those in air stored fruit. After 9 weeks of storage in CA at 0^{o}C then transferred to air resulted in a slight increase in putrescine (PUT) and spermidine (SPD) except a major decrease in spermine (SPN). The rates of respiration and ethylene production of fruit stored in air were higher than those of fruit stored in CA treatments. The increase in CO_2 production by avocado stored in CA at 0^{o}C was probably due to the increased ethylene production stimulated by chilling. Higher CO_2 concentration in the atmosphere appears to suppress ethylene production.

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