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Inhibition of ACC_O (1-aminocyclopropane 1-carboxylic Acid oxidase) Activity of Mangoby Modified Atmosphere Storage

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Abstract: Inhibition of ACC Oxidase Activity of Mango by Modified Atmosphere Storage is aimed to extend the storage life were carried out at Food Technology Laboratory, University of Mataram from by using Completely Randomized Design and continued with Least Significant Different at five percent significance level. Mangoes were stored at Polyethylene (PE) bags; PE + $KMnO_4$; Polypropylene bags (PP); PP + $KMnO_4$ and unpacked (Control) for three weeks. The physical properties of fruit such as weight loss and decay percentage were determined, while physiological properties such as the rate of respiration, ethylene production including ACC_O activity. Inactivation of ACC_O occurred to almost half-time of its activity in mango stored at MAS as compared to unpacked mango. Therefore, paralleled the rate of respiration and production of ethylene at MAS leads to extend the storage life of mangoes. Weight loss and decay percentage of mango kept in MAS for 3 weeks were lower than unpacked.

Key word: ACC_O, Ethylene Control, Fruit Ripening Inhibition, KMnO4, MAS, Mangoes.

I. Introduction

In Indonesia, especially Nusa Tenggara Barat (NTB) Province, the area and production of mangoes increase gradually every year, from 27,187 tones in 1997 to 71,958 tones for financial year 2001 (Diperta NTB, 2002), indicated that recently horticultural commodities have been developed rapidly. It produces in almost all regency in NTB province. The most cultivated mangoes are the highly economic value varieties such as Madu, Arumanis, Manalagi, and Golek (Yuniati dan Suhardjo, 1996). However they have short time storage at ambient temperature (Pesis, 2000) which one of the important constraint that should be managed. Besides, other postharvest factor (pest and pathological decay) which developed rapidly during storage.

One of the method to inhibit the fruit ripening by using polyethylene/polypropylene bags packed with ethylene adsorbent (KMnO4) have been applied in bananas. The experiment on banana's storage by KmnO4 (400g/L) with vermiculite could extend the shelf life up to three weeks (Wills *et al*, l998). It application was combined with Plastic Polyethylene bags that created modified atmosphere which retard the physiological properties that leads to inhibit the activity of enzyme (Kader, 1993). The enzyme was involved in fruit ripening by process ethylene biosynthesis (S-adenosyl methionine (SAM) → 1-aminocyclopropane-1-carboxylic acid (ACC) → ethylene), called 1-aminocyclopropane 1-carboxylic acid oxidase (ACC_O). The activity of ACC_O involved in the process from ACC to produce ethylene in climacteric fruit affected by oxygen concentration inside the bags (Smith *et al*, 1994). Its activity is catalyzed by enzyme and required oxygen, highly regulated and closely parallels the level of ethylene biosynthesis (Kieber and Ecker, 1993; Kende, 1993).

Modification Atmosphere Storage of Mangos is not only applicable methods in order to extend the shelf life, but also finding out the level of oxygen and carbon dioxide that inhibited the activity of ACC_O. Sitrit, *et al*. (l986) explained that ACC_O is influenced by fruit phase ripening, type of packaging, and storage temperature. In this study was examined the effect of modified atmosphere in plastic bags by using ethylene absorbent in relation to activity of ACC_O. The aims of this study were to find out ACC_O activity, the rate of respiration, ethylene production, weight losses and decay percentage during three weeks storage of mangoes in MAS.

II. Material and Methods

This study was conducted at The Laboratory of Agricultural Product Technology and Analytical Laboratory, University of Mataram using Completely Randomized Design which consists of five treatments and three replications as follows:

PE	:	Plastic Polyethylene bags (PE)+ corrugated fiber board
PE+ $KMnO_4$	:	PE + $KMnO_4$ (450g/L)+ corrugated fiber board

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PP	:	Plastic Polypropylene bags (PP)+ corrugated fiber board
PP+ $KMnO_4$	:	PP + $KMnO_4$ (450g/L) + corrugated fiber board
K0	:	Without Packaging (Controlled)

Data was performed with Analysis of Variance (ANOVA) five percent significance level continued by Least Significant Difference LSD) (Hanafiah, 1985). Fresh harvested mangoes var Madu from West Lombok was sorted for weigh uniformity,dipped in 0.2 % fungicide solution, dried at room temperature for about 30 minutes and then enclosed in Plastic bags (stored by MAS) at ambient temperature (25-28ºC) for three weeks. Represented samples were transferred for each MAS to air after 1,2,3 weeks. Parameters to be recorded were weight losses (Syarif and Irawati, 1988) and decay's percentage (Standar Nasional Indonesia, 2000). The rate of respiration and ethylene production during ripening days phase (Jobling, 1993). The respiration rates were measured interm of CO2 (mL/kG/H). CO2 was measured by withdrawing 1 mL samples of gas by syringe from plastic bags and injecting them into a Gas Chromatography. The chromatograph was fitted with a stainless steel column (2 m X 0.3 mm ID) packed with 80 -100 Mesh porapak Q (Supelco, USA) and thermal conductivity with detector. The carrier gas was Helium with a flow rate at 28 mL/min. Chromatograph was calibrated with standard mixed containing 5 % CO2 in air. The peaks was separated gas were recorded on strips chart recorder. Ethylene productions was expressed as micro µL/kG/H. Ethylene concentrations was measured by withdrawing 1 mL sample of gas by syringe from plastic bag and injecting them into a GC fitted with a stainless column (2 m X 0.3 mm ID) packed with activated alumina (80 Mesh) and flame ionisation detector. The retention time on concentrations of ethylene were calibrated against an ethylene mixtured containing 1.9 µL/L in Nitrogen. Peaks separated gasses were recorded on a strips charts recorder. Operated condition were collumn temperature 110 oC , detector temperature 110 oC, injecting port temperature 80 oC, air 300 mL/min. Hydrogen 24 mL in Nitrogen carrier gas 28 mL/min. (Jobling, 1993). The activity of ACC_O was determined in pulp tissue section according to Bufler methods (1986) with following modification. The pulp of tissues was taken from equatorial region with a cork borer and sliced with a razor blade yielding 1 g FW. Then the samples was placed in 25 X 180 mm test tubes containing 10 mL of solution comprising 0.1 mM ACC dissolved in 0.4 M Sucrose and 0.02 M $CaCl_2$ in distillated H_2O. After 30 minutes the pulps were removed from the solution and they were quickly blotted dry with tissue paper and placed in a 10 mL plastic syringe. Immediately after enclosure in the syringe, CO_2 was added to establish 5 % concentration. After 30 minutes the accumulated ethylene was measured by removing 1 mL gas sample and analyzed with Gas Chromatography (Varian 3300) fitted with PID detector. Saturating concentrations of ACC were supplied to ensure that ACC_O activity was not limited by substrate availability. ACC_O activity (the ability to convert ACC to ethylene) was expressed in ηL $C_2H_4.g^{-1}$ of fruit tissue (Jobling, 1993; Basuki, 2001).

III. Results and Discussion

Inactivation of ACC_O

After 3 weeks storage of mangos ACC_O activity was measurable although the concentration was very low. The activity of ACC_O in MAS packed fruit was inhibited in comparison with unpacked fruit. The activity of ACC_O following removal from PE and PP bags every week stored were non significant (Figure 1). These data indicated that inactivity of ACC_O occurred in PE/PP bags with or without $KMnO_4$, otherwise in air storage fruit ACC_O activity was normal. Inactivation of ACC_O occurred to almost half of its activity in mangoes stored in MAS as compared to air storage. ACC_O activity in mangoes also paralleled to the changes of ethylene and be correspondent the change of the rate of respiration. This observation agree Gorny and Kader (1997) who showed that induction of ACC_O is suppressed in CA treatments of apples compared to storage in air (Kalra, *et al*., 1995). Similar parallel changes in ACC_O activity and ACC concentration have been reported in apple (Jobling, 1993), avocado (Basuki, 2001) and tomato (de Wild *et al*., 2005). ACC_O activity in freshly harvested fruit ripened at ambient temperatures rises constantly throughout the lag period increasing substantially at the onset of ethylene production in climacteric fruit (Sitrit, *et al*, 1986; Cua and Lizada, l990; Smith, *et al*., 1994; Starrett and Laties, 1991).

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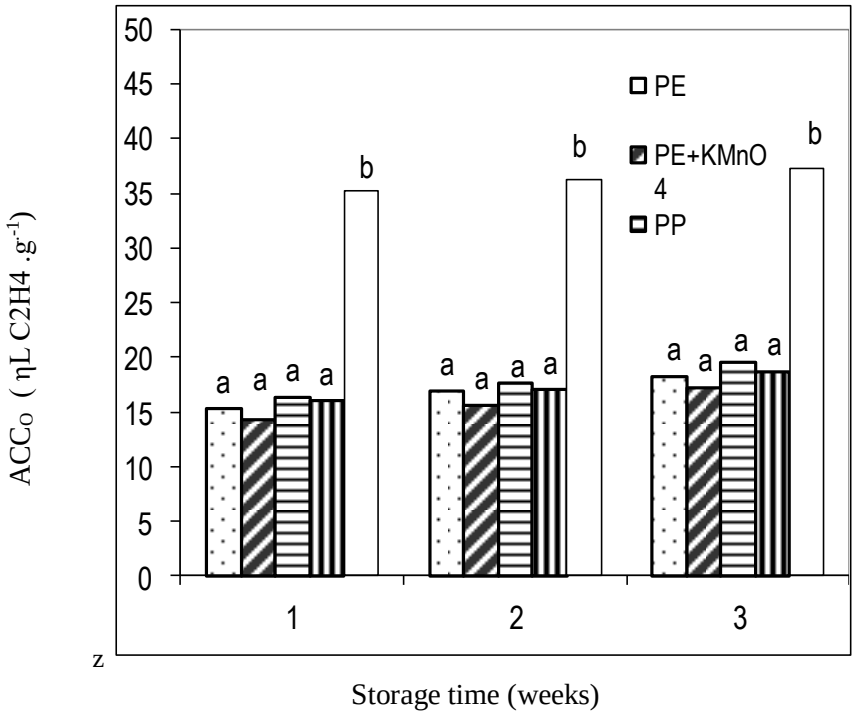

Fig. 1. ACC_O activity at climacteric peak of mangoes during 1, 2, and 3 weeks storage in MAS. The graph that followed by the same letter at the same time storage indicated non significant differences according to LSD 5 %.

The rate of respiration and ethylene production

Climacteric patterns of CO_2 and ethylene production in freshly harvested fruit expressed clearly with peak recorded on the 5th days during this experiment (Figure 2). The rate of respiration and ethylene production of fruit stored in air (control) was higher than fruit stored in MAS with or without ethylene absorbent. The lowest ethylene production found in fruit kept in PE bags with $KMnO_4$, this indicated that MAS inhibited the production ethylene, therefore fruit climacteric time would be extended. MAS of mangoes by using PE/PP bags with ethylene absorbent ($KMnO_4$) decrease the rate of respiration and ethylene production (Figure 3). Application of $KMnO_4$ inside PE bags have been conducted in banana Cavendish that delayed the ripening up to three weeks (Wills *et al.*, l998).

The respiration pattern and ethylene production of mangoes during ripening (Singh, 2000) seem to be similar with respiration pattern of avocado (Basuki, 2001). The climacteric rise and ethylene production during mangoes ripening was accelerated by temperature and type of packaging, it seem that the production of CO_2 of mangoes stored in air higher than in MA. The rate of respiration was related to ethylene production, if high ethylene production which accelerated respiration rate and ripening (Harris *et a.*,1997). Furthermore, high CO_2 concentrations in MAS reduced the rate of respiration of banana (Liu *et al*., 2004). CO_2 production was lower in avocado pre treated in low O_2 atmosphere (3 % O_2 and 97 % N_2) during storage at 2 ^oC and 17 ^oC (Pesis *et al*., 1994). The rate of respiration and production of ethylene measured in sample after 2 weeks and monitored every day during 6 days (Figure 3). The increase of rate respiration following stored in CA or MA indicated that specific type of climacteric fruit (Wang, 1990). Lange and Kader (1997a+b) reported that avocado stored in air had higher respiration rates than fruit treated with high CO_2 concentration.

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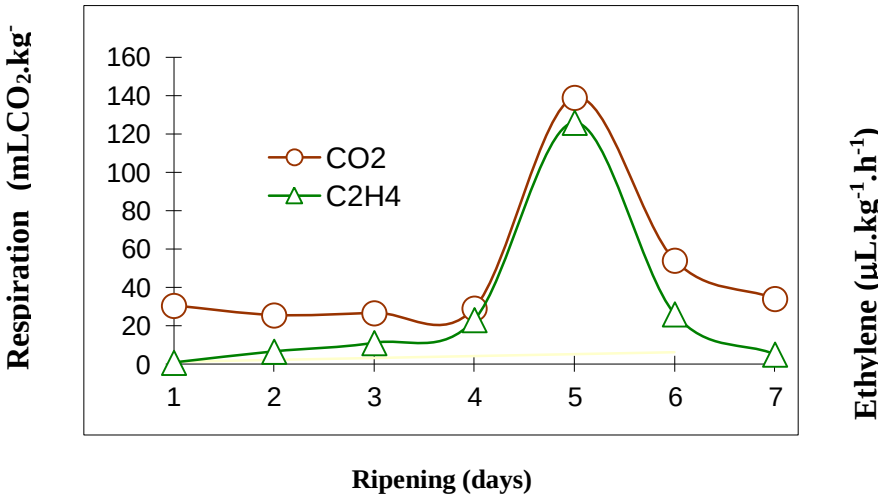

Fig. 2. The rate of respiration and ethylene production of freshly harvested Mangoes during ripening phase at ambient temperature.

Weight losses

Figure 4 showed that all treatments indicated non significant difference. After one weeks storage mangoes packed by PE + $KMnO_4$ showed the lowest weight losses, while Ko showed the highest percentages compared to MAS treatments. Then, after removal from package all treated fruit indicated similar in weight losses. However, PE bags showed the lowest weight loss after 3 weeks storage in MAS. Therefore, the rate of respiration was inhibited as wells as weight losses. Meanwhile, unpacked fruit showed the highest weight loss due to normal respiration rate and transpiration

These indicated that application of KMnO4 as ethylene absorbent is suitable for inhibit respiration process. This result agrees Kader (1986) that MAS combined with KMnO4 would delay the rate of respiration and transpiration of fruit (Karikari *et al*, 1988; Artés-Hernandéz *et al*., 2004

Decay's percentage

The decay's percentage of mangoes during storage increase, only fruit kept in PE plastic bags after three weeks indicated the lowest decay (Figure 5). It assumed that the lowest value because of PE plastic bags provide suitable condition of storage lead to low water release by respiration/ transpiration (Pantastico, 1997). The symptoms of decays in mangoes were notice on the skin changes from green to brownish green lesions after 2 weeks storage. Barmore (l987) stated that the advantage of MAS with KMnO4 was delayed ripening process due to low external ethylene in package. Supported by Porat *et al.*, (2004) who found that MAS reduced postharvest rind disorders in citrus fruit.

IV. Conclussion

Inactivation of ACC_O occurred to almost half of its activity in MAS as compared to air storage.The rate of respiration and production of ethylene at MAS was inhibited leads to extend the storage life of mangoes.Weight losses and decay's percentage of mango kept in MAS for 3 weeks were lower than control.

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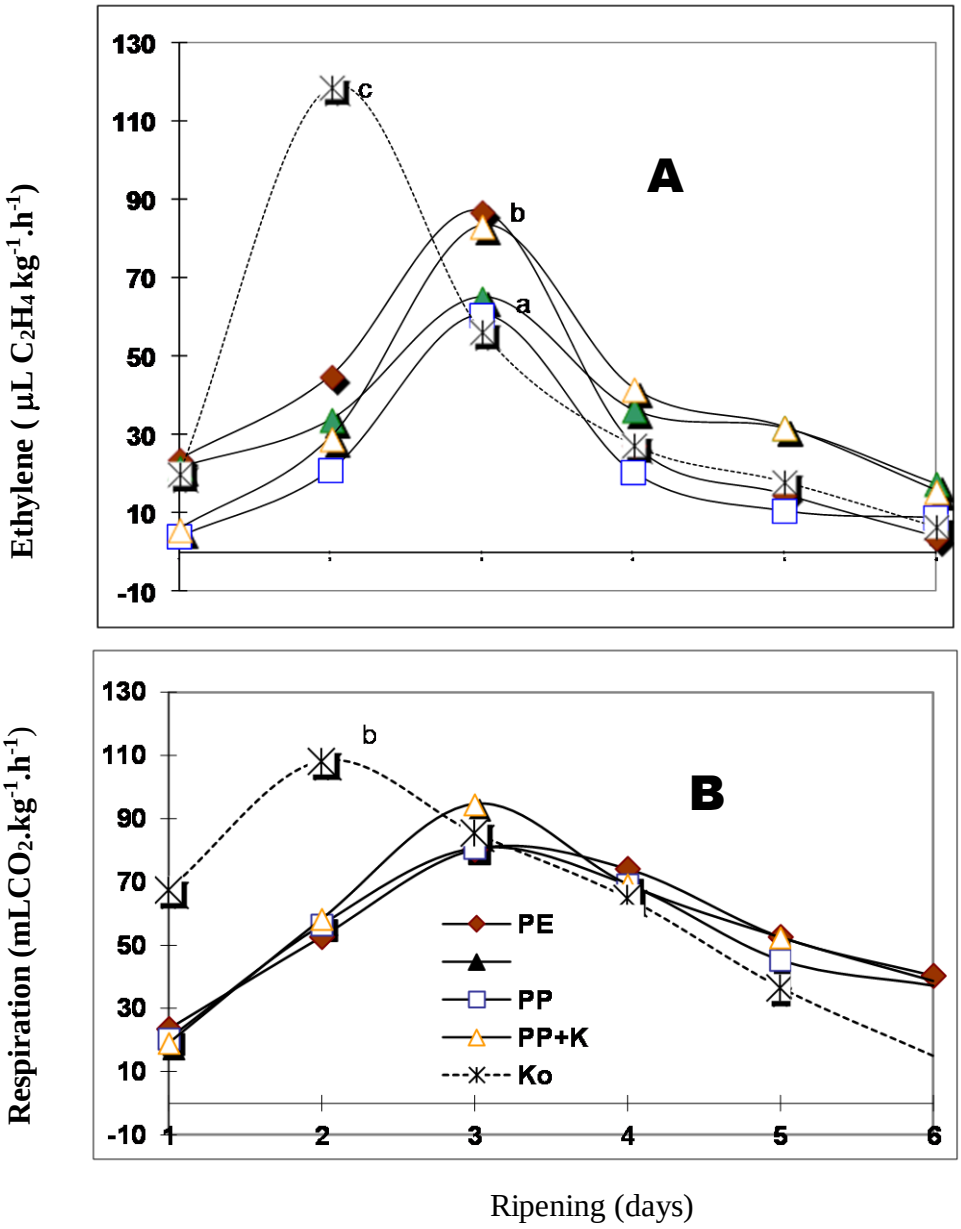

Fig. 3. Ethylene production (A) and the rate of respiration (B) of mangoes during ripening process following transfer from MAS after 2 weeks storage. The graph that followed by the same letter at the same time storage indicated non significant difference according to LSD 5 %.

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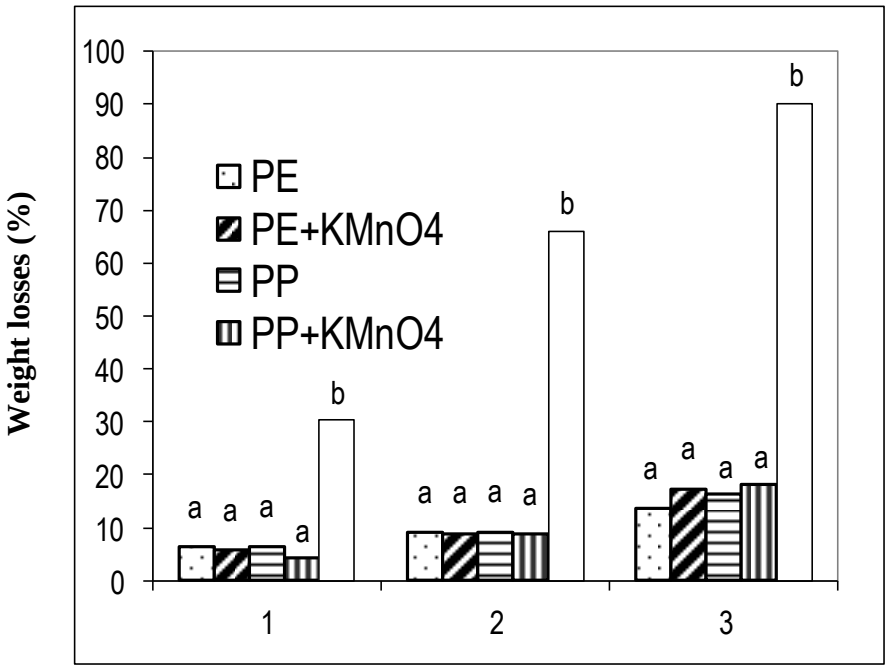

Fig. 4. Weight losses of mangoes stored in MAS for 1, 2 and 3 weeks. The graph that followed by the same letter at the same time storage indicated non significant according to LSD 5 %.

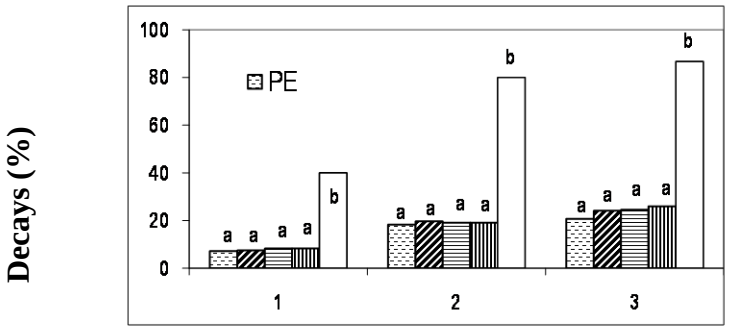

Fig.5. Decays percentage of mangoes stored in MAS for 1,2 and 3 weeks. The graph that followed by the same letter at the same time storage indicated non significant according to LSD 5 %.

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ISSN 2278-2540 | DOI: 10.51583/IJLTEMAS | Volume XII, Issue X, October 2023

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