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Inhibition of Ethylene Biosynthesis in Modified Atmosphere Stored Bananas

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ABSTRACT. *Cavendish bananas were stored in different modified atmosphere (MA) conditions using low density polyethylene bags. The MA conditions were created by sealed Polyethylene bags (0.05 mm, SPEB), evacuated and sealed Polyethylene bags (0.05 mm, EPEB), thick Polyethylene bags (0.1 mm, TPEB) and a control using perforated polyethylene (0.05 mm) bags at 14°C for 30 days. Gas composition of the MA, levels of 1-aminocyclopropane-1-carboxylic acid (ACC), and Malonyl ACC (MACC) which is an ACC conjugate and ACC oxidase activity were measured in order to understand how MA affected ethylene biosynthesis. Reduced oxygen and increased carbon dioxide levels were measured in MA conditions while no ethylene was recorded. A different gas composition was associated with each treatment depending on thickness of the film. Control fruits ripened 20 days after storage, while fruit held under different MA conditions remained green and firm after 30 days of storage. The ACC levels accumulated during the storage in MA held bananas while in control fruits ACC levels remained more or less constant for up to 20 days of storage. The MACC levels increased in all the bananas during storage indicating that MA had no effect on conversion of ACC to MACC. The ACC oxidase activity of pulp disks declined in all MA stored bananas. The data indicate that the extension of the storage life of bananas under MA conditions was due to delay in ethylene production as a result of inactivation of the ACC oxidase enzyme.*

INTRODUCTION

Storage of banana in Polyethylene bags (PEB) and the establishment of modified atmosphere (MA) conditions has been extensively reported to extend the storage life of fruit appreciably at ambient temperature (Scott *et al.*,

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1968; Liu, 1970; Fuchs and Gorodeisky, 1971; Sarananda, 1992). This is a low cost technique which could assist banana producers to transport their fruits for a short duration without the need for costly refrigeration (Scott *et al.*, 1983). Keeping MA stored bananas at refrigerated temperature can further extend the storage life. MA together with low temperature storage would therefore enable to transport more mature bananas which give better flavour.

A decline in respiration and delay in ethylene production has been reported when bananas are stored under MA conditions (Scott, *et al.*, 1983). Respiration can be expected to be lower due to low oxygen concentration of the MA environment. Mapson and Robinson (1986) have observed that the delay in onset of ripening under less than 7.5% oxygen was due to inability of the fruit to synthesize ethylene.

Accumulation of ACC (1-aminocyclopropane-1-carboxylic acid) which is an immediate precursor of ethylene has been observed under stress conditions (Yang, 1985). Rapid onset of ripening when fruits were transferred to air, which were stored in high carbon dioxide and low oxygen, provide strong evidence to support the hypothesis that MA has increased the synthesis of ACC (McGlasson and Wills, 1972). The reason why ACC is not converted to ethylene under MA conditions has not been reported. Therefore, the objective of this paper was to investigate as to the reasons why ACC is not converted to ethylene in MA storage.

MATERIALS AND METHODS

Mature green Cavendish bananas exported from Jamaica, stored at 14°C for two weeks during transportation to UK were used for the study. Damage free hands were selected, divided into 2 clusters, washed in tap water and were allowed to air dry at 25°C. Low density polyethylene was used to create modified atmosphere. Eighteen individual clusters were enclosed in Polyethylene bags (0.05 mm low density) 40 x 24 cm and the bags were sealed using an impulse sealer. Similarly gas in another 18 bags was evacuated using a low pressure vacuum pump and sealed. Another 18 clusters were placed in similar size PEB but 0.1 mm thick polyethylene and were sealed. The remaining 18 clusters were enclosed in PEB similar to that used in SPEB but with 40 perforations each of 0.5 cm in diameter and used as controls. All the bags were transferred into a cold room which was maintained at 14°C and 90% RH.

Three bags from each treatment were randomly taken out daily on initial 3 days of storage and then at 10 day intervals up to 30 days. Oxygen and carbon dioxide concentrations of bags were measured using gas chromatograph fitted with alumina column and thermal conductivity detector. Column temperature was maintained at 90°C and nitrogen gas was used as a carrier. ethylene concentration was measured by using Photovac gas analyzer.

ACC oxidase activity

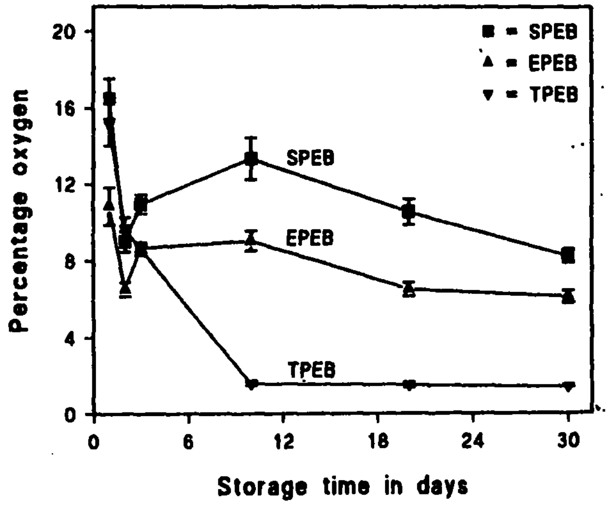
ACC oxidase activity was measured *in vivo* according to Dominguez and Vendrell (1993). A piece of pulp tissue was removed by passing a sterilized cork borer (10 mm diameter) through a finger and pulp disks of 2 mm thickness were transferred into a sterilized 25 ml flask. Two ml of sterilized water was added to the flask and sealed. A tissue of similar size from the same finger was placed in another flask of same volume and 2 ml of 5 mmole ACC solution was added. After a three hour incubation period, 1 ml of gas sample was withdrawn from the head space and the ethylene concentration was measured using Photovac gas analyzer. Activity of ACC oxidase was expressed as nmole C_2H_4 gFW^{-1} h^{-1}.

ACC and MACC assay

Pulp tissue for ACC and MACC assay was frozen in liquid nitrogen. The frozen tissue was ground into a fine powder using a coffee grinder and stored at -20°C. ACC content of tissue was measured following the method of Lizada and Yang (1979). ACC was extracted by homogenizing 1 g tissue with 2 ml of ethanol and the mixture was centrifuged. Supernatant was allowed to dry in an air flow. Ethylene liberated in the presence of Hg^{+2} and a mixture of NaOCl : NaOH at a ratio 1:2 was measured using a Photovac gas analyzer. MACC was measured from the aqueous extract hydrolysed with 2N HCl in a water bath maintained at 100°C for 3 h (Hoffman, *et al.*, 1982). Rest of the procedure was similar to the ACC assay. Results were expressed as nmol MACC gFW^{-1}.

RESULTS

Oxygen, and carbon dioxide concentrations of MA are illustrated in Figure 1a and 1b, respectively. Oxygen levels of all the MA treatments declined while carbon dioxide levels increased. The maximum level of carbon



SPEB - Sealed Polyethylene Bags
TPEB - Thick Polyethylene Bags
EPEB - Evacuated and Sealed Polyethylene Bags

Figure 1a. **Oxygen concentration of modified atmosphere during storage.**

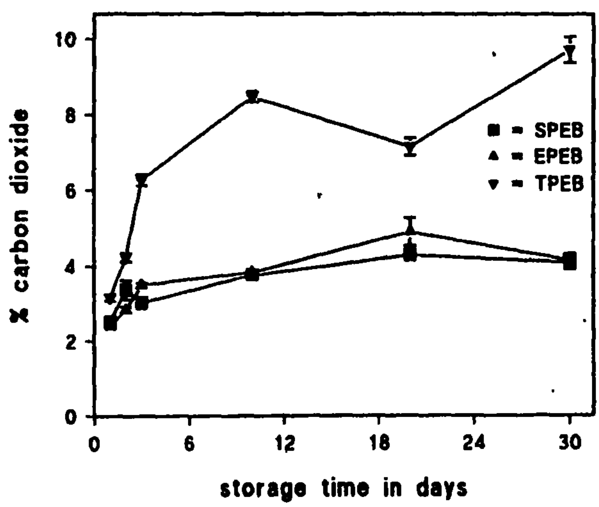


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Figure 1b. **Carbon dioxide concentration of modified atmosphere during storage.**

dioxide (6.7%) and the minimum level of oxygen (1.6%) were observed in TPEB at 20 days of storage. Although carbon dioxide level was maintained in more or less equal concentrations in SPEB and EPEB, the oxygen concentration was lower in the EPEB than SPEB. The maximum concentration of ethylene was 0.6 ppm observed during the first 3 days of storage and was not detectable later part of the storage.

ACC and MACC levels

ACC levels of the pulp of bananas packed in different modified atmosphere packages are illustrated in Figure 2a. The ACC levels in all the MA treatments remained approximately the same as in control for up to 20 days of storage. Control fruits ripened before 30 days of storage hence data were not included. ACC levels in the pulp for all MA stored bananas increased after 20 days of storage. The highest ACC level was recorded in bananas stored in EPEB and the lowest level was associated with bananas stored in SPEB.

A rapid increase in MACC level was observed in all the treatments during first 3 days of storage (Figure 2b) but then the MACC level levelled off. There was no significant difference observed among treatments at any stage of storage indicating that synthesis of MACC was not affected by MA.

ACC oxidase activity

ACC oxidase activity of bananas stored in MA compared to control was given in Figure 3. An initial higher ACC oxidase activity was observed in bananas stored in TPEB. ACC oxidase activity of control fruits which were stored in perforated PEB remained unchanged throughout the storage period. However, no observation was made at 30 days of storage due to early ripening of bananas stored in perforated PEB. A decline in ACC oxidase activity was associated with all MA stored bananas.

Although bananas used for the experiment were not freshly harvested, gas compositions developed in all MA were able to keep the fruits green and firm for 30 days. Moreover, the eating quality of ripe fruits which were stored in all MA remained acceptable. In contrast, control fruits ripened 20 days after storage even at 14°C.

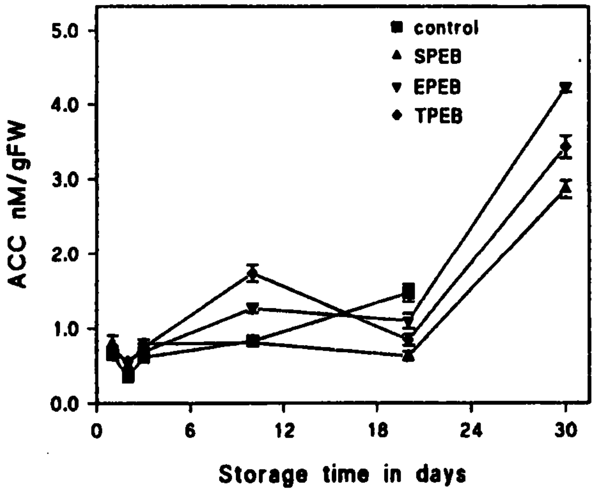

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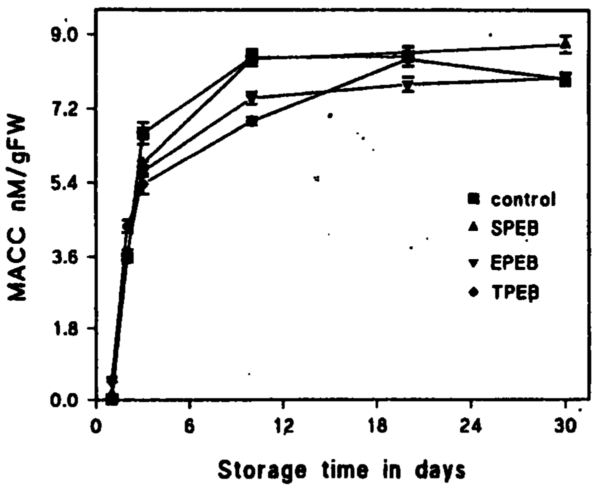
Figure 2a. **1-aminocyclopropane-1-carboxylic acid (ACC) levels of modified atmosphere stored bananas.**



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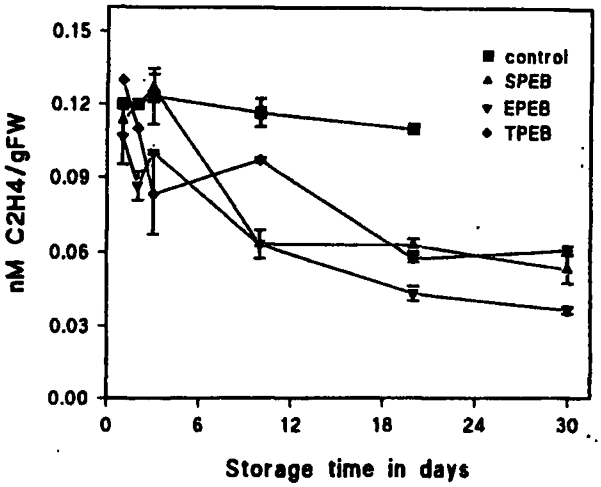
TPEB - Thick Polyethylene Bags
EPEB - Evacuated and Sealed Polyethylene Bags

Figure 2b. **Malonyl ACC (MACC) levels of modified atmosphere stored bananas.**



SPEB - Sealed Polyethylene Bags
TPEB - Thick Polyethylene Bags
EPEB - Evacuated and Sealed Polyethylene Bags

Figure 3. **1-aminocyclopropane-1-carboxylic acid (ACC) oxidase activity of modified atmosphere stored bananas.**

DISCUSSION

The variable gas composition achieved by MA was probably due to different levels of permeability and thickness of the film. The low levels of oxygen in combination with higher levels of carbon dioxide extended the storage life of MA stored bananas. The initial trace amount of ethylene observed in MA may be due to stress induced ethylene (Yang, 1985) resulting from exposure to high carbon dioxide, low oxygen and low temperature. Since ethylene is a highly volatile gas it must have escaped through the film. Absence of ethylene observed in MA stored bananas during subsequent storage may have been due to inhibition of ethylene biosynthesis mechanism.

The conversion of S- adenosyl methionine (SAM) to ACC is the main site of control of ethylene biosynthesis pathway in plant tissues (Yang, 1980). If it was the same case for MA stored bananas, ACC levels would expected to be lower during the storage in order to inhibit ethylene production. However, results showed that ACC levels increased in MA stored bananas at latter part of storage. No ethylene was detected, although the ACC levels were increased. Therefore, conversion of SAM to ACC was not inhibited by storing bananas under MA. The fact that stress conditions accelerate the ACC synthesis (Yang,

1985) confirms the higher levels of ACC accumulated in MA stored bananas at latter part of the storage.

ACC can either be oxidized to ethylene or converted to MACC as an ACC conjugate which is a irreversible reaction (Hoffman *et al.*, 1982).

Results showed that activities of ACC oxidase in all MA stored bananas kept on declining throughout the storage period. Conversion of ACC to ethylene is a oxygen dependant reaction (Yang, 1985). Therefore, at low levels of oxygen in MA, activity of this enzyme was not detected. The key factor which regulate the autocatalytic ethylene production in bananas has been reported to be the increase in ACC oxidase activity (Dominguez and Vendrell, 1993). The results of the present experiment confirms the decline in ACC oxidase activity in MA stored bananas is the main factor which inhibit ripening.

The increase in MACC gave evidence that part of the ACC has got converted to MACC which was a ACC conjugate. However, the similar levels of MACC in both control and MA stored bananas showed the increase was not due to MA. Early ripening of control fruits showed there was no barrier to convert ACC to ethylene. Therefore, the non-conversion of ACC to ethylene in MA stored bananas can only be explained through the possible mechanism of inactivation of ACC oxidase.

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