Postharvest Biology and Technology, 3 (1993) 183–190

POSTEC 01053

Effects of CO_2 on ethylene biosynthesis in 'Bartlett' pears *

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(Accepted 7 May 1993)

ABSTRACT

Mature-green 'Bartlett' pear (*Pyrus communis* L.) fruit discs (8 mm diameter and 10 mm thick) were placed under a continuous flow of air (control) or air enriched with 0.1, 0.5, 1, 5, 10, or 20% CO_2 for up to 10 days at 20°C. Pear discs kept in air+5 to 20% CO_2 exhibited lower ethylene production rates, lower activities of 1-aminocyclopropane-1-carboxylic acid (ACC) synthase and ACC oxidase (ethylene forming enzyme = EFE) and remained greener than fruit discs stored in air. ACC oxidase activity (both in vivo and in vitro) and ethylene production rate increased in fruit tissue exposed to air+1% CO_2. Depending on its concentration within the pear fruit tissue, CO_2 can either stimulate or inhibit ACC oxidase activity, and consequently ethylene production rate. At concentrations above 1%, CO_2 retards ethylene action.

Key words: *Pyrus communis*; Ethylene; Controlled atmosphere; ACC synthase; ACC oxidase

INTRODUCTION

Ethylene is generally considered to be the hormonal regulator of the ripening of climacteric fruits, but the exact mechanism by which the rise in the rate of ethylene synthesis takes place is not known.

Adams and Yang (1979) described the pathway of ethylene production in higher plants as follows: Methionine → SAM → ACC → C_2H_4. ACC synthase, which converts SAM to ACC, is known as the rate limiting enzyme in this pathway. The content of ACC is low during the preclimacteric stage of many fruits, but increases greatly during the climacteric, and then decreases in the postclimacteric stage (Yang and Hoffman, 1984).

Ethylene-forming enzyme (EFE) activity in vitro has been difficult to study because its activity completely disappeared during extraction (Yang and Hoffman,

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* Research supported, in part, by BARD grant no. US-1746-89.

1984; Kende, 1989). Very low EFE activity was retained in isolated vacuoles (Guy and Kende, 1984) and by kiwifruit membrane vesicles (Mitchell et al., 1988), and their activity was greatly reduced when membrane structure was destroyed. From this it has been assumed that EFE is membrane bound (Guy and Kende, 1984; Porter et al., 1986). However, recently complete recovery of in vitro EFE activity in a cell free soluble fraction from melon fruits has been obtained by Ververidis and John (1991). Extraction of a viable enzyme from Golden Delicious apple fruit was improved by adding insoluble polyvinylpolypyrrolidone (PVPP) to bind polyphenols which otherwise inactivate the enzyme (Kuai and Dilley, 1992).

Carbon dioxide can reduce, promote, or have no effect on ethylene production or action in fruits, depending on the commodity, variety, physiological age, initial quality, CO_2 concentration, temperature, and duration of exposure to such conditions (Kader, 1986; Kubo et al., 1990). Both beneficial and detrimental effects of CO_2 have been demonstrated, but the mode of CO_2 action on plant tissues is still not well understood.

Cheverry et al., (1988) reported that 20% CO_2 at 20°C quickly inhibited climacteric ethylene production in 'Granny Smith' apples and 'Fuerte' avocados but did not modify the content of 1-aminocyclopropane-1-carboxylic acid (ACC). Therefore it seems that high CO_2 levels inhibit conversion of ACC to ethylene.

The promotive effect of CO_2 on ethylene production has been demonstrated in seeds (Kato and Esashi, 1975) and leaves (Aharoni et al., 1979). Philosoph-Hadas et al. (1986) found that CO_2 stimulates the synthesis of the ethylene-forming enzyme (EFE) in leaf discs. It appears that low concentrations of CO_2 exert their effect by promoting the conversion of ACC to C_2H_4.

The inhibitory effect of CO_2 on autocatalytic ethylene production in climacteric fruits could be due to a competition for the same active site (Burg and Burg, 1967). When CO_2 concentration is low (about 1%) this may promote ethylene formation in climacteric fruits (Bufler, 1986); however, at 5 to 20% CO_2 has the opposite effect (Cheverry et al., 1988).

Ethylene exerts its effect whether it is biosynthesized by the plant tissue or supplied externally. It is thought that ethylene needs to bind to a receptor to form an activated complex to trigger the primary response. This primary reaction once started can lead to a chain of reactions, including modification of gene expression, thereby leading to a wide range of physiological responses (Yang, 1985). Sisler and Wood (1988) stated that the effect of CO_2 is complex with both stimulation of C_2H_4 production as well as an inhibitory effect which does not appear to directly involve the binding site.

This study was designed to examine the effects of various levels of CO_2 added to air on the biosynthesis of ethylene in pear fruit discs.

MATERIALS AND METHODS

Plant material and treatments. Mature-green 'Bartlett' pears were stored at 0°C for 2 weeks before the experiments were initiated. This cold treatment induces more uniform ripening upon transfer to 20°C. Fruits were selected for uniformity

of size and freedom from defects. Skin color was measured with a Minolta colorimeter (Minolta, Ramsey, NJ) using 'a' color value (green to red). Fruits with similar color and ethylene production rate were dipped in a solution of commercial bleach (5% chlorine) for one minute, then washed with a detergent and distilled water. Plugs were extracted from one side on the equatorial zone to obtain discs at uniform maturity stage. Fruit discs were placed in tissue culture plates (16 discs per plate) and ventilated with air or air + CO_2 (0, 0.1, 0.5, 1, 5, 10, or 20%) using a continuous flow-through system (10 ml/min.), and were left at 20°C overnight before measurements were made to avoid wound ethylene. The experiments were continued up to 10 days at 20°C. The atmosphere inside the plates was equilibrated after 10 h, and then each plate was closed (air-tight) during 10 min and gas samples were taken with a 1 ml syringe to measure ethylene. A Carle flame ionization gas chromatograph (Model 211, EG & G Chandler Engineering, Tulsa, Okla.) was used to determine ethylene concentration in these gas samples.

Preparation of extracts. Nine pear discs per treatment were taken for analysis of ACC content and activities of EFE, and ACC synthase. During the preparation of extracts the temperature was kept below 3°C at all times.

Assay of intermediates and enzymes. ACC synthase was extracted from the tissue and assayed following the method of Yu et al. (1979). ACC extracted in 80% ethanol was determined as described by Lizada and Yang (1979). Determination of EFE activity was elaborated by measuring the conversion of ACC to ethylene, as described by Hoffman and Yang (1982).

Preparation of EFE extracts. Four pear discs per treatment were used to obtain enzyme extracts for EFE activity in vitro. During the preparation of extracts the temperature was kept below 3°C at all times and 10% PVP was added. Assays were performed using the methodology of Ververidis and John (1991). In a preliminary test, enzyme extracts were performed inside of a plastic bag filled with nitrogen gas, and under a constant flow of nitrogen gas. EFE activity 'in vivo' was higher in discs kept in air and no significant difference was found between N_2-filled plastic bag and extraction under a continuous flow of N_2 gas. Thus, all subsequent extractions were done in air. Adding polyvinylpyrrolidone (PVP) during extraction to bind phenolic compounds improved the EFE activity in vitro.

Optimum pH in vitro and in vivo. Optimum pH curves for EFE activity in vitro of preclimacteric and climacteric pears exhibited two peaks one at pH 7.0 (MOPS buffer and the other at pH 8.0 (Tris-HCl). EFE activity in vivo using pericarp discs showed a maximum activity at pH 8.6. Smith et al. (1992) reported a pH optimum at about pH 7.5 for EFE partially purified from melon.

RESULTS AND DISCUSSION

Effects of CO_2 on ethylene production. Pear discs exposed to air + 0.5% CO_2 had a higher ethylene production rate than those kept in air. Air enriched with 1% CO_2 resulted in a two-fold increase of ethylene production relative to discs kept in air. The air + 5% CO_2 treatment reduced ethylene production rate during the first 5 days by about 50% in comparison with the air control. Under air + 20% CO_2,

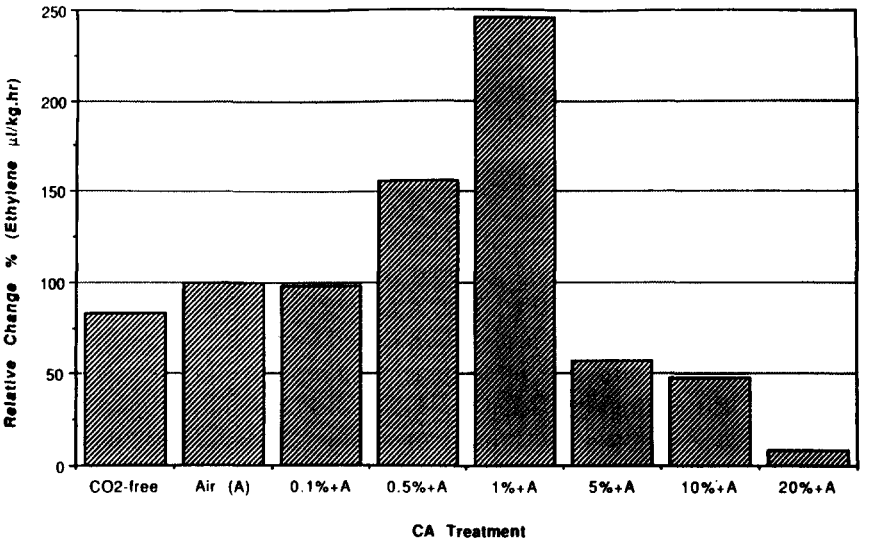

Fig. 1. Relative change (%) of ethylene production rate (μl/kg.hr) at climacteric peak of pear discs under air + CO_2 (0, 0.1, 0.5, 1, 5, 10, and 20%).

ethylene production was reduced by more than 5 times during the first 7 days and more than 2 times during the last 3 days of storage at 20°C. There were no significant differences among the control (air), CO_2-free air and air + 0.1% CO_2.

Figure 1 summarizes all CO_2 treatments tested and their relative change (% of ethylene production rate) was calculated in relation to the maximum climacteric peaks of air (control) treatment. Air + 1% CO_2 and air + 20% CO_2 treatments

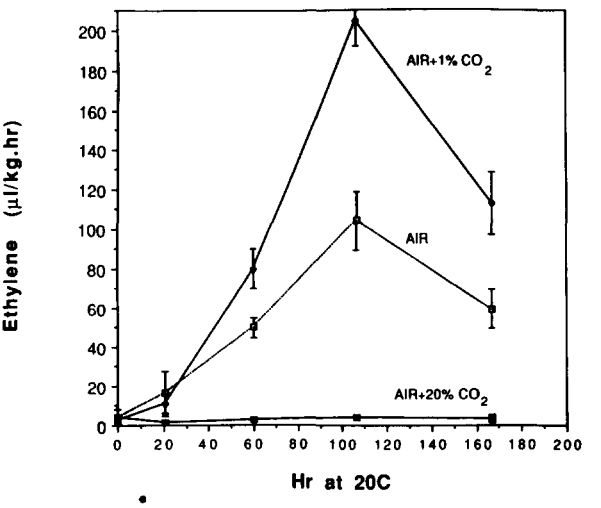

Fig. 2. Ethylene production rates (vertical bars represent ± SD) of 'Bartlett' pear fruit discs kept in air and in air + 1 or 20% CO_2 at 20°C.

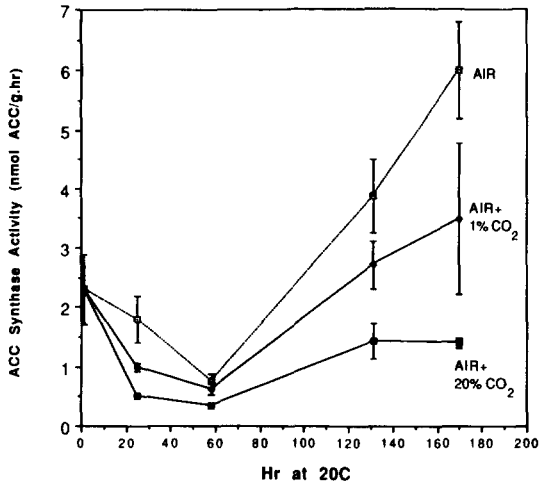

Fig. 3. ACC synthase activities (vertical bars represent ±SD) in 'Bartlett' pear fruit discs kept in air and in air + 1 or 20% CO_2 at 20°C.

were selected to study the changes in activities of the key enzymes and in intermediates of ethylene biosynthesis.

Effect of CO_2 on ethylene intermediates and enzymes. The opposite effect of 1% and 20% CO_2 added to air on the ethylene production rate was very clear in pear discs used for analysis of key intermediates and enzymes (Fig. 2).

The activity of ACC synthase in discs exposed to air + 20% CO_2 was the lowest during 7 days at 20°C (Fig. 3); under air + 1% CO_2 ACC synthase activity was lower than the control.

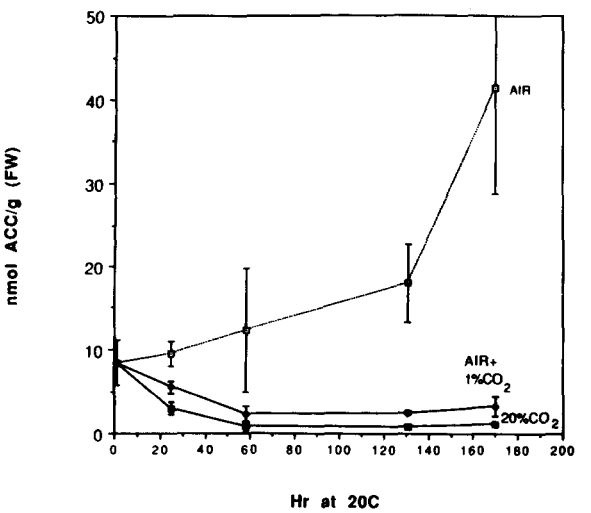

Fig. 4. ACC contents (vertical bars represent ±SD) in 'Bartlett' pear fruit discs kept in air and in air + 1 or 20% CO_2 at 20°C.

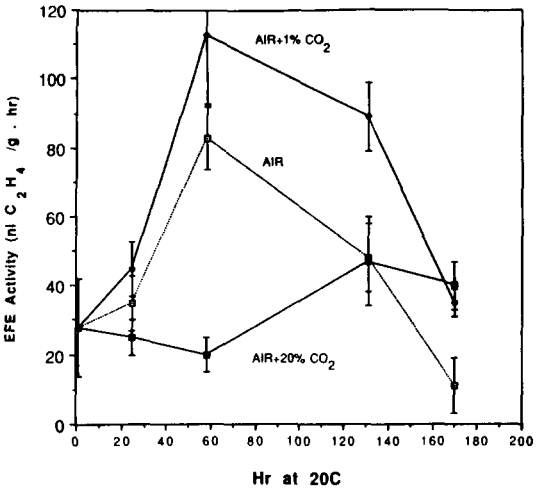

Fig. 5. 'In vivo' ethylene-forming enzyme (EFE) activities (vertical bars represent ±SD) of 'Bartlett' pear fruit discs kept in air and in air + 1 or 20% CO_2 at 20°C.

The ACC content (Fig. 4) decreased when CO_2 was added at either 1% or 20% to air. The activity of EFE in vivo (Fig. 5) and in vitro (Fig. 6) was stimulated by the 1% CO_2 + air treatment but was reduced by the 20% CO_2-enriched air treatment.

EFE activity in vitro (Fig. 6) was lower than in vivo (Fig. 5) probably because the crude enzyme extract was used for the assay without purification; however both

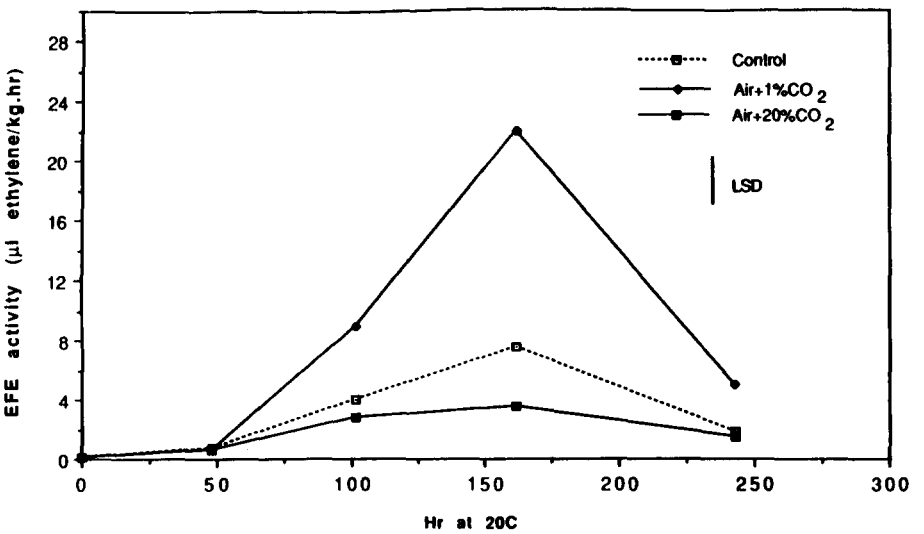

Fig. 6. 'In vitro' ethylene-forming enzyme (EFE) activity of 'Bartlett' pear tissue extracts obtained from discs kept under a continuous flow of air (control), air + 1% CO_2, and air + 20% CO_2 at 20°C.

methods exhibit higher EFE activity under air + 1% CO_2. All the assays were performed at optimum pH of 7, which was close to 7.2 used by Ververidis and John (1991). Optimum pH for EFE activity in vivo was 8.6.

The results reported here show that 5 to 20% CO_2 levels inhibit the production of ethylene. Reduced ethylene production by 'Bartlett' pears maintained in air + 10% CO_2 was reported by Kerbel et al. (1988). Kubo et al. (1990) reported that CO_2-enriched atmospheres either reduced ethylene production or caused no change depending on the commodity.

Bufler (1986) found that 1% CO_2 can increase ethylene production in apple while higher concentrations inhibited such production. ACC synthase induction and the EFE are inhibited by high CO_2 levels in the atmosphere (Bufler, 1984). Cheverry et al. (1988) reported that EFE induction (de novo synthesis) in apple and avocado discs was inhibited by CO_2, but the EFE activity was increased by this gas. ACC synthase was not affected by 20% CO_2 in tomato discs (Zamponi et al., 1990).

CONCLUSIONS

We conclude that CO_2 had two different effects on ethylene biosynthesis in pear discs depending on its concentrations: all elevated CO_2 levels inhibit activity of ACC synthase, while EFE activity is differentially affected by CO_2, being stimulated at low CO_2 levels and inhibited at high CO_2 levels.

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