J Plant Growth Regul (1983) 2:81–87

Journal of
Plant
Growth
Regulation

Effect of Low Oxygen and High Carbon Dioxide on the Levels of Ethylene and 1-aminocyclopropane-1-carboxylic Acid in Ripening Apple Fruits

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Received January 5, 1983; accepted May 2, 1983

Abstract. The association of the level of ACC and the ethylene concentration in ripening apple fruit (*Malus sylvestris* Mill, var. Ben Davis) was studied. Preclimacteric apple contained small amounts of ACC and ethylene. With the onset of the climacteric and a concomitant decrease in flesh firmness, the level of ACC and ethylene concentration both increased markedly. During the postclimacteric period, ethylene concentration started to decline, but the level of ACC continued to increase.

Ethylene production and loss of flesh firmness of fruits during ripening were greatly suppressed by treatments with low O_2 (O_2 1–3%, CO_2 0%) or high CO_2 (CO_2 20–30%, O_2 15–20%) at the preclimacteric stage. However, after 4 weeks an accumulation of ACC was observed in treated fruits when control fruit was at the postclimacteric stage.

Treatment of fruit with either low O_2 or high CO_2 at the climacteric stage resulted in a decrease of ethylene production. However, the ACC level in fruit treated with low O_2 was much higher than both control and high CO_2 treated fruit; it appears that low O_2 inhibits only the conversion of ACC to ethylene, resulting in an accumulation of ACC. Since CO_2 inhibits ethylene production but does not result in an accumulation of ACC, it appears that high CO_2 inhibits both the conversion of ACC to ethylene and the formation of ACC.

Abbreviations: SAM, S-adenosyl-L-methionine; ACC, 1-aminocyclopropane-1-carboxylic acid.

Ethylene is a plant hormone initiating the ripening processes of fruits. (Pratt and Goeschl 1969, Mapson 1970, Abeles 1973). In climacteric-type fruits such as apple, a massive amount of ethylene is produced, which is accompanied by physiological and biochemical changes during ripening. Thus, the level of ethylene in fruit tissue is closely related to ripeness. Providing fruits have neither entered the ethylene climacteric nor have begun to ripen, any manipulation that reduces or delays ethylene production will retard ripening and thereby improve the quality of apple fruits (Liu et al. 1979). It is a commercial practice to store the apple fruits with low O_2 and high CO_2, which significantly reduces ethylene production, thereby reducing respiration and inhibiting ripening and senescence of fruits.

Adams and Yang (1979) and Lürssen et al. (1979) established the following sequence for the pathway of ethylene biosynthesis in plant tissues: Methionine → SAM → ACC → ethylene. In this study we examined the effect of low O_2 and high CO_2 on ethylene production and ACC accumulation in apple fruit during ripening.

Materials and Methods

Plant Material

Apple fruits (*Malus sylvestris* Mill, var. Ben Davis) were harvested from orchards in Haiyang County, Shanton Province, China, and treated either with low O_2 or high CO_2 at both preclimacteric (5 days after harvest, internal ethylene concentration $<0.1\ \mu l \cdot l^{-1}$) and climacteric stages (27 days after harvest, internal ethylene concentration $\sim 1500\ \mu l \cdot l^{-1}$).

Treatment with Low O_2 and High CO_2

Fruits (20 kg each) were enclosed in PVC-film (0.14 mm in thickness) bags. For high CO_2 treatment, the concentration of O_2 and CO_2 in the bag were maintained in the ranges of 15–20% and 20–30%, respectively, by addition of CO_2, O_2, or air. In low O_2 treatment, O_2 concentration was maintained between 1–3%, and CO_2 evolved from fruit was absorbed by a cup of concentrated KOH solution placed within the bag. Both CO_2 and O_2 were monitored by Orsat Apparatus. The experiment was performed at room temperature (15–20°C).

Determination of Ethylene Concentration and Firmness of Fruit

Ethylene concentration within the fruit tissues and the firmness of fruit were measured according to Liu et al. (1979). At each of the specified times, ten fruits were removed from each gas treatment. Holes 0.8 cm in diameter were punched to the core of each apple and sealed with rubber serum stoppers. After 20 min, gas samples were withdrawn by syringes from holes for ethylene determination on a gas chromatograph with a 3 × 2000 mm activated alumina column and a flame ionization detector. During this time, apples did not show pronounced wound ethylene production. Then flesh firmness of fruits was measured with a penetrometer (tip diameter 1.11 cm). After ethylene concentration and firmness measurement, those apples were sacrificed for ACC assay.

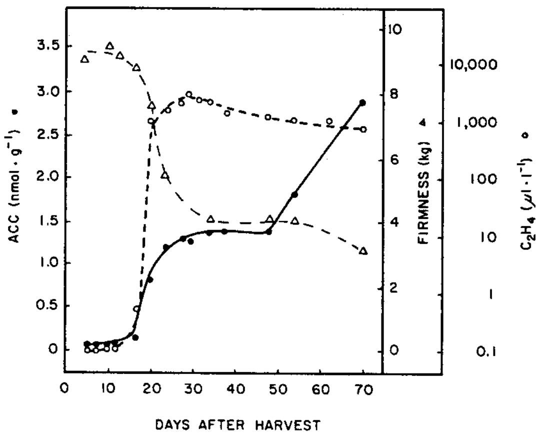

Fig. 1. Changes in ACC (●), internal ethylene concentration (○), and firmness (△) of apple fruit during ripening. open figures: ethylene; closed figures: ACC; control (○, ●); high CO_2 (△, ▲); low O_2 (□, ■). Arrow denotes point at which the fruits were transferred from air to low O_2 or high CO_2 treatment.

ACC Assay

Fruit tissue disks (0.8 cm in diameter × 0.2 cm) punched from ten fruits in each treatment were ground by glass mortar and pestle in 20 ml of 5% sulfosalicylic acid for each 10 g of tissue. After centrifugation for 15 min at 10,000 × *g*, the supernatant solution was passed through a column of cation exchange resin (732 type, Shanghai Resin Factory, H^+ form), ACC was eluted with 2N NH_4OH, and the eluate was concentrated under vacuum at 40°C. The residue was dissolved in 2 ml of distilled water, and 0.5 ml of the solution was used for ACC assay according to Lizada and Yang (1979). The efficiency of ACC conversion to ethylene ranged between 50% and 60%.

Results

Changes in Ethylene Concentration and ACC Content of Apple Fruits During Ripening

The changes in ACC content and flesh firmness in relation to internal ethylene concentration are shown in Fig. 1. Ethylene concentration was lower than 0.1 $\mu l \cdot l^{-1}$ and the flesh firmness was higher than 9.1 kg until 13 days after harvest, indicating that the fruit was at the preclimacteric stage (Liu et al. 1979). During this period the content of ACC was also very low (0.2 $nmol \cdot g^{-1}$). Between 15 and 20 days after harvest, ethylene production increased rapidly followed by a decrease in flesh firmness, showing that the fruit had entered the climacteric and that ripening had begun, at which time ACC content increased slightly. Afterward ethylene concentration continued to rise and reached a maximum (2500 $\mu l \cdot l^{-1}$) around the 30th day after harvest, while ACC level increased remarkably to 1.2–1.4 $nmol \cdot g^{-1}$ and flesh firmness decreased rapidly. On the 34th day, when the fruit was at the stage of postclimacteric with reduction in firmness at a minimum (ca. 4.1 kg), the internal ethylene concentration decreased slowly and steadily, while the ACC content remained higher, 1.2–1.4 $nmol \cdot g^{-1}$. In overripe fruits (approximately 50 days after harvest), the level of ACC increased rapidly once again but without a concomitant increase in internal ethylene concentration.

Table 1. Effect of high CO_2 or low O_2 on C_2H_4 concentration, ACC content, and firmness of fruit during ripening.

Days after harvest	Days of treatment	C_2H_4 ($\mu l \cdot l^{-1}$) Air	high CO_2	low O_2	ACC ($nmol \cdot g^{-1}$) Air	high CO_2	low O_2	Firmness (kg) Air	high CO_2	low O_2
7[a]	2	0.1	0.18	0.06	0.16	0.20	0.25	9.6	9.5	9.4
20	15	1040	0.14	0.09	0.79	0.15	0.24	7.9	10.1	9.5
34	29	1457	0.10	0.34	1.47	5.0	18.66	4.1	9.3	9.4

[a] 7, 20, and 34 days after harvest correspond to preclimacteric, climacteric, and postclimacteric stages, respectively, in control fruits.

Effects of Low O_2 and High CO_2 on Ethylene Production and ACC Formation in Preclimacteric Fruits

Ethylene production was markedly inhibited when preclimacteric fruits were stored under either low O_2 or high CO_2. Ethylene concentration remained as low as 0.2 $\mu l \cdot l^{-1}$ for 2 weeks after treatment (20 days after harvest), whereas rather high concentrations of ethylene (1000 $\mu l \cdot l^{-1}$) were found in the control fruits (Table 1), indicating that ripening of the control fruits had progressed. When ethylene concentration in the control fruits reached 1500 $\mu l \cdot l^{-1}$ 34 days after harvest, concentrations of ethylene in fruits treated with low O_2 or high CO_2 were only 0.34 and 0.10 $\mu l \cdot l$, respectively (Table 1). Moreover, in the fruits treated with either low O_2 or high CO_2, few changes in firmness were observed throughout this period, the value being about 9.5 kg (Table 1).

As shown in Table 1, from the 7th to the 34th day after harvest, the ACC level in the control fruit increased from 0.2 to 1.5 $nmol \cdot g^{-1}$. Although there was a trace amount of ACC (below 0.2 $nmol \cdot g^{-1}$), in both low O_2- and high CO_2-treated fruits 2 weeks after treatment, ACC level was found to increase markedly 4 weeks after treatment (34 days after harvest). In the case of low O_2-treatment, ACC content was as high as 18 $nmol \cdot g^{-1}$, which was nearly 100 times higher than that in fruits sampled 20 days after harvest and 10 times higher than that in the control at the same chronological age. Increase in ACC level was also observed in fruits receiving high CO_2 treatment. These data indicate that at the preclimacteric stage low O_2 and high CO_2 inhibited ethylene production and associated biochemical changes (e.g. reduction in flesh firmness), but resulted in accumulation of ACC. Increase in ACC level, however, was delayed by both low O_2 and high CO_2 treatments. It is noteworthy that in the case of high CO_2, ACC content was much lower than that in low O_2.

Effects of Low O_2 and High CO_2 on Ethylene Production and ACC Formation in the Climacteric Fruits

When the concentration of ethylene increased to 1500 $\mu l \cdot l^{-1}$, as concomitant accumulation of ACC was observed in control fruits (Figs. 1 and 2). As in preclimacteric fruits, low O_2 and high CO_2 exhibited a great inhibitory effect

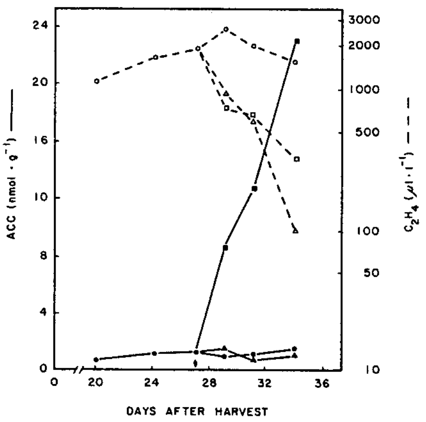

Fig. 2. Effect of low O_2 or high CO_2 treatment at climacteric stage on internal ethylene concentration and ACC level in apple fruits. open figures: ethylene; closed figures: ACC; control (○, ●); high CO_2 (△, ▲); low O_2 (□, ■). Arrow denotes point at which the fruits were transferred from air to low O_2 or high CO_2 treatment.

on ethylene production in climacteric fruit (Fig. 2). The concentration of ethylene decreased gradually after treatment with low O_2, while ACC content increased sharply to 23.2 nmol · g^{-1} within a week, which was 15 times higher than that found in the control. Similarly, ethylene concentration dropped rapidly as the apple fruit was treated with high CO_2; however, in contrast to low O_2, high CO_2 did not affect ACC level significantly during the 1 week subsequent to transferring the fruits to CO_2.

Discussion

The patterns of changes in ACC content and ethylene production in apple fruits during ripening are similar to those in avocado, banana, and tomato fruits reported by Hoffman and Yang (1980). It has been known that the formation of ACC is the rate-limiting reaction in ethylene biosynthesis in plant tissues (Yang 1980, Cameron et al. 1979, Yu et al. 1979). In this experiment the significant positive relationship between ACC level and ethylene concentration in ripening apple fruit was observed. In overripe fruits, however, the increase in ACC content was not accompanied by an increase in ethylene production. This observation was interpreted to indicate that the membrane-associated enzyme system catalyzing the conversion of ACC to ethylene was impaired as the tissue senesced, while the cytosol enzyme responsible for ACC synthesis (Boller et al. 1979, Yu et al. 1979) was not affected, and thereby, ACC was accumulated (Hoffman et al. 1980).

It has been observed in this experiment that although low O_2 and high CO_2 are able to inhibit ethylene production and ripening processes (e.g. decrease in firmness) in preclimacteric fruit, ACC synthesis is delayed, but not prevented, and eventually ACC is accumulated in large quantity.

Although both low O_2 and high CO_2 are effective in inhibiting ethylene production in apple fruits, the modes of their action seem to be different. Low O_2 applied at preclimacteric or climacteric stages inhibited ethylene production, but increased ACC content remarkably. It has been shown that low O_2 does not inhibit the formation of ACC, but inhibits the conversion of ACC to ethylene, since the latter reaction requires oxygen (Adams and Yang 1979, Boller et al. 1979). In nitrogen, ethylene production ceased, but a surge of ethylene production was observed when the fruits were returned to air (Burg and Burg 1965). Low O_2 should therefore limit the conversion of ACC to ethylene.

It has been reported that pretreatment with high CO_2 is effective to delay and/or inhibit ripening processes and ethylene production in apple fruits (Couey and Olsen 1975, Liu et al. 1979). Data reported in this paper also show that at either the preclimacteric or climacteric stage high CO_2 is able to inhibit ethylene production; however the ACC content is increased slightly (treated at preclimacteric) or unchanged (at climacteric), which is unlike the effect of low O_2. It is not known whether treatment of the climacteric fruits with high CO_2 beyond 7 days would result in accumulation of ACC as was found in preclimacteric fruits after treatment for 29 days. These observations indicate that high CO_2 may inhibit both the conversion of ACC to ethylene and ACC biosynthesis. Alternatively, high CO_2 may promote the metabolism of ACC to its conjugated form, which is not detectable by the Lizada and Yang assay (Amrhein et al. 1981, Hoffman et al. 1982). Since ripe apples do not form conjugate ACC (Amrheim et al. 1982), we feel that the former possibility is more likely. Another possibility is that high CO_2 or low O_2 may inhibit the onset of ripening and thereby inhibit ACC biosynthesis indirectly, since ACC synthesis may be simply the consequence of ripening.

Acknowledgments. The authors gratefully thank Professor S. F. Yang (Department of Vegetable Crops, University of California, Davis, USA) for a gift of ACC and for his critical reading of this manuscript.

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