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Changes of 1-Aminocyclopropane-1-carboxylic Acid Content in Ripening Fruits in Relation to their Ethylene Production Rates[1]

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ABSTRACT. Changes in the level of 1-amino cyclopropane- Icarboxylic acid (ACC) were compared to ethylene production during fruit ripening of avocado *(Persea americana* Mill.) banana *(Musa sapientum L.)* and tomato *(Lycopersicon esculentum* Mill.). Preclimacteric tissues contained less than 0.1 nmol/g of ACC in all tissues. In avocado, the level of ACC increased to 45 nmol/g in the later stage of the climacteric rise, then decreased to 5 nmol/g, and later increased to over 100 nmol/g in overripe fruit. In banana ACC increased to 5 nmol/g during the climacteric, decreased to 2 nmol/g several days after the climacteric peak, and increased up to 5 nmol/g in overripe fruit. Levels of ACC in tomato ranged from 0.1 to 10 nmol/g and were significantly correlated with ethylene production rates in all but overripe fruits. The correlation between the ACC content and the production of ethylene is discussed.

In climacteric-type fruits the dramatic increase in ethylene production is closely associated with an increase in respiration and with other ripening phenomena. The essential role of this increased ethylene production in initiating fruit ripening has been established (1). Adams and Yang (3) have studied ethylene biosynthesis in apple tissues and established the following sequence: Methionine → S-adenosylmethionine (SAM) → ACC → C_2H_4. In vegetative tissues ethylene production greatly increases following application of exogenous ACC (8). It is now clear that it is the conversion of SAM to ACC, but not the conversion of ACC to ethylene, which restricts ethylene production (16, 17). In contrast, preclimacteric apple, muskmelon, and avocado fruit tissues exhibited only slight increases in ethylene production following the application of ACC (D. 0. Adams, M. L. Bliss, and N. E. Hoffman, unpublished observations). These observations, coupled with the knowledge that preclimacteric fruits are unable to convert SAM to ACC (2), suggest that both the conversion of SAM to ACC and the conversion of ACC to ethylene are restricted in preclimacteric fruit tissues.

ACC was first identified in perry pears and cider apples by Burroughs (6) in 1957. Although Burroughs observed that the amount of ACC in perry pears increased during

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storage, he did not find ACC in all varieties of apple and pear he examined (7). To understand the regulation of ethylene production in ripening fruits, information with respect to the changes in ACC levels during the ripening process is needed. The present communication describes the changes in ACC levels in relation to ethylene production in ripening avocado, banana, and tomato fruits.

Materials and Methods

MATERIAL. 'Fuerte' avocados at the preclimacteric stage were harvested at the University of California, South Coast Field Station, in March and May, 1979. 'Tropic' tomatoes at the mature green stage were picked in March 1979 from a local greenhouse. Green ungassed bananas were purchased from a local shipper. Fruits were allowed to ripen naturally at 20°C in 10-liter glass jars through which humidified air was passed. ACC was purchased from Calbiochem.

ETHYLENE DETERMINATION. Ethylene production of each fruit was monitored individually during the course of ripening until it was sacrificed for ACC assay. Each fruit was placed in a separate enclosed jar (1 to 2 liter) for 1 hr, after which a 1-ml gas sample was withdrawn by syringe for ethylene determination by gas chromatography.

ACC EXTRACTION AND ASSAY. Fruit at a specified stage of ripening were individually diced and blended in a Waring blender, either in 2 ml of 4.5% (weight/volume) sulfosalicylic acid solution per g of avocado or in 1 ml of 6% sulfosalicylic acid per g of tomato or banana. After standing overnight at 0°C, the homogenates were centrifuged for 10 min at 10,000 x g. Supernatants were filtered through glass wool to remove lipids and nonpelletable insoluble materials. The supernatant was passed through a column of cation-exchange resin (Dowex 50, H^+) as described previously (12). Amino acids, including ACC, were eluted with 2N NH_4OH. After concentration under reduced pressure at 50^o, the eluates were assayed for ACC by using the NaOCl reagent as described in Lizada and Yang (12). In this assay the efficiency of ACC conversion to ethylene in each sample was determined by adding a known amount of ACC as an internal standard (12). The efficiencies ranged between 70 and 80% in all fruit extracts. In some cases the fruit tissues were extracted with $HClO_4$ at a final concentration of 5%. After neutralization with NaOH, the extract was directly subjected to ACC assay with the NaOCl reagent (12) without prior purification by ion exchange resin. Although the efficiency of ACC conversion to ethylene in these extracts was sometimes lower (70-80% for avocado, 60-70% for tomato, and 50-60% for banana extracts) than those which were purified with ion-exchange resin prior to assay, the quantities of ACC found in the 2 preparations were very similar. Extracts of tissues containing high levels of ACC, such as avocado, are less likely to need ion-exchanging because very little plant tissue is necessary for the assay.

Results

AVOCADO. Although the maximum ethylene production at the climacteric peak of fruits harvested in March was lower (100-120 $nl\bullet g^{-1}\bullet hr^{-1}$) than that of those harvested in May (250-300 $nl\bullet g^{-1}\bullet hr^{-1}$), the patterns of change in ACC levels in relation to ethylene production were similar. Avocado fruits are known to possess a sharp rise and fall in rate of ethylene production during the course of ripening (11). The relative ripening stage of each avocado was established by normalizing their rates of ethylene production to a typical ethylene production curve obtained from a single fruit which was monitored continuously for 10 days after harvest. Preclimacteric fruits produced very little ethylene and contained only trace amounts of ACC (less than 0.05 nmol/g fresh weight). The ACC level increased markedly during the climacteric rise, however, and then declined sharply. The highest level of ACC found during the climacteric rise was 45 nmol/g for the fruit harvested in March (Fig. 1). These data indicate that the increase in ACC accumulation is accompanied by the onset of ethylene production. Fruits sampled 1 to 2 days after the ethylene peak showed consistently lower ACC levels (about 5 nmol/g), but a fruit sampled about 3 days after the ethylene peak had a very high ACC concentration. This phenomenon was further investigated with fruit harvested in May. The pattern of ethylene production by each fruit was individually recorded so that the stage of ripening was expressed in days after the ethylene climacteric peak. As the ethylene production rate declined, the level of ACC also declined (Fig. 2). ACC content decreased to a minimum 1 to 2 days after the climacteric peak but thereafter accumulated again dramatically and reached more than 100 nmol/g on day 9 after the peak. Ethylene production continued to decline throughout this period.

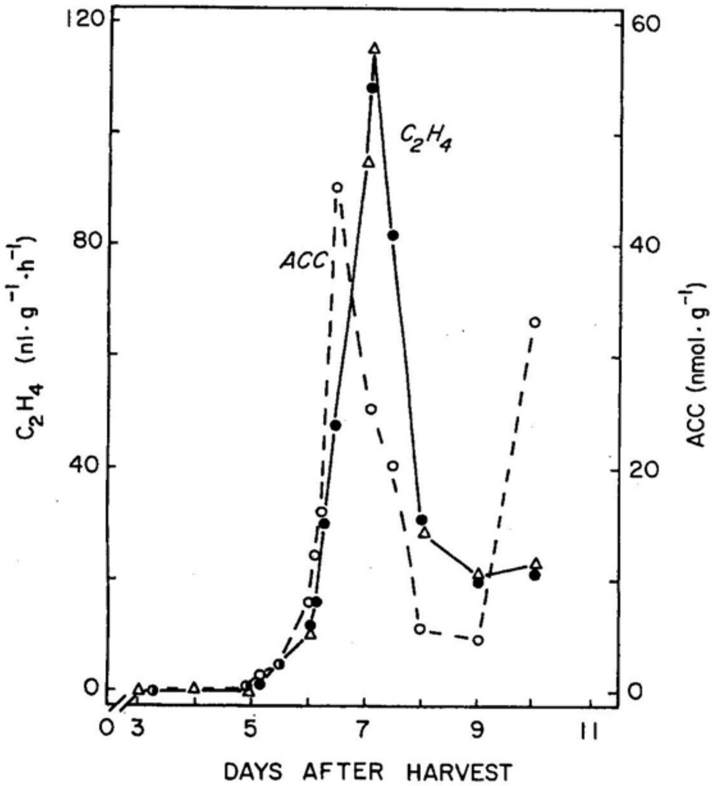

Fig. 1. Change in ACC content of avocado fruits during ripening. Fruits were harvested at mature unripe stage in March and were kept at 20°C in a humidified air stream. The ethylene curve (Δ—Δ) represents a single fruit plotted as a function of days after harvest. Ethylene production was individually determined for the other 14 fruits of the same lot before each was taken for analysis at the desired ripeness stage. The ethylene production datum for each such individual (●) was placed on the ethylene production curve at the time when the value matched the curve. The ACC content (o) for that same individual was then plotted at that same time.

BANANA. The ripeness of the fruit can be visually categorized according to an index of skin color (14). Stage 1 refers to green preclimacteric and stage 8 to overripe bananas. Fruit at the same color index generally produced similar amounts of ethylene, exceptstages 2 and 3, where the color index is of little use. During this period, the climacteric rise and the succeeding fall in ethylene production occurs too rapidly to be reflected by color change. To provide a more accurate portrayal of the ripening stages, ethylene production rates from fruits on the rise, peak, and fall were grouped, averaged, and plotted as color index 2, 2-3, and 3, respectively (Fig. 3). As in avocado, preclimacteric banana contained trace amounts of ACC (less than 0.05 nmol/g). As ethylene production increased so did the content of ACC. In banana, ACC levels remained high for 3 days after the ethylene peak (i.e., between stages 3 and 5) and then declined to low levels at stages 6 and 7. In overripe fruit (stage 8) the levels of ACC were repeatedly found to be higher than those in stages 6 and 7.

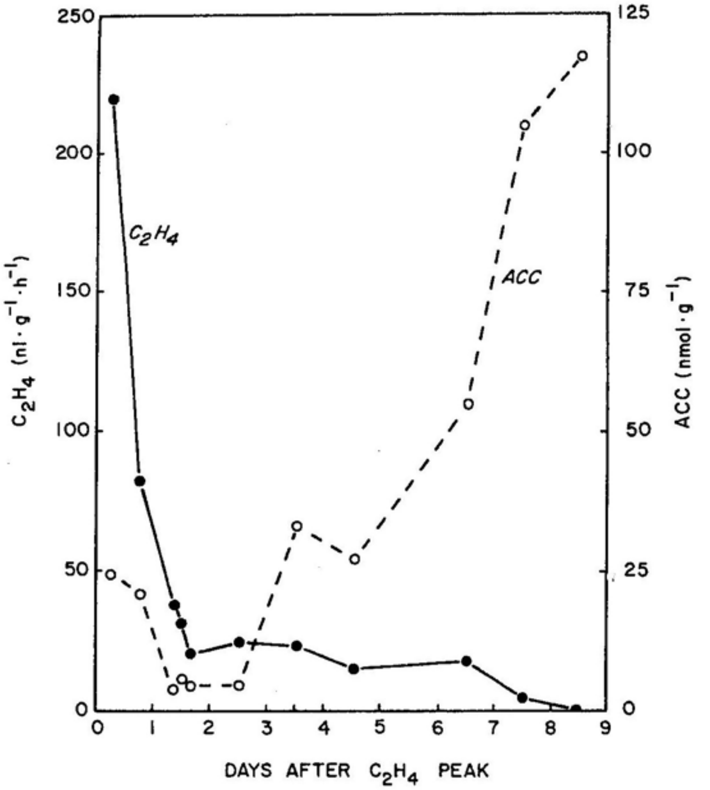

Fig. 2. Change in ACC content of avocado fruit during the post-climacteric stage. Each fruit was individually monitored for ethylene production during the course of ripening up to the time of ACC assay. The ACC content (○) of an individual fruit and its ethylene production rate (●) determined prior to ACC assay were plotted as a function of days after the ethylene climacteric peak. Fruits were harvested unripe in May and handled as in Fig. 1.

TOMATO. Tomatoes were classified as to degree of ripeness according to the following standard color system: 1, mature green; 2, breaker; 3, turning; 4, pink; 5, light red; 6, red. In Fig. 4, values of ethylene production and ACC levels from 2 to 3 fruits at the same stage were averaged and plotted against a color index. Both ACC level and ethylene production were low in mature green tomatoes, but increased at stage 2. Ethylene production continued to increase through stage 5 while ACC levels remained roughly constant. In very ripe fruit, stage 6, ACC levels were significantly higher than in previous stages. A similar relationship between color index and ACC level was also found for vine ripened tomatoes (data not shown). For the data in Fig. 4, stages 1 to 5, where C_2H_4 is increasing, ACC and ethylene are significantly correlated at 0.01 level, r = 0.756. When ACC levels and ethylene production rates are expressed in logarithm, their correlation was even more significant, r = 0.872.

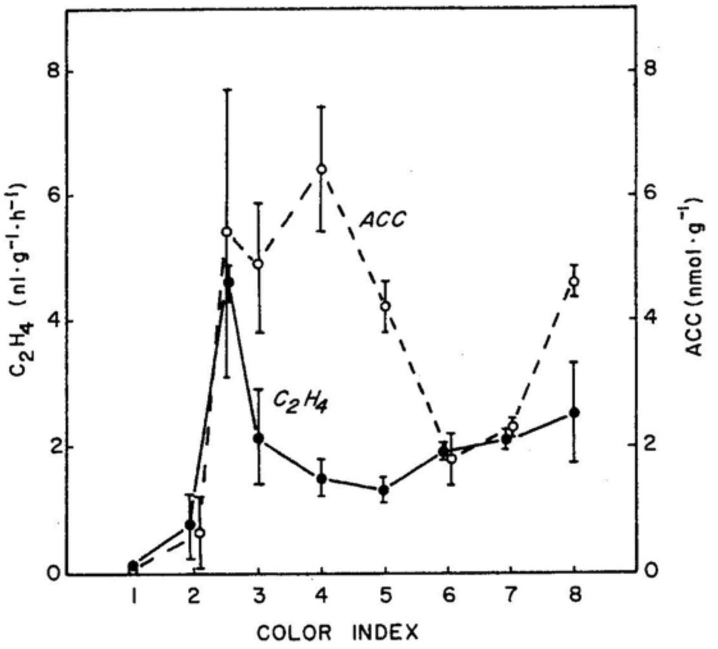

Fig. 3. Change in ACC content and ethylene production rates of banana as a function of a color index of ripeness. Stage 1 indicates preclimacteric, and stage 8 overripe fruit. Each fruit was monitored for ethylene production up to the time of ACC assay. For each fruit the ACC content (○) and its ethylene production rate (●) measured immediately before ACC assay were plotted as a function of color index. For fruits categorized in stages 2 and 3, a further distinction in the index of maturity was made based on ethylene production rate as described in the text. Error bars represent 1 SD based on average of 2 or more fruits.

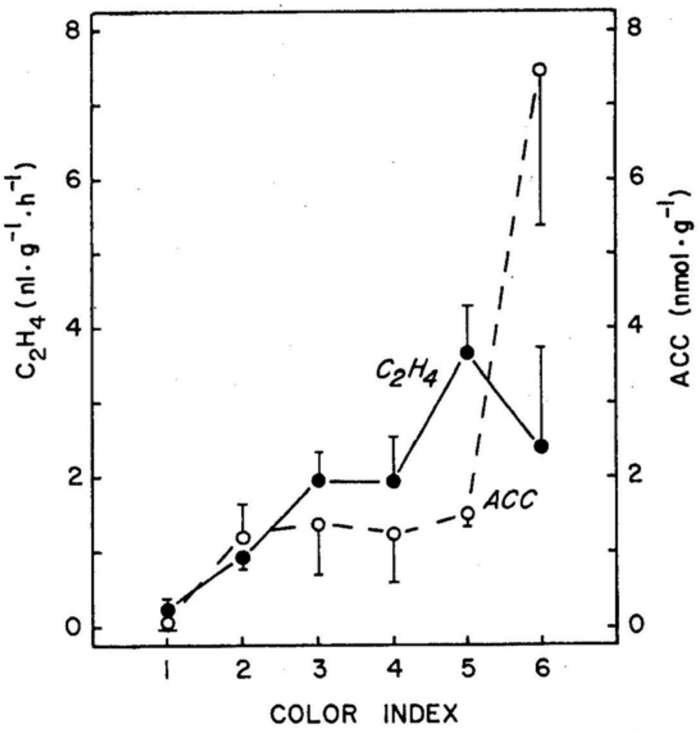

Fig. 4. Change in ACC content of tomato fruit during ripening. Each fruit was individually monitored for ethylene production up to the time of ACC assay. For each fruit the ACC content and its ethylene production rate measured immediately before ACC assay were plotted as a function of color index. Error bars represent 1 SD based on average of 2 or more fruits.

Discussion

Although avocado, banana and tomato fruits differ in the quantity of ACC which they accumulate during ripening, their patterns of ACC accumulation are similar. In the preclimacteric stage, there was very little ACC in any of these fruits. At the onset of ethylene production, a concomitant accumulation of ACC was observed. In overripe fruits, ethylene production was reduced, but high levels of ACC were found in all 3 species.

The low levels of ACC in preclimacteric fruit suggest that availability of ACC is a limiting factor regulating ethylene production. However, addition of ACC to these tissues causes only a few-fold increase in ethylene production (2). These observations suggest that preclimacteric tissue lacks capability not only for the synthesis of ACC but also for the conversion of ACC to ethylene. Inhibitor studies with cycloheximide on preclimacteric tissue suggest that protein synthesis is required for the onset of increased ethylene production during the ripening process (9). The initial rise in ACC may be due, therefore, to new synthesis of ACC synthase, though the possibility of activation of this enzyme cannot be ruled out.

Except for overripe fruits there was a positive correlation between the level of ACC and the rate of ethylene production. These data further suggest that once ACC starts to accumulate as a result of increased activity of ACC synthase, the enzyme responsible for the conversion of ACC to ethylene is also synthesized or activated at a rapid rate. Such a view is supported by the observation that the ability to convert ACC to ethylene is much greater in postclimacteric than in preclimacteric apples (2, 3).

Change in the level of ACC is determined by the rate of ACC synthesis relative to its rate of utilization or degradation. It has been shown that in apple tissue the conversion of ACC to ethylene represents the major, if not the sole, fate of ACC (2). In avocado and banana, the rapid accumulation of ACC which occurs during the onset of ripening indicates that the rate of ACC formation is greater than the rate of ACC utilization. A decrease in the level of ACC indicates that the rate of ethylene synthesis is greater than that of ACC synthesis. In avocado the level of ACC drops rapidly during and immediately after the ethylene peak. Although the level of ACC in banana eventually decreases, it remains high during and for several days after the ethylene peak. This difference might be attributed to the fact that avocado has a much higher rate of ethylene production than banana, and thus utilizes more ACC. The second increase in ACC occurs as fruit become overripe and is accompanied by low ethylene production. This suggests that the ethylene-forming enzyme is impaired as the tissue senesces. In this post-ripening state, ACC would accumulate as long as ACC synthesis is not impaired to the same extent. Imaseki and Watanabe (10), Anderson et al. (4), and others have shown that ethylene production is sensitive to osmotic shock, cold shock, and homogenization. These observations are interpreted to indicate that some part of the ethylene-forming system is associated with membrane. ACC synthase has been isolated from tomato by Boller et al. (5) and Yu et al. (15) and appears to be localized in the cytosol. If one part of the ethylene-forming system is membrane-associated, it is reasonable to assume that the enzyme involved in the conversion of ACC to ethylene is membrane-associated. Ultrastructural studies of ripening avocado revealed that membranes began to deteriorate approximately 3 days after the ethylene peak (13), which coincided with the stage at which ACC began to accumulate (Fig. 1 and 2).

Burroughs was unable to find ACC in all cultivars of apple and pear that he examined (7). Undoubtedly, this was due to the paper chromatographic technique he employed which was not sufficiently sensitive to detect the small quantity of ACC present in the extract. It is now clear that ACC is present in all ethylene-producing tissues which have been examined and plays a most significant role in fruit ripening by serving as the immediate precursor of ethylene.

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