## Short Communication

## Ethylene and Ripening of Mangoes

A. K. Mattoo and V. V. Modi

Department of Microbiology, M.S. University of Baroda, Baroda, India

Received October 8, 1968.

Enough evidence (3) has accumulated to show that ethylene is a fruit ripening hormone. Ethylene evolution in fruit tissues accompanies processes of maturation and aging. Although work on the metabolic changes caused by artificial doses of ethylene has appeared (2,4,7), the real nature of ethylene reactions and its relation to metabolites in inducing fruit ripening are still not clearly understood. With the multitude of changes that occur in ripening fruit, no one change has been shown to be initiated directly by ethylene.

In mangoes not much is known about the exact role of ethylene action. Mangoes, which were thought to be nonclimacteric (1), have been found to have a normal pattern of ethylene evolution which coincides with their respiratory peak (2). We have observed that Alfanso mangoes do produce ethylene (6) using gas chromatographic method (5). Together with the ethylene evolution and respiratory climacteric in mangoes, the catalase and peroxidase activities were found to increase considerably, due to the disappearance of a heat-labile and nondialyzable inhibitor of these enzymes. Using mango slices further investigations were carried out to study the effect of ethylene on these enzyme activities and also on their endogenous inhibitor.

Alfanso mangoes (Mangifera indica) were used in these investigations. The mangoes used in the experiments were surface sterilized, and then cut into uniform slices.

Slices of unripe (preclimacteric) tissue (2 g fr wt) were placed in 5 liter containers. Ethylene mixtures of 10 ppm and 50 ppm were introduced into these chambers using the evacuation method (9). The chambers were washed and rinsed with alcohol before use. A beaker containing solid NaOH was kept in the center of the container to absorb CO<sub>2</sub> evolved. Unripe tissue slices incubated in a similar container in the absence of ethylene served as controls. The containers were sealed and incubated at 25°. At various time intervals (17 hr, 24 hr, 45 hr, and 70 hr) the slices were removed for analysis. Containers were unsealed at these times and exposed to air for 1 or 2 hr, and then once again the required amount of ethylene was introduced.

The treated slices and their controls were, after removal, cooled to 0° and immediately extracted for the determination of enzyme activities and the inhibitor concentration.

Methods employed for the preparation of cell free extracts, estimation of catalase and peroxidase activities and their inhibitor, were essentially those reported earlier (6).

One unit of catalase is that amount of the enzyme which decomposes 1 micromole of  $H_2O_2$  per hr at  $0^{\circ}$  and 1 unit of peroxidase is that amount of the enzyme which decomposes 1 micromole of  $H_2O_2$  per min at  $25^{\circ}$ .

Inhibitor was estimated by adding graded amounts of treated and nontreated tissue extracts to the ripe enzyme extract and after a preincubation of 15 min, the residual activity of the particular enzyme was

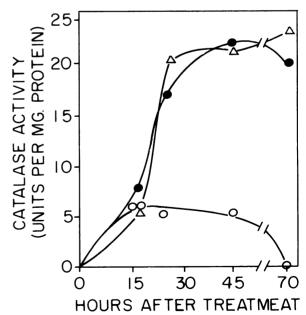


Fig. 1. Time-course changes in effects of ethylene on catalase activity in Alfanso mango slices incubated at 25° for 70 hr.  $\bigcirc$  = Controls;  $\triangle$  = 10 ppm ethylene; and  $\bullet$  = 50 ppm ethylene.

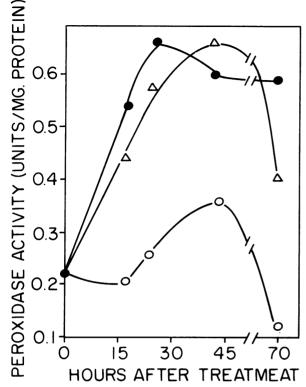


Fig. 2. Time-course changes in effects of ethylene on peroxidase activity in Alfanso mango slices incubated at 25° for 70 hr.  $\bigcirc$  = Controls;  $\triangle$  = 10 ppm ethylene; and  $\bullet$  = 50 ppm ethylene.

estimated. One unit of the inhibitor is that amount of the unripe extract which blocks the activity of 1 unit of catalase or peroxidase, as described above.

The effects of various concentrations of ethylene on the catalase and peroxidase activities in the slices incubated over a wide range of time at 25° are shown in figures 1 and 2. Ethylene stimulates catalase and peroxidase activities 4 and 3 folds respectively over control slices. Ethylene stimulation reaches a maximum level at 45 hr. From the results it is evident that at 0 hr the preclimacteric mango slices have no detectable catalase activity but appreciable peroxidase activity.

Burg and Burg (2) have earlier suggested that approximately 0.05 ppm ethylene present in a mango at the time of preclimacteric minimum is sufficient to influence metabolic activity in the fruit. Moreover, in apple tissue slices, the evolution of ethylene has been found to increase by increasing the surface area by cutting the tissue in smaller slices (8). In our experiments, in the untreated preclimacteric mango slices also there is low but significant increase in the activities of the enzymes, catalase and peroxidase, along with a slight decrease in the activity of the inhibitor of these enzymes, suggesting that ethylene produced in these slices gives rise to these changes. These results indicate clearly that ethylene

promotes the activities of these oxidative enzymes, catalase and peroxidase in the slices prepared from preclimacteric mangoes. Coincident with the ethylene stimulation of enzyme activities is the disappearance of the inhibitor of these enzymes, from the treated slices. This effect of ethylene is shown in figure 3 and table I. After 45 hr exposure to ethylene the inhibitor of catalase and peroxidase is undetectable in treated mango slices. Preliminary results indicate the similar effect of ethylene on the enzyme amylase.

Table I. Disappearance of the Peroxidase Inhibitor in Unripe Mango Slices on Exposure to Ethylene

	Specific activity of inhibitor				
Ethylene	$0\mathrm{hr}$	17th hr	24th hr	45th hr	70th hr
ppm	units per mg protein				
0	0.500	0.200	0.138	0.138	0.160
10	0.500	0.170	0.022	0.015	0.000
50	0.500	0.190	0.002	0.000	0.000

The present and our earlier data (6) indicate that before the onset of the climacteric in mangoes ethylene, synthesized by the fruit, stimulates respiratory enzymes catalase and peroxidase and inactivates the inhibitor(s) of these enzymes. All this together and possibly other factors vigorously initiate part of the metabolic system eventually resulting in the ripening of the fruit.

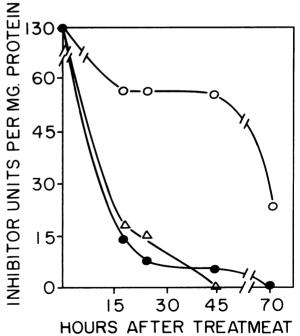


Fig. 3. Effect of ethylene on disappearance of catalase inhibitor from Alfanso mango slices incubated at 25° for 70 hr.  $\bigcirc$  = Controls;  $\triangle$  = 10 ppm ethylene; and  $\bullet$  = 50 ppm ethylene.

## Acknowledgments

This investigation was supported by a grant P1-480/FG-In-276 from the Agricultural Research Service of the United States Department of Agriculture.

## Literature Cited

- Biale, J. B., R. E. Young, and A. Olmstead. 1954. Fruit respiration and ethylene production. Plant Physiol. 29: 168-74.
- 2. Burg, S. P. and E. A. Burg. 1962. Role of ethylene in fruit ripening. Plant Physiol. 37: 179-89.
- 3. Burg, S. P. and E. A. Burg. 1965. Ethylene action and the ripening of fruits. Science 148: 1190-96.
- 4. KAR, B. K. AND H. K. BANERJI. 1939. Effect of ethylene on *Mangifera indica*. Nature 144: 597-98.

- 5. LIEBERMAN, M., A. T. KUNISHI, L. W. MAPSON, AND D. A. WARDALE. 1966. Stimulation of ethylene production by methionine in apple slices. Plant Physiol. 41: 376–81.
- 6. Mattoo, A. K., V. V. Modi, and V. V. R. Reddy. 1968. Oxidation & carotenogenesis regulating factors in mangoes. Ind. J. Biochem. 5: 111-14.
- Pratt, H. K. 1961. The role of ethylene in fruit ripening. Recent advances in botany. Vol. II. 1160– 65.
- 8. Shimokawa, K. and Z. Kasal. 1966. Biogenesis of ethylene in apple tissue. I. Formation of ethylene from glucose, acetate, pyruvate, and acetaldehyde in apple tissue. Plant Cell Physiol. 7: 1-9.
- Umbreit, W. W., R. H. Burris, and J. F. Stauffer. 1957. Manometric techniques. Burgess Publishing Company, Minneapolis, Minnesota.