THE EFFECT OF LOW OXYGEN ON THE ACTIVITIES OF PECTINMETHYLESTERASE AND ACID PHOSPHATASE DURING THE COURSE OF RIPENING OF BANANAS

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Introduction

The practice of controlled atmosphere storage of fruits has developed without a corresponding increase in the understanding of the mode of action of high CO_2 or low O_2 concentrations. It was suggested by Kidd and West (6) that low O_2 and/or high CO_2 concentrations may interfere with the action of ethylene. Burg and Burg (3) demonstrated later that in pea sprouts CO_2 acted as a competitive inhibitor of ethylene action, and further that O_2 was required for ethylene to exert its physiological effects. Beyer (2) has also shown that the metabolism of ethylene, which is deemed necessary for its action requires the presence of O_2.

Respiration is not the sole biological activity which utilizes O_2 in plant tissues. Further, since senescence can proceed with no concomitant increase in respiration, the retarding effects of low O_2 concentrations on fruit ripening must involve processes other than respiration. Thus it is necessary to carry out experiments with a view to identifying the metabolic steps which utilize O_2 and whose activity is diminished at O_2 levels less than those in air.

The effect of low O_2 concentration on fruit ripening is two-fold. Firstly, it delays the onset of the climacteric rise in ethylene evolution and respiration, and secondly it retards the rate of ripening in fruits where senescence has been induced by previous treatment with exogenous ethylene (4, 9). In apples, O_2 concentrations delay fresh softening and the increase in the levels of soluble polyuronides (7). The mechanism by which O_2 concentration delay the onset of the rise in ethylene evolution is completely unknown. Oxygen is indeed required for the conversion of ACC to ethylene (10). However the total suppression of ethylene production requires levels of O_2 which are below those used in CA storage.

Thus the mechanism by which low O_2 levels decrease the rate of ripening of initiated fruits is still speculative in nature. Softening is a ubiquitous phenomenon of fruit ripening. This softening is always associated with an extensive breakdown of cell walls (1, 5). Further, the activity of several hydrolytic enzymes, which would be expected to degrade cell wall components, increases drastically in the course of ripening (5). Cell walls also contain enzymes other than those

involved in the hydrolysis of complex polysacharides, such as phosphotases (8). However, the role of these enzymes in the degradation of cell walls is not clear (8). In this report we present data concerning the effect of low O_2 (2.5%) on the activities of pectinmethylesterase and acid phosphatase in banana fruits during ripening.

Results and Discussion

The activities of the afore-mentioned enzymes were followed in fruits whose ripening had been initiated by treating them with ethylene until the rate of CO_2 evolution was half-way to its climacteric peak. At this point part of the fruits were transferred to 2.5% O_2. The rates of CO_2 and O_2 evolution were monitored continuously. Figs. 1, 2 and 3 show that, as expected (9), the rate of CO_2 output decreased upon transfer to 2.5% O_2 and showed virtually no change up to eight days. However, when the fruits were transferred to air (Fig. 3), there was a sharp increase in CO_2 evolution but it never reached the peak values attained in air. Low O_2 treatment appreciably delays the development of the yellow color (Fig. 4). The delay was statistically significant at 0.1% level, even at the early stages of transfer to 2.5% O_2.

Fig. 1 shows that the activity of PME declines gradually in the fruits kept in air while it remained constant in those which were transferred to 2.5% O_2 for six days. Fig. 2 also shows that initially the activity of PME is higher in the fruits kept under 2.5% O_2 than in those kept in air. However, it eventually decreased even at 2.5% O_2. It should be pointed out that in this experiment sampling procedures introduce larger variability than in the first experiment since for each sampling point a separate fruit was used instead of the same, contrary to the procedure used in experiment 1 where a single fruit was used throughout.

The activity of acid phosphatase increased several-fold during ripening in the fruits kept in air (Figs. 1-3). However this increase was suppressed almost completely in the first 6-9 days (Figs. 1-3) in the fruits which were transferred to 2.5% O_2. The slight increase in the acid phosphatase, observed initially kept under 2.5% O_2 (Fig. 2). may reflect the variability in the individual fruits than in the actual increase. It should be pointed out, however, that the activity of this enzyme was always lower in fruits kept under 2.5% O_2 than those held in air. Further, Fig. 3 shows that the inclusion of 18 p.p.m. ethylene in the 2.5% O_2 gas mixture did not alter the effect of 2.5% O_2 on acid phosphatase. Fig. 3 shows that if the fruits were returned to air after being kept at 2.5% O_2 for six days, the activity of acid phosphatase increased rapidly but it never reached the value observed in fruits kept in air during the three days tested.

In summary, the present results indicate that the delay in fruit ripening caused by 2.5% O_2 affects not only the overt changes of ripeness in bananas but also the underlying biochemical events.

LITERATURE CITED

1. Ahmed, E. A. and M. Labavitch 198C. Cell wall metabolism in ripening fruit. I. Cell wall changes in ripening 'Bartlett pears'. Plant Physiol. 65:1009-1013.

2. Beyer, E. M. 1975. $^{14}C_2H_4$ - its incorporation and metabolism by pea seedling under aseptic conditions. Plant Physiol. 56:273-278.

3. Burg, S. P. and E. A. Burg. 1967. Molecular requirements for the biological activity of ethylene. Plant Physiol. 42:144-152.

4. Hesselman, C. W. and H. T. Freebairn. 1969. Rate of ripening of initiated bananas as influenced by oxygen and ethylene. J. Amer. Soc. Hort. Sci. 94:635-637.

5. Hobson, G. E. 1981. Enzymes and texture changes during ripening. In recent advances in the biochemistry of fruits and vegetables. J. Friend and M. J. C. Rhodes pp. 123-132. Academic Press, London.

6. Kidd, F. and C. West. 1934. The influence of the composition of the atmosphere upon the incidence of the climacteric in apples. Gt. Brit. Dept. Sci. Ind. Res. Rept. Food Invest. Bd. 1933: 51-57.

7. Knee, M. 1982. Physiological responses of apple fruits to oxygen concentrations. Ann. appl. Biol. 96-243-253.

8. Lamport, D. T. A. and J. W. Catt. 1981. Glycoproteins and enzymes of the cell wall. Encycl. Plant Physiol. 13B:133-165.

9. Quazi, M. H. and H. T. Freebairn. 1970. The influence of ethylene, oxygen and carbon dioxide on the ripening of bananas. Bot. Gaz. 131:5-14.

10. Yang, S. F. and N. E. Hoffman. 1984. Ethylene biosynthesis and its regulation in higher plants. Ann Rev. Plant Physiol. 35:155-189.

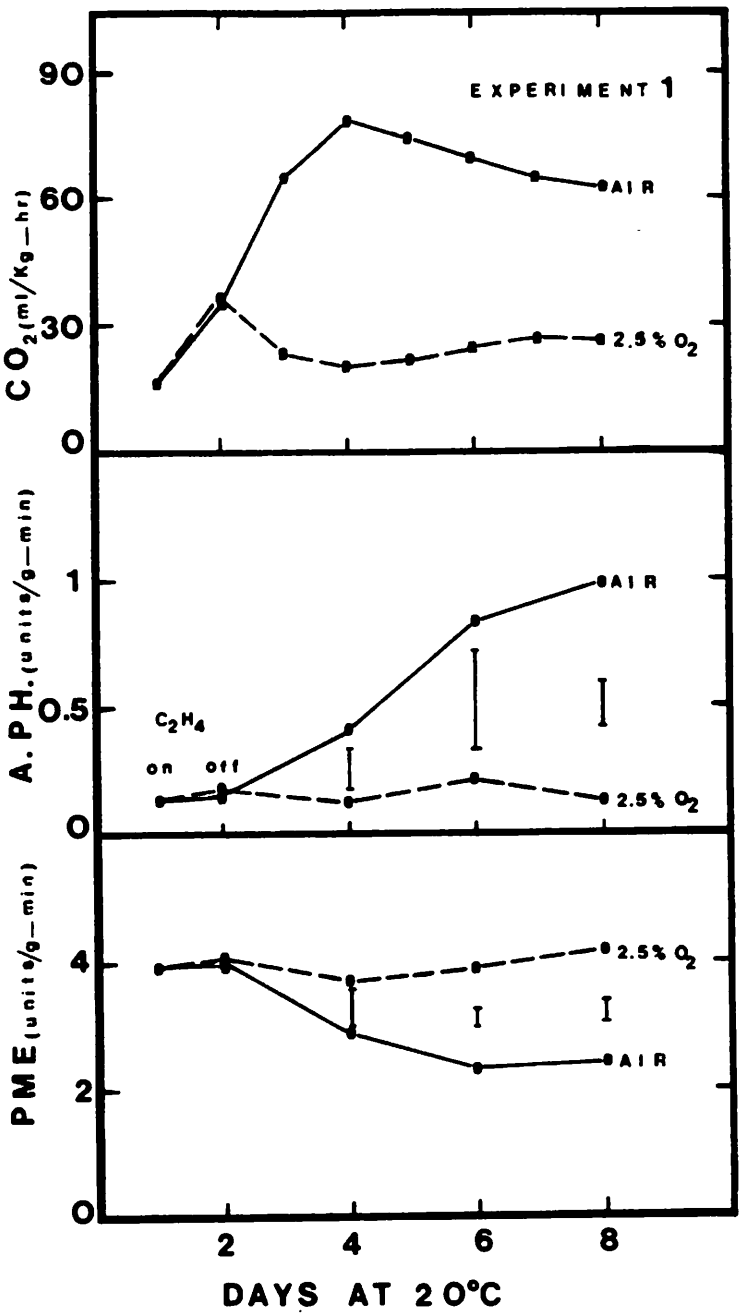

Fig. 1. Changes in respiration rates and activities of acid phosphatase (μ moles p-nitrophenyl-phosphate hydrolyzed/g-min) and PME (μ equivalent ester hydrolyzed/g-min) of initiated fruits in the air and in 2.5% O_2. Bars represent LSD (p=0.001 for acid phosphatase and p=0.05 for PME). Each value is the mean of the 3 single fruits for each sampling time.

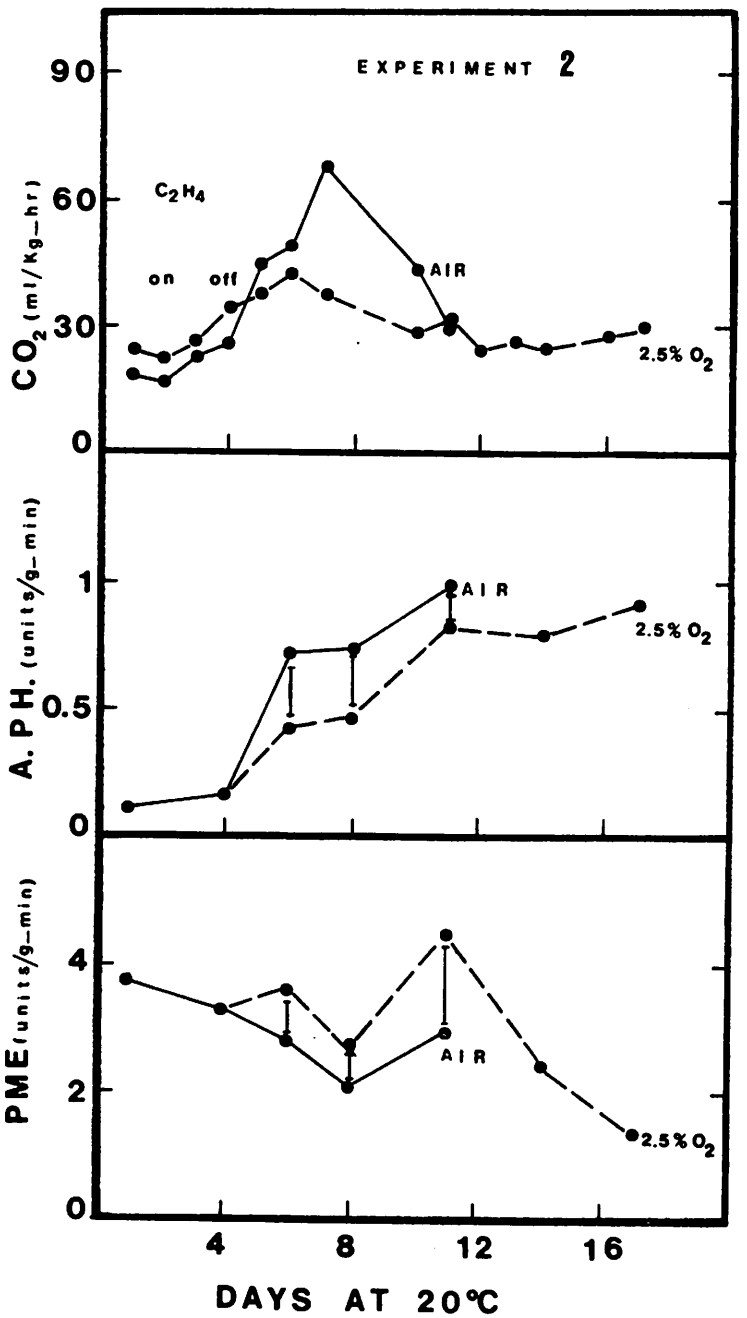

Fig. 2. Changes in respiration and activities of acid phosphatase (μ moles p-nitrophenyl-phosphate hydrolyzed/g-min) and PME (μ equivalent hydrolyzed/g-min) of initiated fruits in the air and in 2.5% O_2. Bars represent LSD (p=0.01 for a. phosphatase and p=0.05 for PME except for the last day where p=0.01). Each point represents the average value of 3 single fruits which were removed from each treatment at the sampling time.

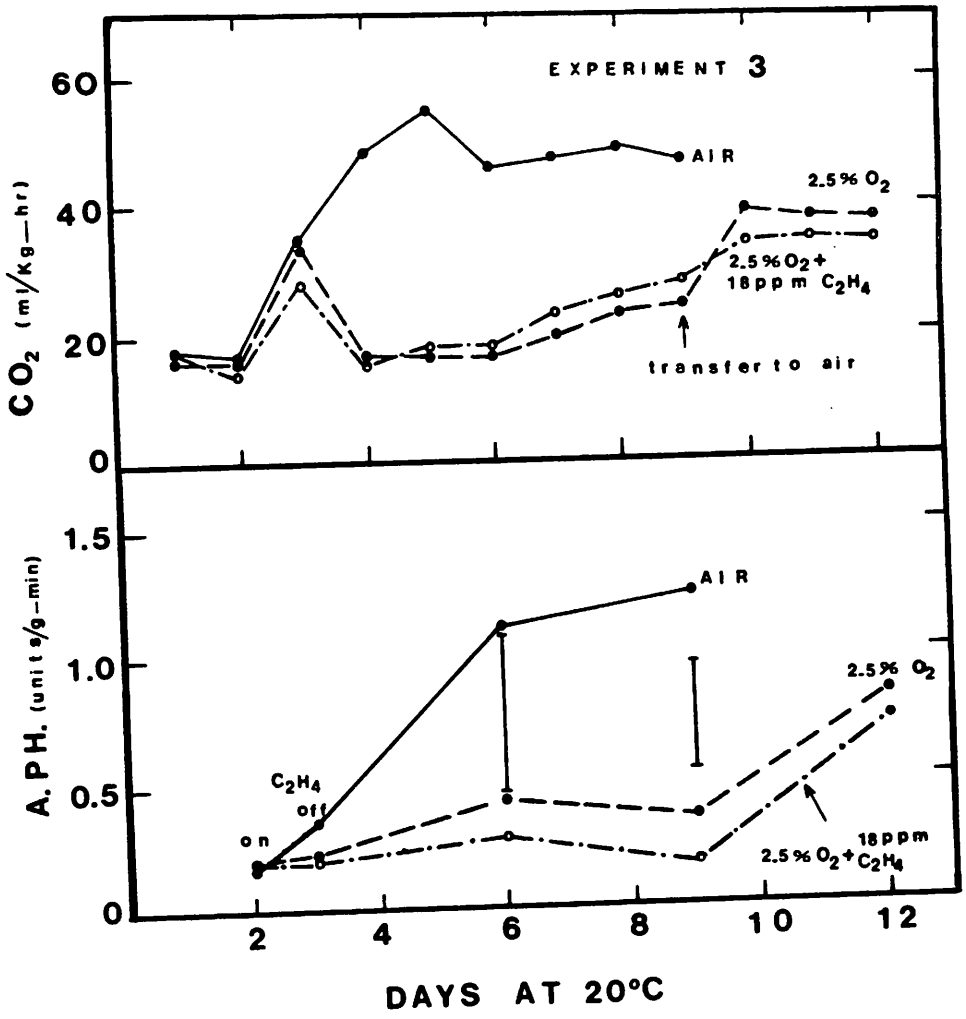

Fig. 3. Changes in respiration and activities of acid phosphatase (μ moles p-nitrophenyl-phosphate hydrolyzed/g-min) of initiated fruits in the air and 2.5% O_2. Bars represent LSD (p=0.01). Each value is the mean of the 3 single fruits for each sampling time.

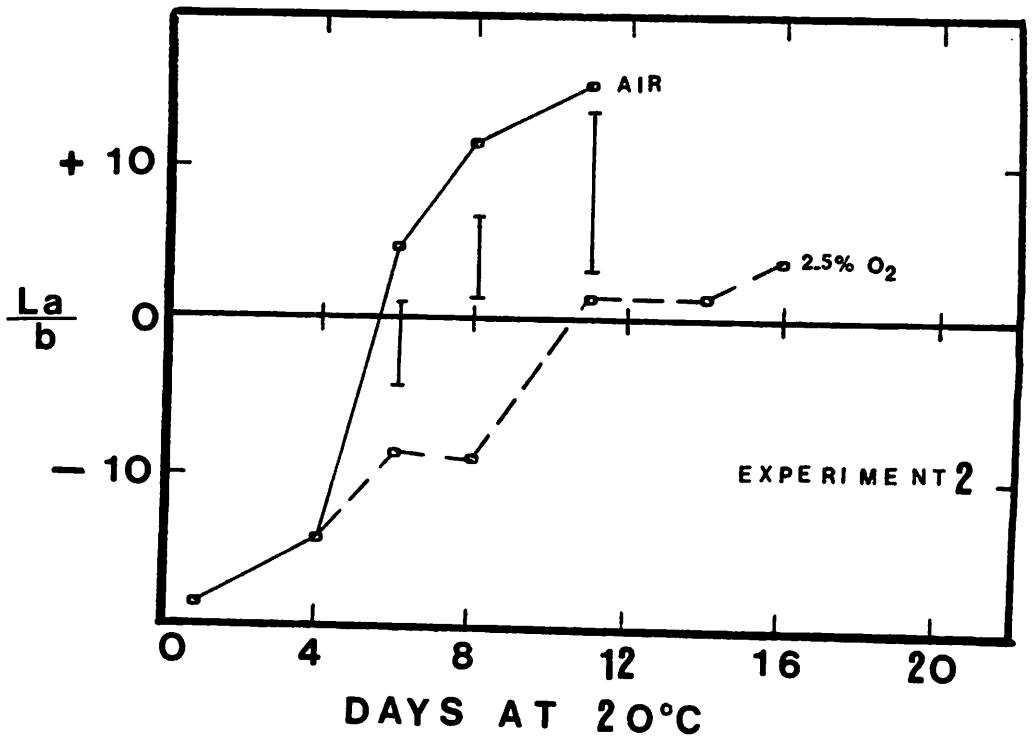

Fig. 4. The effect of 2.5% O_2 on color change during a 12-day period. Vertical bars represent LSD values (p=0.001). Color is expressed in terms of L, a, b Hunter values. Each point represents the average value of 3 single fruits which were removed from each treatment at the sampling time.