Plant Physiol. (1989) 90, 251–258
0032-0889/89/90/0251/08/$01.00/0

Received for publication August 10, 1988
and in revised form December 23, 1988

# Changes in Sugars, Enzymic Activities and Acid Phosphatase Isoenzyme Profiles of Bananas Ripened in Air or Stored in 2.5% $O_2$ with and without Ethylene[1]

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## ABSTRACT

**This study investigates the effect of 2.5% $O_2$, both alone and in combination with ethylene, on respiration, sugar accumulation and activities of pectin methylesterase and acid phosphatase during ripening of bananas (*Musa paradisiaca sapientum*). In addition, the changes in the phosphatase isoenzyme profiles are also analyzed. Low oxygen diminished respiration and slowed down the accumulation of sugars and development of the yellow color. Furthermore, low $O_2$ prevented the rise in acid phosphatase activities and this suppression was not reversed by the inclusion of 100 microliters per liter ethylene in 2.5% $O_2$ atmosphere. Gel electrophoresis of both the soluble and particulate cell-free fractions under nondenaturing conditions revealed the presence of 8 and 9 isoenzymes in the soluble and particulate fractions, respectively. Low $O_2$ suppressed the appearance of all isoenzymes, and the addition of 500 microliters per liter ethylene to the low oxygen atmosphere did not reverse this effect. Similarly, the decline in pectin methylesterase that was observed in air-ripened fruits was prevented by 2.5% $O_2$ alone and in combination with 500 microliters per liter ethylene.**

Ripening of banana fruit is associated with a sharp rise in respiration and ethylene evolution, a massive breakdown of starch concomitant with a rise in simple sugars, total degradation of Chl, and softening (26). Banana cell walls contain pectin, cellulose, and hemicellulosic substances (26). During ripening, the insoluble 'pectins' decrease from 0.5% to 0.2% fresh weight with a corresponding rise in soluble pectins (26). The level of cellulose (2–3%) decreases only slightly during ripening. Hemicelluloses appear to constitute the largest fraction of cell wall materials (8–10%); they decrease to about 1% in ripe fruits (4, 26). In addition, the activities of a number of enzymes change during the ripening of climacteric fruits in general, and bananas in particular (4, 26). The role(s) of a number of enzymic changes in the ripening process is not yet understood.

Low $O_2$ levels delay for long periods the onset of ripening in bananas (11, 23, 28), and in initiated fruits slow down the rate of Chl destruction and sugar accumulation. Even 10% $O_2$ delays the appearance of the yellow pigments (5).

Low $O_2$ levels are extensively used commercially to prolong the storage life of fruits, especially apples and pears. The precise mode of action of low $O_2$ in fruit ripening is not well understood (14, 15). Kidd and West (19) suggested that the beneficial effects of $O_2$ on the longevity of fruits may be related to its interference with production and/or action of ethylene. Burg and Burg (6) provided evidence that for ethylene to exert its biological effects, $O_2$ is required. Furthermore, this effect of $O_2$ is exerted at levels which do not affect respiration (6–8). In other words, the inhibitory effects of low $O_2$ on ethylene action are not an indirect effect of the diminution of respiration. Liu and Long-Jum (21) have shown that the inclusion of 500 $\mu$L/L $C_2H_4$ in 1.5% $O_2$ delays the softening of 'McIntosh' apples. In the present reports (17) we have attempted to study the effects of 2.5% $O_2$, both alone and in combination with ethylene, on the changes of the activities of pectin methyl esterase, acid phosphatases and its isoenzymes, as well as respiration, sugar accumulation, and color during ripening of these fruits. The results showed that during ripening of banana fruit low $O_2$ suppressed the changes in the activities of the above enzymes, and delayed the accumulation of sugars and the appearance of the yellow color. Inclusion of ethylene in 2.5% $O_2$ indicated a differential effect on the magnitude of the aforementioned changes.

## MATERIALS AND METHODS

### Plant Material

Mature green banana (*Musa paradisiaca sapientum*) fruits of uniform size and free from physical defects were purchased from a local wholesale market. They were placed individually in respiratory jars of appropriate size with minimum void space (usually 1 L), and held at 20°C in the dark under a continuous and constant flow of $CO_2$-free humidified air. The flow rates were selected to ensure that $CO_2$ accumulation did not exceed 0.3%. The rates of $CO_2$ and ethylene evolution by each fruit were determined daily. Gas samples were withdrawn from the outlet and injected into a gas chromatograph equipped with either thermal conductivity ($CO_2$) or flame ionization (ethylene) detectors. Measurement of initial rates of $CO_2$ and ethylene evolution enabled assessment of the

[1] Scientific Article No. A-4918, Contribution No. 7961 of the Maryland Agricultural Experiment Station.

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physiological stage of the fruits. Only those green fruits that hardly evolved ethylene were used for the experiments. These preclimacteric fruits were treated with ethylene by passing over them a continuous stream of $CO_2$-free air containing 5 to 10 $\mu$L/L ethylene. When the respiration rates reached 30 to 40 mL $CO_2 \cdot Kg^{-1} \cdot h^{-1}$, *i.e.* one-third to one-half of their climacteric peak value, the exogenous supply of ethylene was discontinued. The fruits were then separated into three batches, each containing an equal number of fruits (usually 3). One batch was kept in air, the second was subjected to 2.5% $O_2$, while the third was treated with a mixture of 2.5% $O_2$ and either 18 $\mu$L/L ethylene (experiment 1) or 500 $\mu$L/L ethylene (experiment 2). The fruits were kept under the respective gaseous environment for 6 d, and then transferred to air.

### Sampling Method

To diminish the variations between different fruits, samples of mesocarp tissue were withdrawn from a single fruit by the method of Awad and Young (1). The method involves removal of cylindrical plugs (2–3 g) with a stainless steel cork borer 10 mm in diameter. One plug of tissue was withdrawn from each fruit at the preclimacteric stage, at the time of transfer to low $O_2$ atmosphere, or low $O_2$ and ethylene atmosphere, and every 48 h (experiment 2) or 72 h (experiment 1) thereafter up to 6 d, and after 2 d upon return of the fruits to air. After removal of the pulp tissue, the resulting holes in the fruit were immediately sealed with lanolin and the fruits were returned at once to the respiratory jars. Pulp tissue plugs were ground in liquid $N_2$ with mortar and pestle and the fine powder stored at −70°C until used. In preliminary experiments, the effect of wounding on $CO_2$ and ethylene evolution due to the removal of tissue plugs from fruits was evaluated. Awad and Young (1) observed that wounding did not alter the rate of either respiration or ethylene evolution. In preliminary experiments, we verified these observations. In addition, the PME[3] and AP activities in intact fruits, and in those from which plugs of tissue had been removed, were similar in both cases.

### Color

For color change determinations (green to yellow) a different sampling procedure was followed. Green preclimacteric fruits were enclosed in respiratory jars and three fruits from the air control and three from 2.5% $O_2$ atmosphere were removed after 0, 2, 4, 7, 10, and 13 d following the transfer to low $O_2$. Hunter L, a, b, values were determined using the Pacific Scientific Spectrogard Color System[4] (10). Measurements of L, a, b, were taken on six different surface portions of each fruit and the average was used for calculations. The instrument was calibrated with white and black standards. The data are expressed as La/b, which is a measure of the rate of change from green to yellow.

[3] Abbreviations: PME, pectinmethylesterase; AP, acid phosphatase; PVPP, polyvinylpolypyrrolidone.

[4] Mention of specific instruments, trade names, or manufacturers is for the purpose of identification and does not imply an endorsement by the U.S. Government.

### Sugars

One g of frozen fine mesocarp powder was boiled in 70% ethanol for 15 min and left overnight at room temperature. The following day, the mixture was centrifuged at 21,000*g* for 15 min. The supernatant was filtered through 0.45 $\mu$m HA Millipore membrane and 25 $\mu$L were analyzed by HPLC using a carbohydrate column (Waters Associates). The sugars were eluted from the column with 85% (v/v) acetonitrile-water at a flow rate of 2.0 mL/min. A refractive index detector (Waters Associates) was used for the quantification of sugars. The data are expressed as $\mu mol \cdot g^{-1}$ (fresh weight).

### Extraction and Assay of Pectinmethylesterase

One g of frozen ground pulp was thawed in 4 mL of ice-cold isolation medium containing 0.1 M Na-acetate (pH 5), 0.5 M NaCl, 2% PVP, 5 mM DTT, 5 mM $\beta$-mercaptoethanol, 0.5 mM PMSF, 1 mM diethyldithiocarbamic acid, and 0.5% (v/v) Triton X-100. Alternatively, the frozen tissue was thawed in 4 mL of 0.5 M Na-acetate (pH 7.5), 1% polyethylene glycol, 5 mM DTT, 5 mM $\beta$-mercaptoethanol, 0.5 mM PMSF, and 1 mM diethyldithiocarbamic acid. After being thawed, the mixture was vortexed eight times for 5 s, with a 2-min interval between vortexing during which it was cooled on ice. The homogenate was centrifuged at 20,000*g* for 20 min and the supernatant saved for enzyme assays. PME was assayed by the method of Rouse and Atkins (31) with slight modifications. Of the supernatant, 1.5 mL were added to 10 mL of 0.5% (w/v) solution of pectin (Sigma) in 0.1 M NaCl. The mixture was brought rapidly to pH 7.5 with dilute NaOH. The release of carboxyl groups was monitored at 25°C for 10 min with an automatic titrator (Methrom Herisau) using 0.01 N NaOH. PME units are expressed in $\mu$mol of ester hydrolyzed $\cdot g^{-1} \cdot min^{-1}$.

### Extraction and Assay of Acid Phosphatase

Preliminary experiments showed that the buffer used for the extraction of pectinmethylesterase proved to be very satisfactory for the isolation of total AP. The activity of AP in the cleared homogenate was measured in a reaction mixture containing 40 $\mu$L of 30 $mg \cdot ml^{-1}$ of *p*-nitrophenyl phosphate in 0.1 M Na-acetate (pH 5), in a final volume of 5 mL. Fifty $\mu$L of the extract were added to initiate the enzyme reaction. The tubes were incubated in a shaking water bath maintained at 40°C (9). One-mL aliquots of the reaction mixture were removed at intervals, added to 3 mL of 0.02 N NaOH and shaken. The absorbance at 407 nm was measured after 15 min. AP units are expressed in $\mu$mol of *p*-nitrophenyl phosphate hydrolyzed $\cdot g^{-1} \cdot h^{-1}$. The enzyme assays were based on measurements of the zero order rate.

### Extraction and PAGE of Acid Phosphatase Isoenzymes

One g of frozen pulverized pulp tissue was mixed with 1.5 mL of 50 mM Tris-HCl (pH 7.5), containing 5 mM $\beta$-mercaptoethanol, 0.5 mM PMSF, 10 $\mu$M, leupeptin, 1 mM diethyldithiocarbamic acid, 5% (w/w, fresh weight) PVPP, 1 mM EDTA, and 10% (v/v) glycerol. After thawing on ice, AP was

solubilized by vortexing the mixture three times for 5 s with an interval of 5 min while the mixture was left on ice, then centrifuged at 21,000*g* for 60 min. The supernatant was saved for enzyme assays. The pellet was resuspended in 1 mL of 50 mM Tris-HCl (pH 7.5), containing 10 mM β-mercaptoethanol, 0.5 mM PMSF, 10 μM leupeptin, 1 mM diethyldithiocarbamic acid, 1 mM EDTA, 10% (v/v) glycerol, 0.5 M NaCl, and 0.5% (v/v) Triton X-100 and left on ice for 15 min with intermittent vortexing as mentioned above. The mixture was centrifuged at 21,000*g* for 60 min, and the supernatant served as a source of particulate enzyme. Both soluble and particulate fractions were recentrifuged at 45,000 rpm for 60 min in a Beckman 50 Ti rotor. The clear supernatants were divided into 0.5 mL aliquots and were then stored at −70°C until used.

Acid phosphatase isoenzymes were analyzed by a modification (16) of the cathodic system of Reisfeld *et al.* (29), using 5C0.66T-11C0.66 gradient polyacrylamide gels. The stacking gel consisted of 3.75T2.5C polyacrylamide. Electrophoresis was carried out in 0.35 M β-alanine-0.14 M acetic acid (pH 4.3), maintained at 2 to 3°C by connecting the cooling coil with a constant temperature (−4°C) bath. The current was set at 30 mA till the samples moved in the stacking gel and then raised to 50 mA. The current was stopped 1.5 h after the tracking dye had run out of the gel.

### Electrophoretic Blotting Procedure

Following electrophoresis, the gel was equilibrated for 60 min by shaking it in prechilled transfer buffer containing 25 mM Tris-192 mM glycine buffer (35) (pH 8.3) with four changes every 15 min. The proteins were then electrophoretically transferred on to nitrocellulose sheets (0.1 μM pore size, Schleicher and Schuell) in a Bio-Rad transblot cell that had previously been filled with prechilled transfer buffer. The nitrocellulose paper faced the anode and the transfer was completed in 3 h at 80 V. The temperature of the buffer was maintained at 5 to 6 °C by using a cooling apparatus.

### Activity Staining of Acid Phosphatase isoenzymes

The AP isoenzymes were detected on nitrocellulose filters by devising a rapid method (16). In brief, the electrophoretic blots were soaked in 20 mM Na-acetate (pH 5), 0.8% (w/v) NaCl, and 0.02% (w/v) KCl (ABS) with four changes for 5 min and then were incubated in the staining solution at room temperature (20°C) while being shaken. The AP isoenzymes were visualized according to Vallejos (36) with the following modifications: to reduce undesired background, the staining solution (Fast Garnet GBC) was filtered through a Whatman No. 1 filter paper. The amount of substrate (α-naphthyl-acid phosphate) was reduced by 50%. The staining solution (100 mL) consisted of 96 mL of 50 mM Na-acetate (pH 5), 1 mL of 1 M magnesium chloride, 3 mL of 0.5% (w/v) α-naphthyl-acid phosphate dissolved in 50% (v/v) acetone and 75 mg of Fast Garnet GBC. When dark purple bands were visible, the blots were washed with 15% (v/v) methanol-7% (v/v) acetic acid. The stained nitrocellulose filters could be stored in $dH_2O$ for prolonged periods of time without fading. For enzyme staining on the polyacrylamide gels, the procedure described above was followed except that the initial washings in ABS were omitted.

### Protein Determination

Proteins from each sample were precipitated with ice cold tricholoroacetic acid (10% final concentration) and measured according to Lowry *et al.* (22).

All treatments were run with three replicates and each experiment was repeated at least twice.

## RESULTS

### Extraction of Pectinmethylesterase

A two-step procedure using high ionic strength buffer at pH 7.5 has been reported to extract all the PME from banana pulp (3). We found that using the extraction buffer at pH 5 resulted in higher PME activity in a one-step procedure compared to a one-step procedure in which high ionic strength buffer at pH 7.5 was used (Table I). Combinations of salt with Triton X-100 and various protease inhibitors seemed to release all of the bound enzyme and this was true with tissue at all stages of ripeness.

### Color Changes in Response to 2.5% $O_2$

In fruits held under 2.5% $O_2$ atmosphere, the rate of color change from green to yellow slowed down compared to fruits left in air (Fig. 1). Thus, the yellow color of fruits kept for 16 d at low $O_2$ was less intense compared to control samples after 10 d, the differences being significant at 1% levels.

### Changes in Sugar Levels in Response to Low $O_2$ Treatments

Following initiation of ripening induced by a brief exposure to ethylene, the levels of fructose, glucose, and sucrose increased rapidly as the rise in respiration progressed in fruits held in air (Fig. 2). The initial rate of sugar accumulation in the fruits subjected to 2.5% $O_2$ alone or in the presence of 500 μL/L ethylene was much slower than the controls. However, the levels of sugars increased appreciably after 4 d of storage and continued to increase further upon transferring the fruits to air (Fig. 2).

**Table I.** *Effect of Buffer Composition on Extractability of Banana Pectinmethylesterase at different stages of ripening*

Buffer A consisted of: 0.1 M Na-acetate (pH 5), 0.5 M NaCl, 2% PVP, 5 mM DTT, 5 mM β-mercaptoethanol, 0.5 mM PMSF, 1 mM diethyldithiocarbamic acid, and 0.5% (v/v) Triton X-100. Buffer B consisted of 0.5 M Na-acetate (pH 7.5), 1% (w/v) polyethylene glycol, 5 mM DTT, 5 mM β-mercaptoethanol, 0.5 mM PMSF, and 1 mM diethyldithiocarbamic acid.

| Buffer | Banana PME Extractability[a] | | |
|---|---|---|---|
| | Preclimacteric | Climacteric | Ripe |
| A | 8.32 ± 0.29 | 7.70 ± 0 | 5.01 ± 1.12 |
| B | 7.32 ± 0.23 | 6.77 ± 0.07 | 4.65 ± 0.70 |

[a] Data in units·$g^{-1}$ $min^{-1}$ are means of 3 fruits ± SD.

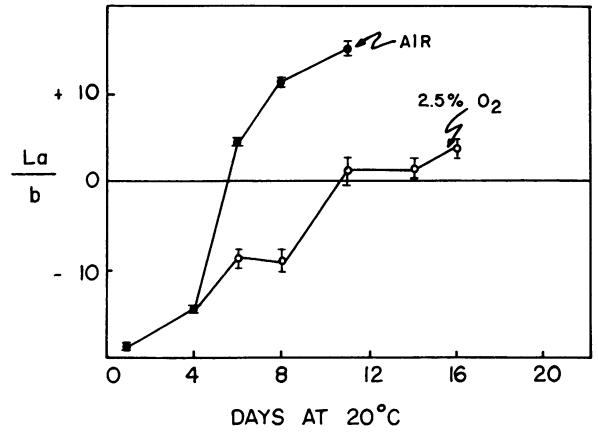


**Figure 1.** Measurements of color changes, from green to yellow in banana fruits ripened in air and in 2.5% $O_2$. Data are the means of three replicates and vertical bars represent the SD. After 4 d differences were significant at 1% level.

### Effect of Low $O_2$ on Respiration

Respiration rates of fruit (Figs. 3 and 4) were typical of earlier work (4, 11, 26, 28). Respiration of fruits ripened in air peaked 3 d after ethylene pretreatment. Once the fruits were transferred to 2.5% $O_2$ atmosphere with or without ethylene, the respiration rates dropped (Figs. 3A and 4A). The rate of $CO_2$ production by the fruits held under 2.5% $O_2$ in the presence of 18 $\mu$L/L ethylene was more or less similar to that of the fruits kept under 2.5% $O_2$ alone (Fig. 3A). In contrast, $CO_2$ evolution was slightly higher in fruits held in an atmosphere of 500 $\mu$L/L ethylene and 2.5% $O_2$ as compared to those held in either 2.5% $O_2$ or 2.5% $O_2$ + 18 $\mu$L/L ethylene (Figs. 3A and 4A). Subsequent to the transfer of these fruits to air on d 7, the respiration rate increased and then decreased. However, this increase in respiration did not reach the values exhibited by the fruits ripened throughout in air.

### Effect of Low $O_2$ on Pectinmethylesterase and Acid Phosphatase Activities

PME activity declined soon after ethylene pretreatment (Fig. 4B). As the ripening progressed, and the respiratory climacteric reached its maximum value (Fig. 4A), PME activity continued to decrease in fruits kept in air (Fig. 4B). However, this rate of decline in PME activity was slowed down in fruits held in 2.5% $O_2$ or 2.5% $O_2$ plus ethylene. Subsequent to the transfer to air, PME activity declined precipitously in these fruits.

In contrast to the trend in PME activity, total AP activity of fruits ripened in air increased considerably (Figs. 3B and 4C). In fruits held in 2.5% $O_2$ atmosphere, this increase in AP activity did not occur (Figs. 3B and 4C). Inclusion of either 18 $\mu$L/L or 500 $\mu$L/L ethylene in 2.5% $O_2$ atmosphere did not markedly alter the pattern of AP activity seen in fruit held in 2.5% $O_2$ alone. However, upon transfer of fruits to air after 6 d of storage in low $O_2$, the AP activity markedly increased (Figs. 3B and 4C) for both low $O_2$ treatments.

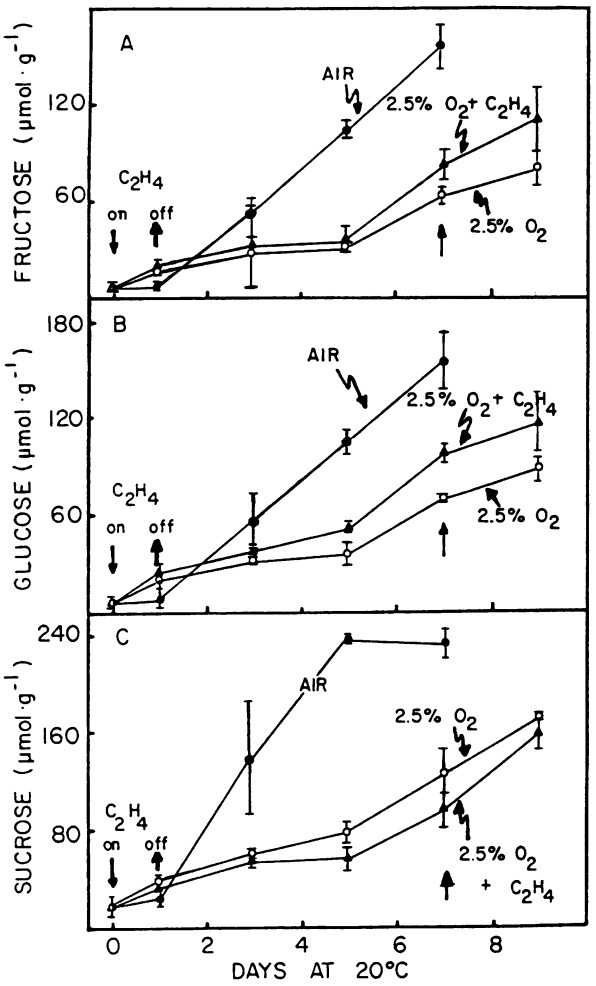


**Figure 2.** Changes in the levels of sugars in banana ripened in air or held in 2.5% $O_2$ or 2.5% $O_2$ + 500 $\mu$L/L ethylene. Single fruits were sampled on d 0 (preclimacteric), d 1 (treated with ethylene), and on d 3, 5, and 7. Fruits held in modified atmospheres were returned to air on d 7 (arrows) and sampled on d 9. Data represent the means of three replicate determinations and vertical bars are the SD.

### Changes in the Profile of Acid Phosphatase isoenzymes

It has been previously reported that the activity of soluble and particulate AP increases by 2- to 4-fold and 26-fold, respectively, in the course of banana fruit ripening (9). The same authors also concluded that the particulate enzyme activity is mainly desorbed from the cell wall. When soluble and particulate fractions of samples extracted from ripe fruit were run on polyacrylamide gels under nondenaturing conditions, the AP activity was resolved into 8 and 9 bands, respectively (Fig. 5). The faster migrating band (No. 9) of the particulate fraction was not detected in the soluble fraction, suggesting its unique association with the particulate fraction.

The changes in AP isoenzymes during ripening of bananas are shown in Figures 6 and 7. No AP isoenzyme was detectable in either the soluble or particulate fraction of green fruits when equal amounts of protein were loaded on the gels (Figs. 6 and 7, day zero). However, AP isoenzymes in the particulate fraction increased first as the fruits ripened (Fig. 7), while

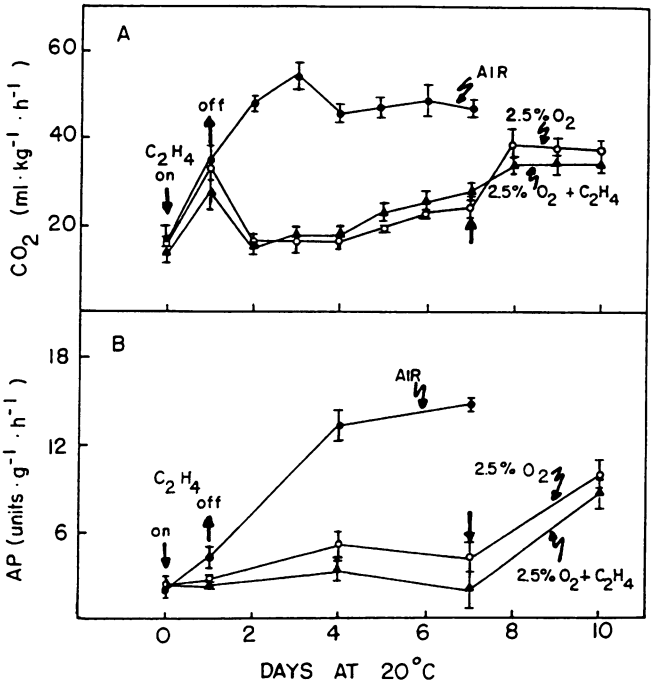


**Figure 3.** Effect of 2.5% $O_2$ or 2.5% $O_2$ + 18 $\mu$L/L ethylene on respiration rates (A) and phosphatase activity (B) of banana fruits. The sampling periods were similar to those given in the legend to Figure 2.

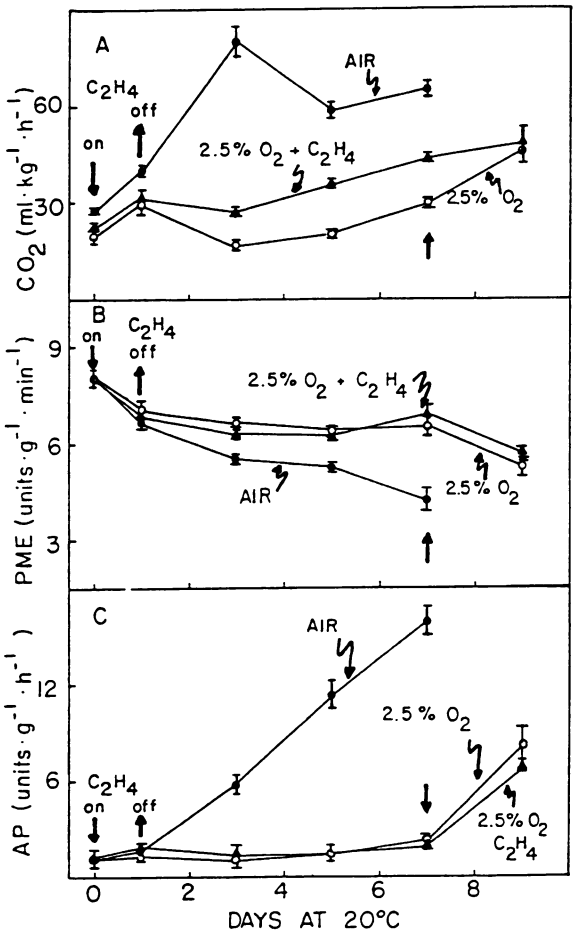


**Figure 4.** Effect of 2.5% $O_2$ with or without 500 $\mu$L/L ethylene on respiration (A), pectinmethylesterase (B), and acid phosphatase (C) activities of single banana fruits. Sampling periods were the same as those described in the legend to Figure 2.

those in the soluble fraction appeared later (Fig. 6). Some of the particulate AP isoenzymes (band Nos. 8 and 9) also appeared late in ripening, suggesting developmental differentiation in the expression of AP isoenzymes (Fig. 7).

The isoenzyme composition of AP extracted from fruit subjected to 2.5% $O_2$ atmosphere showed three interesting features (Figs. 6 and 7). First, no AP isoenzymes were found to be present in the soluble fraction except on the 7th d of storage. The inclusion of 500 $\mu$L/L ethylene did not alter this low $O_2$ effect (Figs. 6 and 7). Second, the particulate isoenzymes tended to stain more weakly in low $O_2$-treated fruits compared to air-ripened fruits. The same was true when ethylene was introduced in the 2.5% $O_2$ atmosphere. Third, AP band No. 9 was selectively absent in fruits held in 2.5% $O_2$ and 2.5% $O_2$ + 500 $\mu$L/L ethylene atmospheres. However, upon transfer of fruits held either in 2.5% $O_2$ or 2.5% $O_2$ + 500 $\mu$L/L ethylene to air, the AP isoenzymes were fully expressed (Figs. 6 and 7, lanes 9), suggesting that the fruits resumed features characteristic of normal ripening.

## DISCUSSION

Ripening of bananas is associated with an extensive breakdown of starch, the main carbohydrate reserve comprising 80 to 95% of the dry matter in the green fruit and 5 to 15% in the ripe fruit (24–26), with a corresponding increase in the levels of soluble sugars, mainly sucrose, glucose, and fructose. Accumulation of sucrose occurred earlier than glucose and fructose. Furthermore, the ratio of the increments of glucose over fructose was close to unity, indicating that the immediate precursor of these sugars is sucrose (37). Ripening of banana fruits was slower in 2.5% $O_2$ or 2.5% $O_2$ + 500 $\mu$L/L ethylene than in air and this was also reflected in the accumulation of the sugars (Fig. 2). During the first 4 d in either 2.5% $O_2$ or 2.5% plus 500 $\mu$L/L $C_2H_4$, the levels of sugars showed hardly any changes. Thereafter, they began to accumulate but within 9 d failed to reach the levels of the air-ripened fruits (Fig. 2). It is interesting to note that this increase in sugars was reflected in a gradual rise in $CO_2$ evolution (Fig. 3). It is also noteworthy that the observed rise in sugar levels and $CO_2$ output was not reflected in either total AP activity or appearance of isoenzymes (Figs. 3, 4, 6, 7), suggesting that these events may not be related either temporally or biochemically.

PME activity in relation to ripening of various fruits has been reported to increase, decrease, or to remain constant (12, 27). In bananas, the information is conflicting. Hultin and Levine (13) reported a very large increase in PME activity during ripening of bananas. Palmer (26) and Brady (3), on the other hand, took precautions to avoid loss of protein due to high content of phenolics in green fruits and found that the activity of PME remains constant in the course of banana ripening. In our experiments, a constant decline in PME activity was observed in fruits ripened in humidified air. The

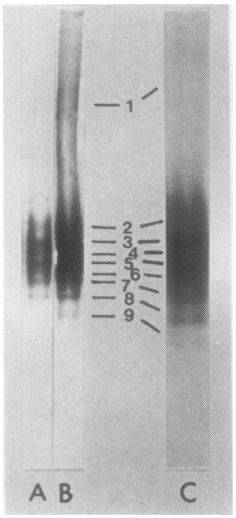


**Figure 5.** Comparison of banana acid phosphatase isoenzymes from soluble (A) and particulate fractions (B, C) of climacteric fruits. Numbers represent different acid phosphatase isoenzymes. Acid phosphatase isoenzymes were stained before (A, B) or after (C) electrotransfer of polyacrylamide gel onto nitrocellulose filter with 25 mM Tris-192 mM glycine buffer.

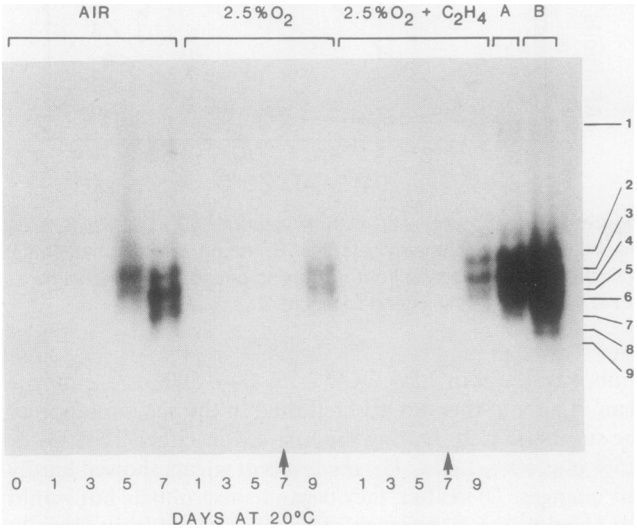


**Figure 6.** Profiles of soluble acid phosphatase isoenzymes from fruit held in either air, 2.5% $O_2$ or 2.5% $O_2$ + 500 $\mu$L/L ethylene. Soluble fruit fractions (equivalent to 8 $\mu$g protein) were electrophoresed in 5T0.66C-11T0.66C gradient polyacrylamide gel and then electrotransferred onto nitrocellulose filters. Blots were stained for acid phosphatase activity as described in "Materials and Methods." Soluble (A) and particulate (B) fractions were run alongside the other samples for comparison. Sampling periods were the same as described in the legend to Figure 2.

discrepancy between these results and those of Brady (3) could be due to one or all of the following reasons: (a) different banana cultivars tested, (b) different extraction procedures used (one step *versus* two steps), and (c) ripening and storage conditions. Brady (3) held bananas continuously in ethylene atmosphere.

The decrease in PME activity observed in air-ripened fruits

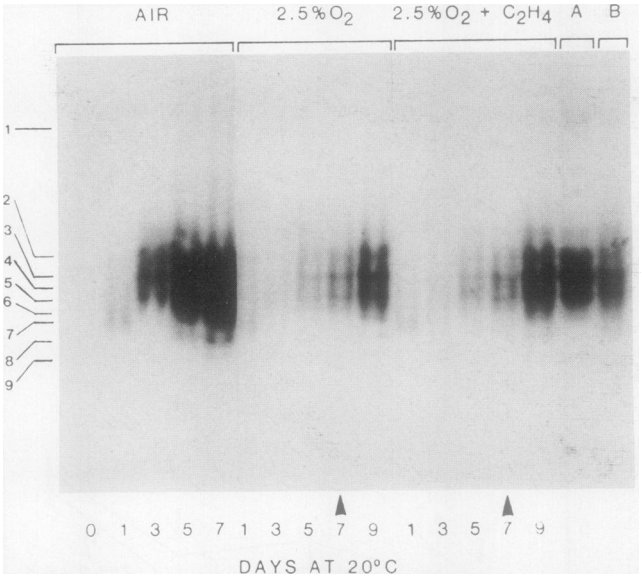


**Figure 7.** Profiles of particulate acid phosphatase isoenzymes from banana fruits ripened in air, and 2.5% $O_2$, both with and without 500 $\mu$L/L ethylene. Methods are described in the legend to Figure 6. A, soluble fraction. Samples run in lane B were from fruits held for 4 d in 2.5% $O_2$, but the sample contained 16 $\mu$g protein as opposed to 8 $\mu$g protein for other lanes.

was arrested by either 2.5% $O_2$ or 2.5% $O_2$ plus 500 $\mu$L/L ethylene. On the basis of the present data alone it is not possible to indicate whether the arrest in the decrease of PME activity is due to changes in its rate of either synthesis or degradation. However, the data indicate that low $O_2$ prevents both the rise (AP) as well as the decrease (PME) in the activities of enzymes seen in air-ripened fruits. The effect of low $O_2$ on PME activity was reversible since upon returning the fruits to air the pattern of decline in PME activity was similar to fruits ripened in air.

The results concerning the changes in total AP activity and appearance of AP isoenzymes indicate that there may be a sequential induction of isoenzymes during ripening of bananas (Figs. 6 and 7). However, the physiological significance of the increase in AP activity and the induction of its isoenzymes in the course of ripening is not clear. Furthermore, there is little information concerning the mechanisms that regulate the synthesis and degradation of AP during fruit ripening. De Leo and Sacher (9) and Sacher (33) suggested that the increased AP may be involved in the hydrolysis of mononucleotides, which may limit the synthesis of RNA necessary for the maintenance of the levels of proteins required to sustain cell integrity. This hypothesis may not be sustainable because in most fruits the levels of ATP increase during the course of ripening (2, 32, 34, 38). Based on the effects of inhibitors of both translation and transcription, De Leo and Sacher (9) concluded that the increase in AP activity was due to the *de novo* synthesis of the enzyme which was dependent upon the synthesis of RNA. Our results tend to support these findings since (a) no AP activity was detected on the blots of preclimacteric fruit extracts, and (b) AP isoenzymes became visible after ethylene treatment, though

traces of total activity could be detected in extracts of preclimacteric fruits.

The transfer of bananas to 2.5% $O_2$ suppressed any further expression of AP isoenzymes as well as the appearance of No. 9 isoenzyme. This suppression of the intensity of the staining of AP isoenzymes was reflected in the diminution of the increase in total AP activity (Figs. 3B, 4C, 6, and 7). From these findings it is evident that the amount of AP protein in extracts of fruits held in low $O_2$ atmosphere was much lower than in the fruits ripened in air, suggesting that low $O_2$ may affect either protein or RNA synthesis, or destabilize preexisting mRNA.

It has been pointed out in the introduction that the delaying effect of low $O_2$ on ripening may be related to processes other than respiration. The results presented here on the low $O_2$ effect suggest a close relationship between respiration and sugar accumulation. However, since the respiratory rise during ripening may be considered a facet of ethylene action (34), it may be expected to be affected by the $O_2$ levels. In other words, the diminution of respiration is not due to the restriction of the terminal oxidases but rather to the decrease by low $O_2$ of the action of ethylene. It is known that the affinity of various processes for ethylene differs (30). In this context, it is interesting that respiration was the only process among those tested which responded with increased rates when higher ethylene concentrations (500 $\mu$L, Fig. 4) were supplemented to low $O_2$ atmosphere, suggesting a higher affinity of this system for ethylene in contrast to processes related to AP induction and accumulation of sugars. Furthermore, while the accumulation of sugars increased gradually after 4 d in low $O_2$, no increase in AP activity was observed throughout the duration of low $O_2$ treatment, suggesting differential effects of low $O_2$ on metabolic processes during fruit ripening.

## ACKNOWLEDGMENTS

We thank Dr. Cindy Tong for skillfully drawing the figures and Steven Altman for his assistance with manuscript preparation.

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