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ABNORMALITIES IN RIPENING AND MITOCHONDRIAL SUCCINOXIDASE RESULTING FROM STORAGE OF PRECLIMACTERIC BANANA FRUIT AT LOW RELATIVE HUMIDITY

NORMAN F. HAARD* and H. O. HULTIN

Department of Food Science and Technology, University of Massachusetts, Amherst, Massachusetts 01002

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Abstract—Green banana fruit ripened at a reduced relative humidity (below 80%) did not undergo the respiratory climacteric or develop any characteristics of normally ripened fruit. Mitochondrial fractions from abnormally ripened fruit showed markedly different succinoxidase activity than the fractions isolated from fruit ripened under standard conditions at 90–95 per cent relative humidity.

INTRODUCTION

RIPENING disorders in banana fruit have been known to result from low-temperature exposure ("chilled"), high-temperature exposure ("boiled"), and from storage in high levels of carbon dioxide.[1] Maintenance of proper relative humidity in the storage atmosphere is also an important factor for the ripening of banana fruit. A relative humidity of 90–95 per cent is generally recommended for green fruit, this being lowered to 75–85 per cent with the ripened fruit to retard fungal infection.[1]

We have ripened banana fruit at a constant temperature of 24° with the relative humidity above 90 and below 80 per cent. Our data show that the respiratory activities of both the intact fruits and the mitochondrial fractions isolated from them are markedly different in the two cases. These results may add to understanding of the relationship between mitochondrial-linked reactions and fruit ripening.

RESULTS AND DISCUSSION

Respiration and Subjective Evaluation of Ripened Fruit

Fruit stored at high relative humidity underwent the upsurge in carbon dioxide evolution characteristic of climacteric fruit and were normal with respect to color, flavor development and texture. Bananas stored in an atmosphere where the relative humidity was maintained below 80 per cent did not undergo the respiratory climacteric (Fig. 1) and showed none of the visual characteristics of normal ripening. The peel color changed from green to grayish-green and finally to mottled gray-black. The green color was subdued by the darkening but did remain, indicating incomplete chlorophyll destruction. The pulp texture of these fruit was firmer than fruit which were normally ripened for the same period of time at higher relative

* Present address: Department of Food Science, Rutgers, The State University, New Brunswick, New Jersey 08903.

[1] H. VON LOESECKE, *Bananas*, 2nd edition, p. 45, Interscience, New York (1950).

humidity, and the characteristic aroma of ripened banana fruit did not develop. These fruit were astringent to the taste and were not as sweet as normally ripened fruit, indicating that starch to sugar conversion was retarded. These abnormalities are in many respects similar to those observed for chilled fruit.[1]

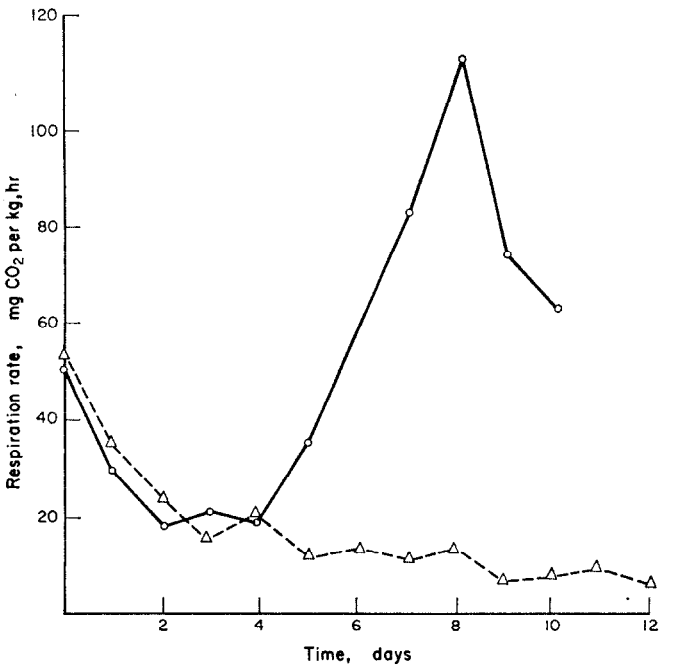

FIG. 1. RESPIRATION OF INTACT BANANA FRUIT AT 24°. RELATIVE HUMIDITY MAINTAINED ABOVE 90% (○) AND BELOW 80% (△).

Succinoxidase Activity of Mitochondria

The yields of protein in the mitochondrial fraction from normally ripened and low relative humidity ripened fruit were nearly identical (Table 1). Considerable differences in the specific activity of succinoxidase of these fractions, however, were found (Table 1, Fig. 2) Mitochondria from normally ripened fruit (HRHM) and from fruit ripened at low relative humidity (LRHM) showed similar initial rates of succinoxidase activity (Fig. 2). HRHM showed a characteristic rapid decline in activity as the time of assay progressed to about 40 min when a lower steady rate was attained. On the other hand, LRHM showed an initial increase in succinoxidase activity up to 20 min followed by a more gradual decrease in activity for the remaining assay. The longer the fruit was stored at the low relative humidity the more gradual was this decrease.

The decline in succinoxidase activity with HRHM was previously shown to be independent of a wide variety of assay conditions.[2, 3] Moreover, when mitochondria were isolated from all postharvest respiratory stages of normally ripened fruit, essentially identical declining rates of succinoxidase activity were observed.

[2] N. F. HAARD and H. O. HULTIN, *Anal. Biochem.* **24**, 299 (1968).
[3] N. F. HAARD, Ph.D. Thesis, Order No. 67-6945 (1967).

TABLE 1. YIELDS AND SUCCINOXIDASE ACTIVITIES OF MITOCHONDRIA ISOLATED FROM BANANA FRUITS STORED AT HIGH AND LOW RELATIVE HUMIDITIES

Ripening conditions	Physical appearance	Mitochondrial protein* extracted (mg/150 g)	$QO_2(N)$*†
24°, above 90% R.H. for 7 days	No indication of abnormal ripening	27·5 24·5	486 499
24°, below 80% R.H. for 7 days	Gray discoloration with green and streaking in pulp	24·0 28·8	828 790
24°, below 80% R.H. for 14 days	Gray to black discoloration with green peel; no signs of normal ripening	25·0 26·6	909 930

* Results of two separate experiments shown.

† $QO_2(N)$=microliters of oxygen consumed per hr by 1 mg of mitochondrial protein-nitrogen with succinate as substrate. Results indicated were corrected for endogenous oxygen consumption. Flasks contained 0·2 ml of 20% KOH in the center well, succinic acid (40 μmoles), K phosphate, pH 7·3 (40 μmoles), sucrose (750 μmoles), $MgSO_4$ (12 μmoles), yeast concentrate, Sigma Chemical Co. (2 mg), bovine serum albumin (3 mg), cytochrome *c*, horse heart, Sigma Chemical Co. (6×10^{-5} μmoles), ATP (3 μmoles), ADP (2 μmoles), and mitochondrial suspension (5 mg) in a volume of 4 ml. Results were based on 1 hr readings (see Fig. 2).

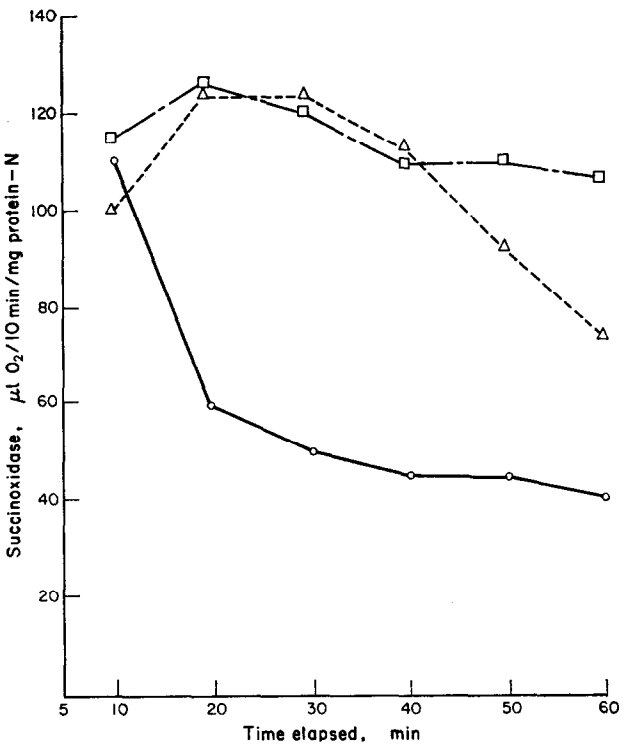

FIG. 2. SUCCINOXIDASE ACTIVITY OF MITOCHONDRIA ISOLATED FROM FRUIT RIPENED AT HIGH RELATIVE HUMIDITY (○), LOW RELATIVE HUMIDITY FOR 1 WEEK (△), AND AT LOW RELATIVE HUMIDITY FOR 2 WEEKS (□). THE CONDITIONS OF ASSAY WERE AS DESCRIBED IN THE LEGEND OF TABLE 1.

The abolition of the respiratory climacteric in fruit ripened at the low relative humidity appears contradictory to the increased succinoxidase activity of the mitochondria from the same fruit. This does not preclude, however, a possible direct relationship between the two phenomena. It is too early to predict what this relationship might be. It is also entirely possible that the relationship is indirect. For example, the increased succinoxidase activity may be a reflection of changed conditions which differentially affect the mitochondria during isolation.

EXPERIMENTAL

Ripening of Banana Fruit

Banana fruits, *Musa cavendishii* (Valery type), were purchased from a local supplier and ripened in a large, ventilated chamber containing a humidifier. The full, solid green fruit was classified between No. 1 and 2, according to the scale of von Loesecke,[1] at the start of these studies.

Respiration of Intact Fruit

A hand of bananas weighing approximately 1500 g was placed in a respirometer as described by Haard.[3] Air from the ripening chamber was scrubbed free of CO_2 and passed over the fruit and into 0·1 N NaOH. CO_2 evolution was then estimated by the double end-point titration described by Braverman.[4]

Isolation and Assay of Mitochondria

The mitochondrial fraction of banana fruit was isolated by the method described by Haard and Hultin.[2] Preparations were assayed for succinoxidase activity with the Gilson differential manometer by a technique similar to that described by Umbreit *et al.*[5] The specific conditions of the assay are given in the legend of Table 1.

[4] J. B. BRAVERMAN, *Introduction to the Biochemistry of Foods*, p. 336, Elsiever, New York (1963).
[5] W. W. UMBRIET, R. H. BURRIS and J. F. STAUFFER, *Manometric Techniques*, 3rd edition, pp. 79–94, Burgess, Minneapolis, Minn. (1959).