

Fruit Storage at Subatmospheric Pressures

Abstract. *The storage life of bananas and other fruits is prolonged by ventilating them with air at less than atmospheric pressure. This procedure accelerates the escape of the ripening hormone ethylene from the tissue; by reducing the oxygen tension it also lowers the fruit's sensitivity to the hormone.*

The storage life of fruits is prolonged when the pressure of air surrounding them is lowered (1). We now describe a series of experiments which help to elucidate the biological mechanism underlying this effect.

All fruit was obtained from local growers, except for Gros Michel bananas which were provided by a Miami supply house (2). Only the method used for bananas will be described; other fruits were handled in essentially the same manner. Duplicate samples consisting of eight hard, green fruits were composed of an equal number of fingers cut from each of several hands of bananas. The fruits were weighed before they were placed in the storage chambers and again when they were removed in order to appraise shrinkage. Both control and treated bananas were stored in 10-liter vacuum desiccators maintained at a constant temperature ($\pm 1^\circ\text{C}$) and continuously evacuated by means of a vented-exhaust, oil-seal pump. Constant ventilation was achieved by admitting air to each desiccator through a vacuum regulator (Matheson model 49) which maintained the selected vacuum (none in case of controls) by allowing air to be bled into the system at the proper rate. Incoming air was brought to the temperature of the desiccator and then saturated by bubbling it through a water tower inserted between the desiccator and vacuum regulator. Air flow through the apparatus was regulated with a needle valve located between

Table 1. Ripening of green Gros Michel bananas in air at 760 mm-Hg and at subatmospheric pressures.

Expt.	Treatment pressure (mm-Hg)	Temp. ($^\circ\text{C}$)	Days to break color	
			Control*	Treated†
1	360	24	10	18
2	360	15	7	15
3	250	15	12	45
4	250	15	14	53
5	150	24	5	>14‡
6	150	15	14	>24‡
7	150	15	10	>40‡

*Normal pressure. †Reduced pressure. ‡Bananas were still green when they were removed after the indicated number of days.

the pump and desiccator; it was measured with a rotameter attached to the inlet of the vacuum regulator. Although the flow was adjusted to 2 cubic feet per hour, the exact setting was not critical. When tank oxygen was used to ventilate the fruits a slight positive pressure of the gas was supplied to the inlet of the vacuum regulator. In a few experiments a known amount of ethylene was added to the ventilating stream by bleeding in a mixture of air and ethylene (1000:1) supplied at constant pressure through a capillary restriction. The concentration of ethylene in the final mixture was checked repeatedly by gas chromatography (see 3).

The storage life of bananas is approximately doubled when they are flushed with air at $\frac{1}{2}$ atm, again doubled when the pressure is reduced to $\frac{1}{3}$ atm (Table 1), and presumably further increased at lower pressures (4). No significant desiccation occurred during the longest storage periods, the fruits ripened normally, and their taste and appearance were satisfactory. Results obtained with other fruits are shown in Table 2.

Lowering the air pressure reduces the amount of oxygen available and this might explain the subsequent delay in ripening, for the same effect is produced when the oxygen content of air is reduced (5). To test this possibility, ripening in air at atmospheric pressure was compared with ripening in pure oxygen at 180 mm-Hg. Thus the fruits were exposed to approximately the same partial pressure of oxygen, but in gas mixtures having total pressures of 760 and 180 mm-Hg, respectively. In six experiments ripening consistently occurred more slowly at the lower pressure, the life of the fruit being increased on the average by 86 ± 19 percent. Therefore it can be concluded that something other than oxygen depletion causes fruits to be preserved in a partial vacuum. This factor is probably removal of the fruit-ripening hormone, ethylene, for when the pressure is reduced any gas synthesized within a fruit escapes more readily and its cellular concentration declines (6). Green ba-

nanas contain about 0.1 to 0.2 part of ethylene per million (1), an amount close to that needed to trigger ripening (1), but when the ambient pressure is reduced by 75 percent the ethylene content should decline by the same amount and the life of the fruit should increase correspondingly (7).

Bananas ripen more swiftly at a pressure of 180 mm-Hg when they are ventilated with oxygen rather than air (8). Since the oxygen partial pressure is higher when oxygen is employed, this result suggests that oxygen depletion enhances storage life in a partial vacuum even though it does not account for the entire effect (9). The partial pressure of oxygen in water-saturated air at $\frac{1}{3}$ atm is approximately 0.065, which is not so low that ethylene formation would be inhibited significantly (10) but is possibly low enough to prevent ethylene from acting on tissues (1). Therefore the sensitivity of the fruits to ethylene at this oxygen partial pressure was investigated. When the oxygen partial pressure was 0.21 atm, ethylene at a partial pressure of 10^{-6} stimulated ripening in 4 days both in air at atmospheric pressure and oxygen at 180 mm-Hg (11). However, when the oxygen partial pressure was reduced to 0.065 (using air at $\frac{1}{3}$ atm), ethylene at a partial pressure of 10^{-6} no longer had its characteristic effect, and the fruit did not ripen for 16

Table 2. Ripening of various fruits in air at 760 mm-Hg and in subatmospheric pressures at a temperature of 15°C .

Treatment pressure (mm-Hg)	Fruits (No.)	Days until 50 percent of fruit ripe	
		Control*	Treated†
<i>Tomato (Homestead No. 2)</i>			
125	40	11	45
<i>Avocado (Pollock)</i>			
200	10	6	12
<i>Mango (Haden)‡</i>			
200	10	3	8
150	10	4	17
<i>Florida sweet cherry (Barbados)</i>			
150	100	5	13
<i>Lime (Persian)</i>			
150	60	10§	>56§
<i>Guava </i>			
150	16	6	>30

*Normal pressure. †Reduced pressure. ‡Taste of treated fruit was poor. §Control figure is days until 50 percent of the fruits had turned yellow and lost their buttons; all sample fruits were still green with intact buttons when the experiment was terminated after 56 days. ||Experiment terminated at 30 days because of fungal contamination. The variety of the guava is uncertain.

days (12). Under these conditions ripening again was effected in 4 days when the partial pressure of ethylene was increased to 6×10^{-6} , so it can be concluded that more ethylene is required to ripen fruits when the concentration of oxygen is lowered. This effect and a reduction in ethylene content are offered as an explanation for increased storage life at subatmospheric air pressures.

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References and Notes

1. S. P. Burg and E. A. Burg, *Science* **148**, 1190 (1965).
2. The bananas were supplied by Banana Supply Co., Miami, Fla.
3. S. P. Burg and E. A. Burg, *Plant Physiol.* **37**, 179 (1962).
4. No attempt was made to see how long bananas could be stored when the pressure was 150 mm-Hg because, after 50 to 60 days, fruits invariably became contaminated with fungus. At low pressures this factor, rather than ripening, limited the duration of the experiments.
5. F. Kidd and C. West, *G. Brit. Dept. Sci. Ind. Res. Rept. Food Invest. Board* **1933**, 51 (1934); R. E. Young, J. Romani, J. B. Biale, *Plant Physiol.* **37**, 416 (1962).
6. S. P. Burg and E. A. Burg, *Physiol. Plantarum* **18**, 870 (1965).
7. For example, there is an exact correlation between the partial pressure of ethylene within an apple and the absolute pressure of the atmosphere in which the apple is situated, even though the rate of ethylene formation does not vary with atmospheric pressure.
8. Ripening is delayed by a factor of more than 4 when fruits are stored in air at 250 mm-Hg instead of 760 mm-Hg (Table 1), whereas in oxygen at 180 mm-Hg the delay is less than a factor of 2 relative to the control in air at atmospheric pressure.
9. Our data do not exclude an effect due to oxygen depletion; they only show that storage life is improved when the pressure is lowered even if the oxygen tension is not altered.
10. S. P. Burg and K. V. Thimann, *Proc. Nat. Acad. Sci. U.S.A.* **45**, 335 (1959).
11. In these experiments reducing the pressure had its characteristic effect, retarding ripening in the absence of ethylene from 18 days in air at 760 mm-Hg to 31 days in oxygen at 180 mm-Hg.
12. In air at atmospheric pressure without ethylene present the fruits ripened in 10 days in this experiment. At a pressure of 250 mm-Hg ripening would occur in approximately 40 days (see Table 1), but under these conditions ripening required only 16 days when ethylene was included. It is clear that the gas enhanced ripening considerably even though it failed to cause its maximum effect.
13. Patent applied for.
14. This research was supported by a grant from the United Fruit Co.

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Inhibition of Protein Synthesis: A Mechanism for the Production of Impaired Iron Absorption

Abstract. Treatment of rats with cycloheximide results in a defect in intestinal iron transport. A similar defect occurs after the parenteral administration of iron. Under both conditions there is impaired uptake of iron into the mucosal cells as well as defective transfer from the intestinal mucosa. It is suggested that the interference in iron transport may be due to a deficiency of an unidentified carrier substance.

The intestinal absorption of iron (1-4) involves at least two steps: (i) mucosal uptake of iron from the intestinal lumen and (ii) transfer of iron from mucosal cells to the plasma. These studies indicate that the intestinal mucosa is important in controlling iron absorption. The actual regulatory mechanisms remain poorly understood, however. Possibly an unidentified carrier substance is important in the transfer of iron from the intestinal mucosa.

Our study shows that inhibition of protein synthesis in the rat by the antibiotic cycloheximide (5) is associated with impairment in the absorption and transport of iron from the intestine.

Female Wistar rats (140 to 160 g) were fasted for 16 hours prior to study. The animals were separated into four groups: (i) controls; (ii) rats given a low-iron diet (Nutritional Biochemicals) for 2 weeks prior to study; (iii)

rats given an intramuscular injection of Imferon (25 mg) 2 weeks prior to study; and (iv) rats given an intraperitoneal injection of cycloheximide (5 mg per kilogram of body weight) 3 hours prior to study. After the animals were lightly anesthetized with ether, the proximal 16 to 20 cm of small bowel was isolated between silk ligatures, care being taken not to obstruct blood flow. The rats received an intraduodenal injection of $\text{Fe}^{59}\text{SO}_4$ (50 μg containing 2×10^6 disintegrations per minute). After 30 minutes the isolated loops were excised, the contents were washed out with 50 ml of 0.9 percent NaCl, and the mucosa was removed by scraping. Portions of the loop contents and the mucosa were assayed for radioactivity in a well-type scintillation counter.

Data on the relative rates of absorption of iron in the four groups of

animals were determined by measuring the mucosal uptake and mucosal transfer of $\text{Fe}^{59}\text{SO}_4$ (Table 1). The mucosal uptake of radioiron (A) was determined by subtracting the amount of radioactivity recovered in the loop contents from the amount injected. The amount of radioiron remaining in the mucosa (B) was measured directly. The mucosal transfer of radioiron (A - B) represents the difference between mucosal uptake and the amount remaining in the mucosa. Mucosal transfer represents net absorption. In control rats there was a mucosal uptake of 65 percent and transfer of 37 percent of the injected dose. In rats previously on a low-iron diet there was a significantly increased mucosal uptake (83 percent) and transfer (56 percent) of radioiron. In contrast, in rats previously injected with Imferon there was a decreased mucosal uptake (52 percent) and a markedly reduced transfer (16 percent) of radioiron. Administration of cycloheximide decreased mucosal uptake (29 percent) and transfer (12 percent) of radioiron.

The amount of radioiron remaining in the mucosa divided by the mucosal uptake, B/A, also reflects the interference in iron transport induced by treatment with iron or cycloheximide. In control animals 42 percent of the iron taken up by the mucosa was still present in the mucosa after 30 minutes, while only 33 percent remained in iron-deficient animals. In contrast, in animals treated with iron or cycloheximide there was a significant accumulation of iron in the mucosa, with 69 and 59 percent, respectively, of the iron taken up by the mucosa still there after 30 minutes.

To confirm that cycloheximide blocks iron absorption, the absorption

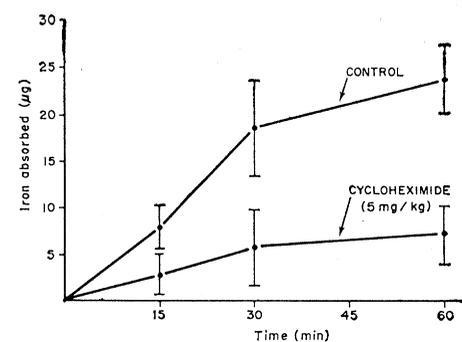


Fig. 1. Absorption of $\text{Fe}^{59}\text{SO}_4$ (50 μg containing 2×10^6 disintegrations per minute) from isolated intestinal loops in control and cycloheximide-treated rats. The values shown represent the mean \pm 1 S.D.