INTERACTIONS BETWEEN O_2 LEVELS, RATE OF RESPIRATION AND GAS DIFFUSION IN 5 APPLE VARIETIES

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Introduction

It is a well established fact that the rate of respiration of fruits and storage organs decreases when the external oxygen concentration decreases below its air value (1, 2, 4, 8, 10, 11, 13). Further, this decrease occurs at levels of oxygen which may not be expected to limit cytochrome oxidase (4). Blackman (1) posutlated that oxygen plays a dual role in apple fruit respiration, i.e. both as an acceptor of electrons in the terminal electron transport chain, and as a regulator of substrate mobilization. He suggested that the terminal oxidase has a high affinity for oxygen, and that it is not inhibited unless the external oxygen concentration drops below 5%, where fermentation is induced. The enzyme(s), on the other hand, which is/are diminished by relatively high oxygen concentrations, is/are not directly involved as an electron acceptor but act(s) as a regulatory enzyme affecting the rate of substrate mobilization. Mapson and Burton (12), however, interpreted their results with potato tubers as being indicative of the existance of two terminal oxidases with appreciably different affinities for oxygen. It should be pointed out that previous work with apples indicates that anaerobiosis begins to be established when the oxygen concentration decreases below a critical level, and storage of apples under these conditions will result in low O_2 injury (9). Chevillote (6), on the other hand proposed that the biphasic mode of changes in the rate of respiration in response to the decrease in the external oxygen level is the result of the restriction of cytochrome oxidase by the diffusion of oxygen.

In addition to the above hypotheses one may propose that the decrease in respiration at relatively high oxygen concentrations may be an indirect effect of a decrease in the metabolic activity of the cells, a decrease which is caused by low oxygen concentrations. This in turn will reduce the demand for ATP and hence diminish respiration. The latter hypothesis may not reflect reality because if the rate of oxygen uptake is measured in a closed system, its diminution is made manifest immediately upon the decrease of oxygen to the appropriate levels (unpublished observations). In other words there is no lag phase which might have been expected when the metabolic homeostasis changed from one level of activity to another.

The hypotheses advanced earlier to explain the biphasic nature of the decrease in the rate of respiration as a function of the external oxygen

concentrations were speculative since there were no analytical data of the actual concentrations of oxygen at the center of the plant organs. This is a preliminary report of two years of research concerning the study of the relationships between rates of respiration, gas diffusion, and the external and internal oxygen concentrations in five apple cultivars.

Results and Discussion

Previous work with apples has shown that the critical O_2 concentrations at which the rate of respiration begins to decrease or partial anaerobiosis is established vary with the cultivars (9). These differences could be either metabolic or physical in nature or both. In order to elucidate this aspect, first the diffusivity of oxygen into the different apples must be known. It was shown previously that the gas exchange in apple fruits follows Fick's laws of diffusion (3, 5). According to his first law, the rate of diffusion of a gas is:

$$J = AD \frac{\partial c}{\partial x} \qquad (1)$$

where J is the flux as μmoles/fruit/sec, A is the area in cm^2, and $\frac{\partial c}{\partial x}$ is the gradient of concentration with distance within the barrier. For the determination of $\frac{\partial c}{\partial x}$ one has to solve the equation of Fick's second law of diffusion which, for a sphere, is

$$\frac{\partial c}{\partial T} = D\left(\frac{\partial^2 c}{\partial r^2} + \frac{2}{r} \frac{\partial c}{\partial r}\right) \pm v \qquad (2)$$

where v is the rate of production (+) or consumption (-) of the gas under consideration (7).

In our work we used steady-state conditions, which were achieved by enclosing the apples in a plexiglass cylinder through which a steady stream of the appropriate concentration of the gas was passed. With a slight modification of Burg and Burg's system (3) we were able to sample 0.2 ml of gas from the center of the fruit. In addition, care was taken that the geometrical shape of the fruits approximated that of a sphere. Under steady state conditions, eq. (1) becomes: (7)

$$c(r) = \pm \frac{v}{6D} r^2 - \frac{A}{r} + B \qquad (3)$$

The barriers to diffusion of gases in apples involve the skin, the intercellular spaces of the flesh, and finally the cell wall and membrane. The diffusion through the skin can be considered similar to that of a hollow sphere, of thickness equal to $r_o - r_i$. The flux of O_2 through the skin can be described by eq. 4.

$$J = AKD \frac{C_o - C_u}{r_o - r_i} \qquad (4)$$

where C_o and Cu are the concentrations of O_2 outside the fruit and under the skin, respectively, and k is a dimensionless number which indicates the portion of the total surface permeable to gases. Since neither K nor $r_o - r_i$ are known with any degree of precision, the overall diffusivity of the gases is calcualted as:

$$D' = \frac{KD}{r_o - r_i} \qquad (5)$$

which also includes the decrease in the diffusion rate due to the tortuosity of the lenticels. It should be pointed out that the latter would be expected to be rather small since inside the lenticels there are only a few parenchyma cells; moreover their packing is very loose.

To determine the diffusivity of gases through the intercellular spaces eq. (3) is solved for a solid sphere:

$$C_i = C_o \pm \frac{v}{6D} r^2 \qquad (6)$$

where v is the rate of gas evolution (+) or absorption (-), and C_i and C_o are the concentrations at the center of the fruit and in the ambient atmosphere respectively. To determine D of eq. (6) C_o and C_i, as well as v, must be measured. For this we peeled the apples, blotted them dry and inserted them into the cylinder through which a constant stream of CO_2-free air was passed.

The results of table 1 show the diffusivity of CO_2 through the flesh of three apple cultivars. It may be seen that Stayman apples are the least permeable to CO_2. The data also show that the tortuosity decreases the diffusivity of CO_2 in air by 185-, 107-, 101-fold for Stayman, York Imperial and Gala, respectively. Once D is determined, the concentration of CO_2 under the skin can be calculated. Table 2 compares the calculated differences in CO_2 concentration between those at the center of the fruit and under the skin with those measured in the peeled apples. It may be seen that the values are in good agreement. Table 3 shows the values of D' for CO_2 for different external O_2 concentrations. It may be seen that the magnitude of D' is similar, although the rate of respiration varies, as is predicted by eq. (4). The results presented in Table 4 compare the observed rates of CO_2 output with those calculated by inserting the values of D', C_u and $C_o{}^{CO_2}$ into eq. (4). It may be seen that the calculated and observed rates of CO_2 output are similar. This in turn indicates the experimental procedures followed and the assumptions made reflect the in vivo gas exchange in apple fruits.

The results of Table 5 show that the diffusivity of CO_2 varies with the five cultivars tested. Further, the internal concentration of CO_2

depends, as would be expected, on the diffusivity of the gas as well as on the rate of its production. For fruits with similar rates of CO_2 output, Gala and Rome Beauty, there is an inverse relationship between the ratios of their respective D' and internal CO_2 concentrations. The results of Fig. 1 show that the rate of CO_2 output of Rome Beauty apples begins to decrease when the external oxygen concentration decreases to less than 8%. This gradual decrease continues until the O_2 concentration is dropped to about 1.5%, to be followed by an increase as the oxygen concentration decreases to zero. The critical external concentrations at which the rate of CO_2 begins to decrease vary with the cultivars (Table 6). However, if the differences in the rate of respiration are taken into consideration, the internal concentration of O_2 may not be different for the five cultivars tested. In other words, the observed external differences in the critical oxygen concentrations are due to differences in diffusivity and rate of respiration.

If the oxygen concentration of the intercellular spaces is in equilibrium with the cellular sap then the concentration of O_2 at the surface of the cell will be about 90μM. Since the affinity of cytochrome oxidase for oxygen is very high, $K_m^{O_2} = 0.05\mu M$ (14), and since the cytoplasm of apple cells is restricted to a very thin layer under the cell walls it is unlikely that this level of oxygen will limit cytochrome oxidase. In Fig. 2 it is shown that in 1.5 mm thick sweet potato slices suspended in air, the rate of oxygen uptake is of zero order until the external oxygen concentration is reduced to about 0.4%. It should also be pointed out that the rate of oxygen uptake of sweet potato slices is 6-10 fold higher than that of the five apple cultivars tested. Collectively the above results indicate that the enzyme(s) which is (are) affected by relatively high oxygen concentrations may not be cytochrome oxidase. The nature of this oxidase(s) is not as yet known. However, preliminary calculations indicate that its apparent $K_m^{O_2}$ is 20-40-fold higher than that of cytochrome oxidase (unpublished observations).

Table 7 compares the range of external and internal concentrations of oxygen below which the rate of CO_2 output ceases to decrease and begins to increase. It should be pointed out that the analytical determination of low internal O_2 concentrations in the presence of air is liable to quite appreciable experimental error. However, if the internal concentration is calculated from the diffusivity of O_2 (data not shown), the internal concentration varies between 0.3 to 0.55% for Gala and Stayman apples, respectively. In view of the differences in the rate of O_2 uptake between individual fruits, as well as between cultivars, and of the differences in the percentage of intercellular spaces, it is anticipated that the range of internal O_2 in which the fruits begin to experience anaerobiosis will vary. Nevertheless, our two-year study indicates that at 15° C, the temperature of the experiments, partial anaerobiosis is not expected at external oxygen concentrations above about 1.5 (Table 7).

In summary, the present results indicate that (a) the differences in the external critical concentrations at which the rate of CO_2 begins to

decrease in the five cultivars are mainly due to variations in the diffusivity of O_2 as well as to rate of respiration, and, (b) the system with low affinity for O_2 may not be cytochrome oxidase but another enzyme with an apparent $K_m^{O_2}$ 20-40 fold higher than that of cytochrome oxidase.

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Table 1. Values of diffusivity of CO_2 through the flesh of apple fruits.

Apple	D cm^2/sec
Gala	1.508×10^{-3}
Stayman	0.826×10^{-3}
York Imperial	1.435×10^{-3}

Table 2. Comparison of the calculated ΔC% between the center of the fruit and under the skin with those observed in peeled apples.

Apple Variety	C% Observed	C% Calculated
Gala	0.232	0.215
York Imperial	0.283	0.254
Stayman	0.510	0.580

Table 3. Relationships between external O_2 concentration and rate of CO_2 evolution and diffusivity in Rome Beauty apples.

Gas Phase %O_2	μl/CO_2/g/h	D' cm/sec x 10^{-4}
Air	6.32	.450
18	5.98	.463
15.04	6.46	.495
12.16	6.01	.453
7.9	5.16	.491
4.70	4.69	.488
3.26	3.77	.473
2.20	3.60	.501
0.90	3.57	.444

Table 4. Observed and calculated rates of respiration in Rome Beauty apples at different external O_2 calculations.

% Oxygen	CO_2 µl/g/h Observed	CO_2 µl/g/h Calculated
Air	6.32	6.01
18	5.98	6.04
15.04	6.46	5.87
12.16	6.06	6.01
7.90	5.16	5.00
4.70	4.69	4.62
3.26	3.77	3.78
3.20	3.66	3.20
0.90	3.47	3.17

Table 5. Rate of CO_2 evolution, % internal CO_2 and O_2 concentration, and diffusivity of CO_2 in five apple cultivars.

Apple Variety	External O_2 Concentration %	ul CO_2/ g/h	% Internal Gas Concentration		D'cm/sec x10^{-4}
			CO_2	O_2	
Gala	Air	6.03	0.93	19.76	1.590
York Imperial	Air	10.20	2.60	18.15	.950
McIntosh	Air	5.30	2.08	18.56	.640
Rome Beauty	Air	6.26	3.25	17.17	.469
Stayman	Air	12.30	7.30	13.80	.402

Table 6. Range of external and internal oxygen concentrations over which the rate of CO_2 output begins to decrease.

Variety	% O_2 External	% O_2 Internal
Gala	5.0-6.0	4.6-5.20
McIntosh	5.5-6.7	4.50-5.10
Rome Beauty	7.5-8.0	5.90
Stayman	9-10	4.45-6.0

Table 7. Range of external and internal O_2 concentrations at which the rate of CO_2 output stops falling

Apple Variety	% O_2 Concentration	
	Ambient Atmosphere	Centre Fruit
Gala	~0.6	~.5
York Imperial	0.8	~.300
Rome Beauty	1.25	.476
Stayman	1.50	~.716

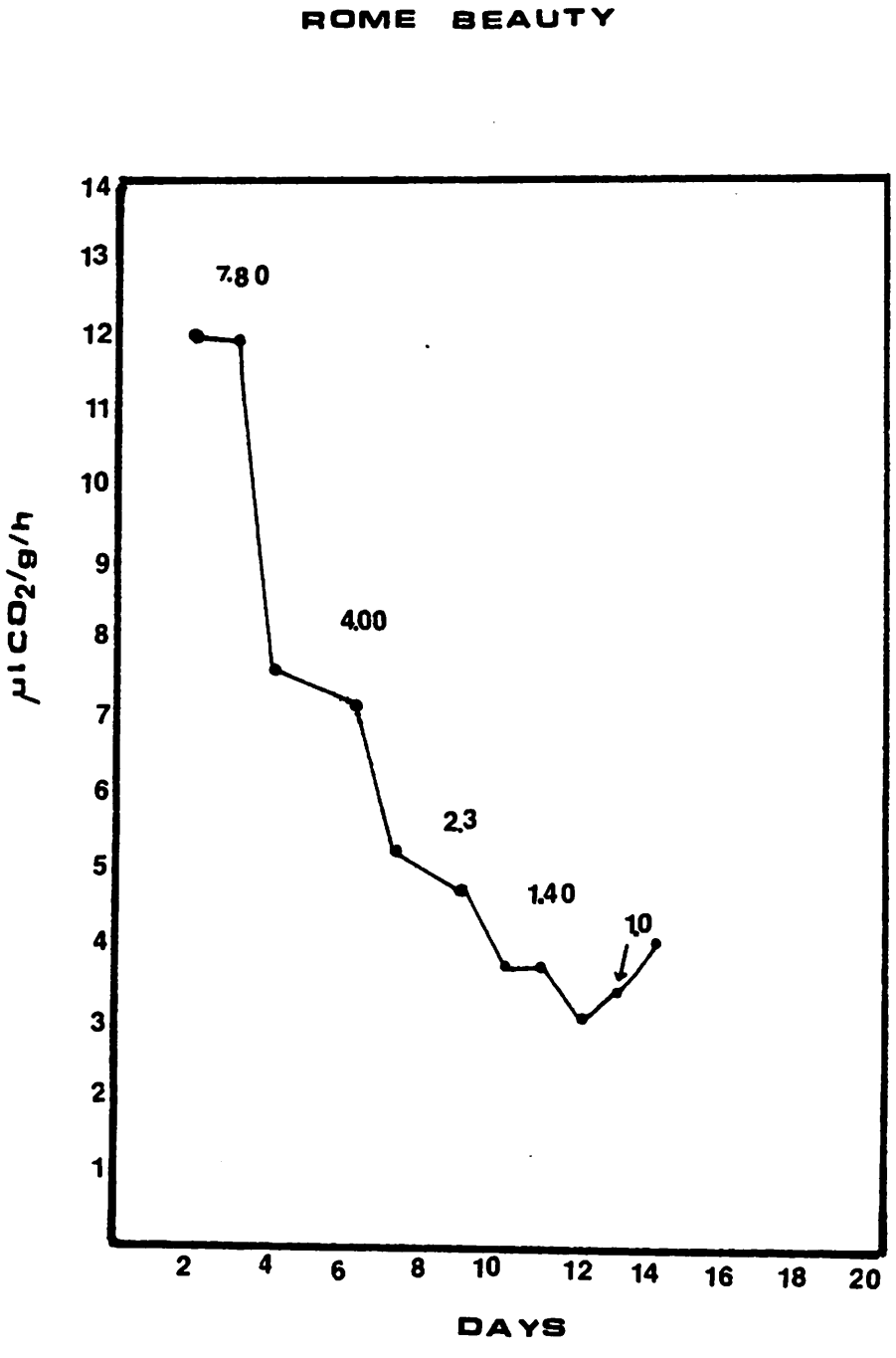

Figure 1. Rate of CO_2 output of Rome Beauty apples as a function of external O_2 concentration.

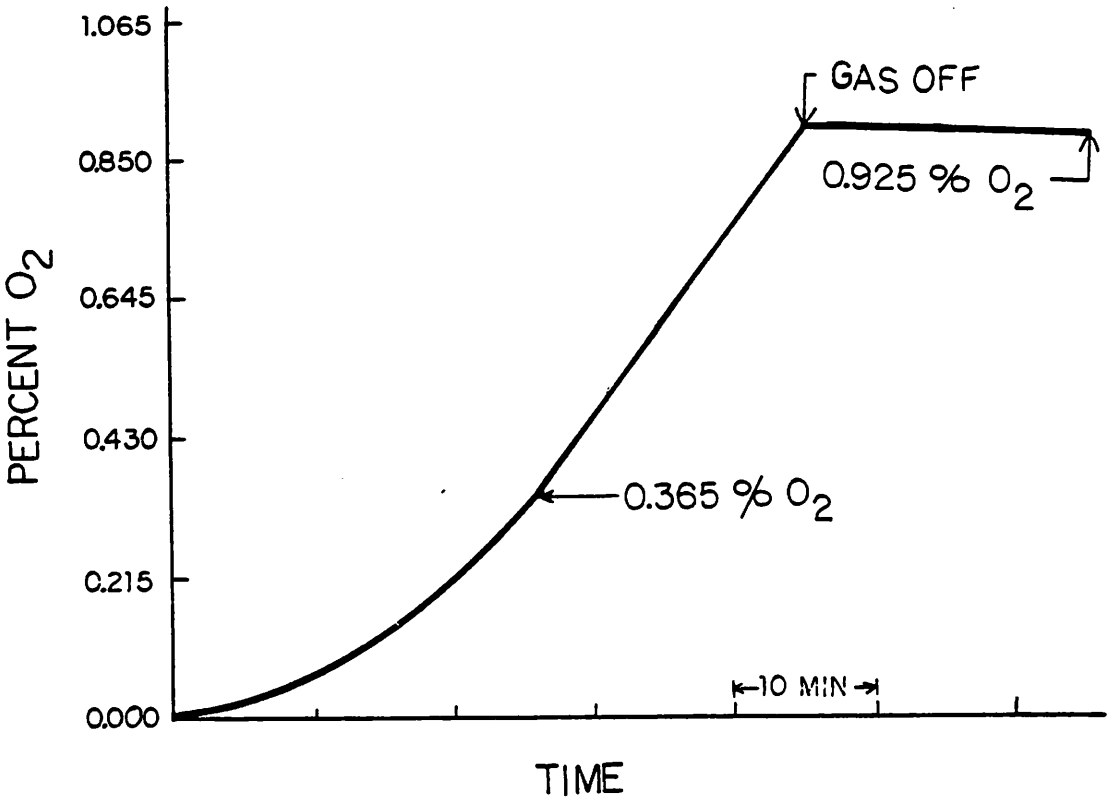

Figure 2. Rate of oxygen uptake in 1.5 mm thick sweet potato slices suspended in air in a closed system. The slices were kept under 0.9% O for 1 hr, and at the arrow the oxygen electrode was sealed air-tight.