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A Tentative Model to Describe the Respiration of Stored Apples

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Abstract. **Using mathematical equations that describe the 0_2 mass-transfer and the enzymatic oxidation of the organic substrates of apples *(Malus domestica* Borkh.), we developed a kinetic model to correlate fruit respiration rate with environmental oxygen partial pressure (PO$_2$. The kinetic determinations were carried out at room temperature using apples stored at 3 to 4C for 11 to 19 weeks. Results show that: 1) the calculated value of the Michaelis-Menten constant related to the enzymatic oxidation of the respiratory substrate ($K_m = 2.1 \pm 0.5.10^{-5}$ mol·kg^{-1}) is close to that reported in the literature for cytochrome-c oxidase; 2) the located range of PO$_2$ levels where 0_2 becomes the limiting factor in the respiration process (near 2.6 kPa at T = 20.5 ± lC) is close to those usually used on a commercial scale for controlled atmosphere storage.**

Although much technical information has been published on the storage of fresh fruits (Biale and Young, 1981; Burton, 1978; Isenberg, 1979; Smock, 1979; Tucker and Laties, 1985), a kinetic model describing the main biochemical changes occurring in the stored fruit as a result of its respiration activity is not available.

From a kinetic point of view, respiration can be simplified into three steps: 1) O_2 mass-transfer between the environmental atmosphere and the cellular solution of the fruits; 2) O_2 use and CO_2 production inside the cells; and 3) the consequent mass-transfer of $C0_2$ from the cellular solution to reach the environmental atmosphere.

The O_2 and CO_2 mass-transfers between the apple and its storage environment have been investigated; the results obtained and the experimental method and hypothesized equations used have been reported (Andrich et al., 1987, 1988, 1989a, 1989b, 1990; Fiorentini et al., 1986). To describe the mass transfer rate of the two gases involved in respiration, the following equation related to the mass-transfer between two heterogeneous phases (gas-and apple) was adopted (Danckwerts, 1970; Sinclair, 1987):

$$V_{mt} = k_{-i}\cdot A\cdot([O_2]_{eq} - [O_2]) = k_{-i}\cdot A\cdot(H\cdot PO_2 - [O_2]) \qquad [1]$$

where: $[O_2]$ = concentration of oxygen present inside 1 kg of apples at a random time t = t (mol·kg^{-1}); k_{-i} = kinetic constant related to the apple-phase (kg·m^{-2}·h^{-1}); A = surface area related to 1 kg of the considered apples (m^2·kg^{-1}); $[O_2]_{eq}$ = concentration of O_2 inside the apples that would be in equilibrium with the partial pressure (PO$_2$) of O_2 present at a random time t = tin the environmental atmosphere (mol·kg^{-1}); H = Henry's constant related to the saturation equilibrium = $[O_2]_{eq}$/ PO$_2$ (mol·kg^{-1}·Pa^{-1}). If the analyzed system has reached the steady state so that the respiration rate equals the rate of mass transfer, Eq. [1] allows us to calculate the amount of O_2 present inside the apple ($[O_2]$). This quantity is closely related to the second step of the hypothesized kinetic model, which concerns the cellular oxidation of the respiration substrates.

If the respiration rate is directly related to the amount of O_2 dissolved in the cellular solution, the Michaelis–Menten equation can be applied and the suitability of the values of the involved constants (Km = Michaelis's constant and k_{res} = kinetic constant) can also be verified.

Materials and Methods

The experimental runs were carried out using 'Golden Delicious' apples, carefully followed during their ripening and harvested at color change (22 weeks from flowering). Only healthy looking apples were collected and stored at 3 to 4C, 85% RH, for 11 to 19 weeks. Beginning the 1lth week of storage, a sample of 13 to 15 healthy apples was taken each week and used for an experimental run carried out at room temperature (20.5 ± 1.OC).

The handling of the fruits and the method used to determine the surface area of the apples have been reported (Andrich et al., 1989a; Fiorentini et al., 1986). The system used to follow the O_2 respired and the CO_2 produced is illustrated in Fig. 1. PO$_2$ in the system was verified by gas chromatography.

The $C0_2$ produced was collected as $BaCO_3$ by continuously washing the gas in the apparatus with an aqueous solution of $Ba(OH)_2$. The $BaCO_3$ precipitated was removed by filtration and the residual concentration of OH^- was evaluated by titration. To maintain a constant gas composition inside the reactor (PO_2 = initial value and $PCO_2 \simeq 0$), the volume of absorbed CO_2 was continuously and automatically replenished by pure O_2, thus allowing the respiration rate of the apples to be calculated. The difference between the CO_2 produced and the O_2 consumed gave a measure of the eventual fermentation activity, a process not detectable at high O_2 concentrations (PO$_2$ > 5 kPa).

The amounts of glucose, fructose, sucrose, glucitol, and malic acid in the fruit were determined using commercial enzyme kits.

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Abbreviations: A, surface area of fruit per unit weight (m^2·kg^{-1}); d, density of fruit (kg·1^{-1}); H, equilibrium constant H involved in mass-transfer of O_2 (mol·kg^{-1}·Pa^{-1}); k_{-i}, kinetic constant involved in mass-transfer of O_2 (kg·m^{-2}·h^{-1}); k_{res}, product of kinetic constant and enzymatic concentration (mol·kg^{-1}·h^{-1}); K_1, equilibrium constant between $[O_2]_{c.s.}$ and $PO_{2(i.s.)}$ (mol·kg^{-1}·Pa^{-1}); K_2, equilibrium constant between $[O_2]$ c.s./$[O_2]$ i.s.; K_m, Michaelis–Menten constant (mol·kg^{-1}); $[O_2]$, concentration of oxygen inside apple at a random time t = t (mol·kg^{-1}); $[O_2]_{c.s.}$, concentration of O_2 dissolved in the cellular soap (mol·kg^{-1}); $[O_2]_{eq}$, concentration of O_2 (mol·kg^{-1}) in apple and in equilibrium with partial pressure of O_2 in the atmosphere; $[O_2]_{i.s.}$, concentration of O_2 present in intercellular space (mol·kg^{-1}); PO$_2$, partial pressure of O_2 in the atmosphere (Pa); $PO_{2(i.s.)}$, partial pressure of O_2 in intercellular space (Pa); R, ideal gas constant (Pa·1·mol^{-1}·K^{-1}); T, temperature (K); $V_{i.s.}$, volume of intercellular space (1); V_{mt}, O_2 mass-transfer rate (mol·kg^{-1}·h^{-1}); V_{res}, respiration rate (mol·kg^{-1}·h^{-1}); w, weight of fruit (kg).

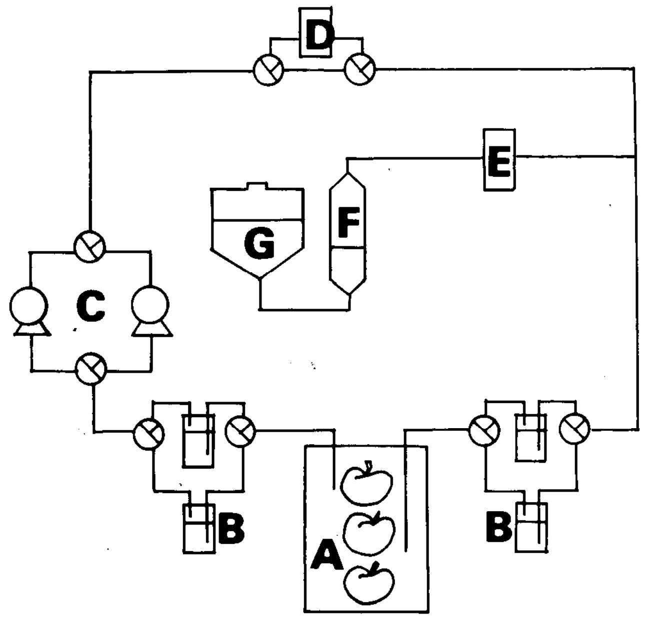

Fig. 1. Simplified scheme of the system used: A = reactor filled with apples; B = traps to collect CO_2 produced; C = circulating pumps; D = gas chromatography; E = one-way valve; F = graduated O_2 tank; G = tank filled with saturated aqueous solution (NaCl).

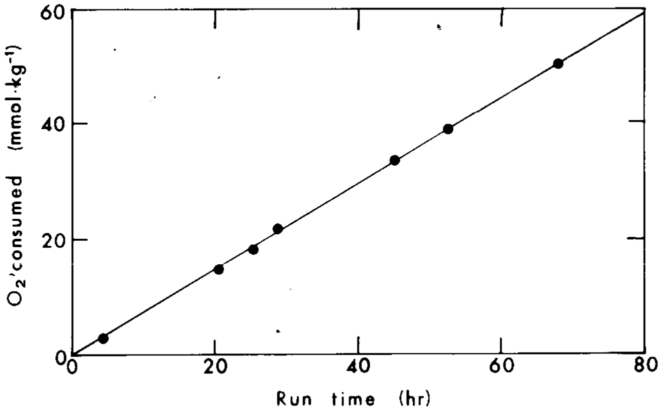

Fig. 2. Oxygen consumed by apples at a constant PO_2 and at $PCO_2 \approx 0$ (T = 20.5C; PO_2 = 16.3 kPa; PN_2 = 83.7 kPa; RH = 85%). The experimental points reflect a random run.

Results

By the linear regression of the experimental data for O_2 consumption, measured at constant PO_2 and at $PCO_2 \approx 0$, as a function of the related run time, it was possible to draw a straight line through the origin of the axes and to obtain a high correlation coefficient (Fig. 2). The slope of this line represents the respiration rate (V_{res}) of the apples under our experimental conditions. The values for V_{res}, determined as a function of the various PO_2 and of the storage time used for the apples are reported in Table 1. The confidence intervals (95%) are a function of the variance connected with the related linear regression (see note to Table 1).

At similar PO_2 levels, results were similar for samples stored for various periods. Under these experimental conditions, therefore, storage time did not seem to significantly influence the respiration rate, as already reported by Knee (1971).

Table 1. Respiration rates (V_{res}) at 21 ± 1C and the related 95% confidence intervals (c.i.) as a function of the PO_2 used and the corresponding storage times (3 to 4C; 85% RH).

Storage time (weeks from harvest)[z]	PO_2 (kPa)	V_{res} (mmol·kg^{-1}·h^{-1})	c.i.[y]
19	1.9	0.28	0.04
19	2.8	0.36	0.03
18	3.2	0.41	0.02
19	3.4	0.43	0.05
17	3.7	0.51	0.06
12	3.8	0.53	0.06
17	3.8	0.45	0.03
12	3.8	0.45	0.05
14	4.3	0.48	0.03
11	4.7	0.47	0.03
18	4.8	0.48	0.06
15	5.1	0.59	0.02
14	6.5	0.55	0.04
11	6.6	0.58	0.06
17	6.6	0.55	0.05
12	7.7	0.53	0.03
17	7.7	0.52	0.02
16	14.2	0.72	0.03
11	15.9	0.74	0.05
16	16.2	0.64	0.06
19	16.3	0.75	0.06
13	16.4	0.69	0.02
18	16.7	0.79	0.07
15	25.1	0.79	0.02
11	26.3	0.63	0.02
15	26.7	0.84	0.05
16	27.3	0.83	0.01
16	27.5	0.74	0.04
13	28.4	0.59	0.04
15	28.4	0.59	0.06

Thirty-ninth week of year.

[y] c. i. = confidence interval of related $V_{res} = t_{0.05} \cdot d_{V_{res}}$ where: $t_{0.05}$ = value tabulated as a function of degree of freedom (df); df = N – 2; N = no. experimental points involved in considered linear regression;

$$d_{V_{res}} = ((N/(N \cdot \sum_{i=1}^{N} X_i^2 - (\sum_{i=1}^{N} X_i)^2)) \cdot (\sum_{i=1}^{N} (Z_i - V_{res} \cdot X_i)^2)/(N-2))^{0.5};$$

X_i = run time related to an experimental determination; Z_i = experimental amount of oxygen respired at considered run time.

When an apple–environment system reaches the steady state, $V_{res} = V_{mt}$ and, knowing the values for the respiration rate (Table 1) and for the parameters k_{-i} and H involved in the mass-transfer Eq. [1], it is possible to calculate the amount of O_2 present inside the apples by:

$$[O_2] = H \cdot PO_2 - V_{res}(k_{-i} \cdot A) \quad [2]$$

where: A = 0.110 ± 0.003 [$m^2 \cdot kg^{-1}$]; k_{-i} = 18.7 ± 1.9 [$kg \cdot h^{-1} \cdot m^{-2}$] (Andrich et al., 1989b); H = 0.14 ± 0.02 [$mmol \cdot kg^{-1} \cdot kP^{-1}$] (Andrich et al., 1989b).

To correlate V_{res} with the O_2 concentration in the cellular solution ($[O_2]_{c.s.}$), the existence of an instantaneous equilibrium between the intercellular O_2 ($[O_2]_{i.s.}$) and $[O_2]_{c.s.}$ has been assumed, in accordance with the hypothesis of our kinetic model (Andrich et al., 1989a). The value of the equilibrium constant K_1 between $[O_2]_{c.s.}$ and the corresponding partial pressure of O_2 in the intercellular space ($PO_{2(i.s.)}T$) can then be calculated if, in accordance with Burton (1974), the cellular solution is assumed to be a 0.4 M sucrose solution:

$$K_1 = [O_2]_{c.s.}/PO_{2(i.s.)} = 1.35 \cdot 10^{-8} (mol \cdot kg^{-1} \cdot Pa^{-1}) \quad [3]$$

Therefore, the K_1 value has been calculated using data on the equilibrium between the O_2 present in the gas phase and that dissolved in a 0.4 M sucrose solution (Burton, 1974).

If one knows the total amount of O_2 present inside 1 kg of apples and the value of the constant K_1 at various environmental PO_2, one can calculate the corresponding amount of O_2 dissolved in the cellular solution. In fact, assuming the intercellular space ($V_{i.s.}$) to be 21% of the total fruit volume (Baumann and Henze, 1983), it is possible to express $IO_2]_{i.s.}$ as a function of $PO_{2(i.s.)}$:

$$[O_2]_{i.s.} = PO_{2(i.s.)} \cdot V_{i.s.}/(w \cdot R \cdot T) = PO_{2(i.s.)} \cdot 0.21/(d \cdot R \cdot T) = 1.02 \cdot 10^{-7} \cdot PO_{2(i.s.)} \quad [4]$$

where: w = weight of apples (kg); R = ideal gas constant ($Pa \cdot liter \cdot mol^{-1} \cdot K^{-1}$); T = temperature (295 K); and d = density of apples ($0.85\ kg \cdot liter^{-1}$).

Combining Eqs. [3] and [4], it is possible to correlate $[O_2]_{c.s.}$ with $[O_2]_{i.s.}$ to obtain the nondimensional constant K_2: $K_2 = [O_2]_{c.s.}/[O_2]_{i.s.} = 1.35 \cdot 10^{-8}/1.02 \cdot 10^{-7} = 0.132$; and as: $[O_2] = [O_2]_{i.s.} + [O_2]_{c.s.}$, the following equation, giving the amount of O_2 dissolved in the cellular solution as a function of the O_2 that has permeated into the fruit, is obtained:

$$[O]_{c.s.} = 0.117 \cdot [O_2] \quad [5]$$

Moreover, the related value of V_{res} being known, it then becomes possible to evaluate directly the amount of $[O_2]_{c.s.}$ as a function of the environmental PO_2:

$$[O_2]_{c.s.} = 0.117 \cdot (H \cdot PO_2 - V_{res}/(k_{-i} \cdot A)) \quad [6]$$

As, under our experimental conditions, the concentrations of the main apple constituents (glucose, fructose, sucrose, glucitol, and malic acid) did not seem to vary with storage time (Table 2) sufficiently to change the total amount of respiratory substrate (assumed to be the molar sum of all the respirable compounds present inside 1 kg of fruit) nor to affect the respiration rate, the-following equation could be adopted (Michaelis–Menten):

$$V_{res} = k_{res} \cdot [O_2]_{c.s.}/(K_m + [O_2]_{c.s.}) \quad [7]$$

where: k_{res} = product of the kinetic constant and the enzymatic concentration corresponding to the maximum respiration rate ($mol \cdot kg^{-1} \cdot h^{-1}$); K_m = Michaelis-Menten constant ($mol \cdot kg^{-1}$).

To determine k_{res} and K_m, the inverse of the respiration rates reported in Table 1 (l/V_{res}) were plotted as a function of the inverse of the related amounts of O_2 dissolved in the cellular solution ($1/[O_2]_{c.s.}$), which were calculated by Eq. [2] (Fig. 3): $1/V_{res} = K_m/k_{res} \cdot 1/[O_2]_{c.s.} + 1/k_{res}$. The slope of the straight line obtained by applying the least squares method to these points gives the $K_m : k_{res}$ ratio, while the intercept with the y axis gives $1/k_{res}$. The value of K_m obtained ($2.1 \pm 0.5.10^{-5}$ $mol \cdot kg^{-1}$) is of the same order of magnitude as that reported by Burton (1974) for cytochrome-c oxidase ($0.35 \cdot 10^{-5}\ mol \cdot kg^{-1}$) and underlines the existence of a great affinity between oxygen and the cellular solution, justifying the low PO_2 used in controlled atmosphere storage.

Table 2. Percentages (means of five determinations ± 1.96 standard deviation) of the main constituents of apples stored under refrigerated conditions for two periods.

	Storage (weeks)^z	
	12	21
Glucose (%)	3.5 ± 0.4	3.1 ± 0.1
Fructose (%)	8.3 ± 0.3	7.8 ± 0.4
Sucrose (%)	2.4 ± 0.2	2.1 ± 0.4
Glucitol (%)	0.7 ± 0.2	0.5 ± 0.1
Malic acid (%)	0.5 ± 0.1	0.7 ± 0.2

^{z}Harvest: 39th week of year.

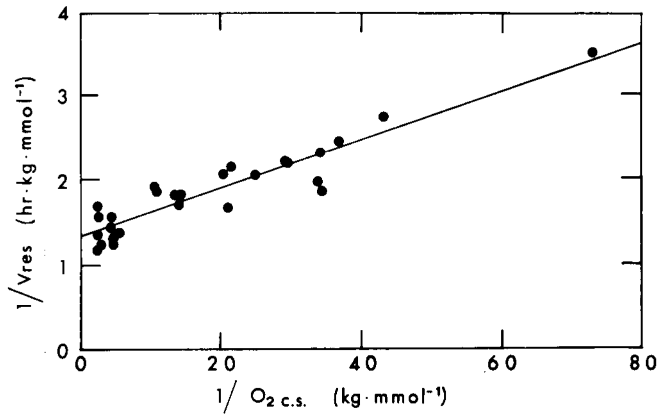

Fig. 3. Linearized form of the Michaelis-Menten equation ($r^2 = 0.86$).

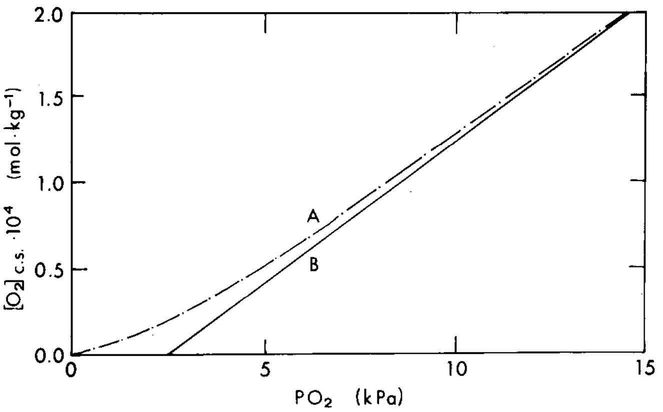

Fig. 4. $[O2]_{c.s.}$ curve as a function of the PO_2, employed (Eq. [2]), assuming the respiration rate (V_{res}): **(A)** constant and equal to the maximum respiration rate (k_{res}); **(B)** variable with PO_2 according to Eq. [7].

To detect the range of PO_2 where O_2 becomes the rate-determining factor, the asymptotic value of the respiration rate (k_{res} = maximum rate = $0.75 \pm 0.06 \cdot 10^{-3}\ mol \cdot kg^{-1} \cdot h^{-1}$) was introduced into Eq. [6]. In this hypothesis, Eq. [6] represents in the plane $PO_2 = [O_2]_{c.s.}$ a straight line whose intercept with the x axis (2.6 kPa) suggests the range of PO_2 where $[O_2]_{c.s.}$ becomes determining for the respiration rate (Fig. 4, line B). This range, evaluated at room temperature, is reasonably close to those usually used on a commercial scale in controlled atmosphere storage. When particularly low PO_2 values are employed ($PO_2 < 3.5$ kPa), the respiration rate greatly differs from the maximum (k_{res}) and the more realistic shape of curve A on Fig. 4 can be calculated by combining Eqs. [6] and [7]. The basic equations and the variables, as well as the values of the constants involved in the hypothesized kinetic model, are summarized in Table 3. When the mass-transfer rate is equal to the respiration rate (steady state condition), the following expression can be obtained: $k_{-i} \cdot A \cdot (H \cdot PO_2 - ([O_2]_{c.s.} + 1/K_2 \cdot [O_2]_{c.s.})) = k_{res} \cdot [O_2]_{c.s.}/(K_m + [O_2]_{c.s.})$. Thus, knowing the value of the constants involved in this expression, it is possible to calculate the amount of oxygen dissolved in the cellular so-

Table 3. Equations, variables, and constants involved in the hypothesized kinetic model.

Basic equations[z]	
1)$V_{mt} = V_{res}$	[at steady state]
2)$V_{mt} = k_{-i} \cdot A \cdot (H \cdot PO_2 - [O_2])$	
3)$[O_2] = [O_2]_{c.s.} + [O_2]_{i.s.}$	
4)$K_2 = [O_2]_{c.s.}/[O_2]_{i.s.}$	
5)$V_{res} = k_{res} \cdot [O_2]_{c.s.}/(K_m + [O_2]_{c.s.})$	
Variables	**Constants**
1)V_{mt}	$k_{-i} = 18.7 \pm 1.9\ [kg \cdot h^{-1} \cdot m^{-2}]$
2)V_{res}	$A = 0.110 \pm 0.003\ [m^2 \cdot kg^{-1}]$
3)PO_2	$H = 0.14 \pm 0.02\ [mmol \cdot kg^{-1} \cdot kPa^{-1}]$
4)$[O_2]$	$K_2 = 0.132$[y]
5)$[O_2]_{c.s.}$	$k_{res} = 0.75 \pm 0.06\ [mmol \cdot kg^{-1} \cdot h^{-1}]$
6)$[O_2]_{i.s.}$	$K_m = 0.021 \pm 0.005\ [mmol \cdot kg^{-1}]$

[z] For the meaning of the symbols, see Abbreviations footnote.

[y] The value of this constant has been calculated on the basis of the data and assumptions reported in literature (Baumann and Henze, 1983; Burton, 1974).

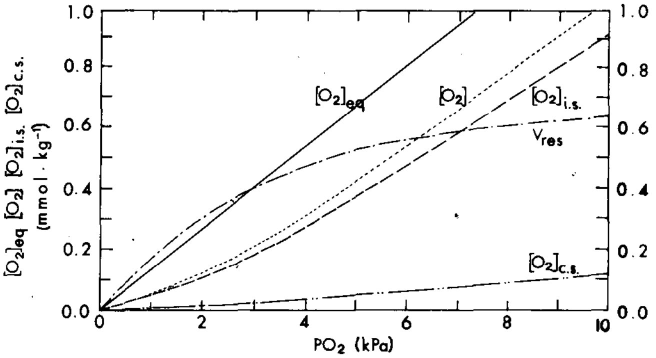

Fig.5. Calculated course of the factors involved inthe hypothesized respiratory model as a function of PO_2 (see equations of Table 3).

lution as a function of the adopted PO_2, by solving the equation: $a \cdot [O_2]^2_{c.s.} + (b - c \cdot PO_2) \cdot [O_2]_{c.s.} - d \cdot PO_2 = 0$, where: $a = k_{-i} \cdot A \cdot (K_2 + 1)/K_2$; $b = k_{-i} \cdot A \cdot K_m \cdot (K_2 + 1)/K_2 + k_{res}$; $c = k_{-i} \cdot A \cdot H$; $d = k_{-i} \cdot A \cdot H \cdot K_m$. As $[O_2]_{c.s.}$ cannot be a negative number, only the following solution can be accepted: $[O_2]_{c.s.} = (-(b - c \cdot PO_2) + ((b - c \cdot PO_2)^2 + 4 \cdot a \cdot d \cdot PO_2)^{0.5})/(2 \cdot a)$. The amount of O_2 dissolved in the cellular solution being known, it is possible to calculate the other variables involved in the adopted kinetic model using the equations given in Table 3.

Figure 5 depicts the theoretical development of V_{res} together with those of the different bases of O_2 involved in the hypothesized respiratory model.

Because of its restricted applicability, this model represents only a preliminary and basic study in a wider research program concerning fruit storage in cold and controlled atmospheres, its aim being to correlate the main quality changes of a fruit with time and storage conditions.

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