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Application of an Enzyme Kinetics Based Respiration Model to Permeable System Experiment of Fresh Produce

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ABSTRACT

An unsteady state method and a steady state method of the permeable system have been developed to measure the respiration rate of fresh produce and to estimate the parameters of enzyme kinetics based respiration model. In the unsteady state method, changes in O_2 and CO_2 concentrations over time inside a permeable package are curve-fitted to appropriate nonlinear equations, respiration rates are calculated by combining the derivatives of the nonlinear equations and the gas permeations through film, and then the parameters of the enzyme kinetics based respiration model are obtained from multiple linear regression of respiration data versus gas composition. In the steady state method, respiration rates are calculated from steady state gas permeations of several packages, and curve-fitting of the data produces the respiration model parameters. The applicability of the two methods was verified successfully using several independent sets of experimental data. The advantage and disadvantage of the methods are discussed.

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NOTATION

$[O_2]$	Oxygen concentration (%)
$[CO_2]$	Carbon dioxide concentration (%)
K_m	Parameter in eqn (7) (% O_2)
K_i	Parameter in eqn (7) (% CO_2)
L	Thickness of polymeric film (mil)
p	Atmosphere pressure (atm)
P_{O_2}	Permeability of film to O_2 (ml mil/m^2 h atm, mmol mil/m^2 h atm or ml mil/in^2 h atm)
P_{CO_2}	Permeability of film to CO_2 (ml mil/m^2 h atm, mmol mil/m^2 atm or ml mil/in^2 h atm)
r	Respiration rate (ml/kg h or mmol/kg h)
r_{O_2}	Respiration rate of oxygen consumption (ml/kg h or mmol/kg h)
r_{CO_2}	Respiration rate of carbon dioxide evolution (ml/kg h or mmol/kg h)
S	Surface area of the package (m^2 or in^2)
t	Time (h)
V	Free package volume (ml)
V_m	Parameter in eqn (7) (ml/kg h or mmol/kg h)
W	Weight of produce in the package (kg)

INTRODUCTION

Modified atmosphere packaging (MAP) is a system in which a modified atmosphere is created and maintained by an intricate interplay between the respiration of the produce and the gas permeation of the package. The respiration rate changes with the atmosphere inside the package, and a knowledge of its dependency on oxygen and carbon dioxide concentrations is required for mathematical modeling of the MAP and for defining the package requirements.

There are three common methods to measure the respiration rate of fresh produce: open system, closed system and permeable system (Lee, 1987). The open system method involves storing the produce in a glass jar that has an inlet port and outlet port through which a pre-mix gas passes. The disadvantage of this method is that it works only with produce of high respiration, and much time and labor are required to obtain data. The closed system method involves monitoring the O_2 and CO_2 concentrations as a function of time inside a closed jar containing the produce. This method is suitable for produce with very low respiration rates, but the data obtained are less accurate than those from the open system method.

The permeable system is similar to the closed system except that a permeable package is used instead of a closed jar. Its advantage is that the experimental conditions are closer to a real package situation, and the time and labor of conducting experiments could be less than for the open and the closed systems (Lee, 1987) providing that the researcher has an intelligent guess of what permeable packages to use. The permeable system is a dynamic system in which the O_2 and CO_2 concentrations change with time before steady state is reached. Both the unsteady state and the steady state

portions could be used to estimate the respiration rate of produce. Recently, Beaudry *et al.* (1992) and Cameron *et al.* (1994) have measured the respiration rate of blueberry, and Talasila *et al.* (1994) have measured the respiration rate of broccoli, all using the steady state portion of the permeable package system. The unsteady state portion of the permeable package is seldom used.

Recently an enzyme kinetics type respiration model has been developed to describe the respiration rate of fresh produce as a function of O_2 and CO_2 concentrations. The model has been shown to be useful for measuring respiration of broccoli (Lee *et al.*, 1991) using the open system method, and respiration of broccoli (Haggar *et al.*, 1992) and blueberry (Song *et al.*, 1992) using the closed system method. However, the applicability of the model to the permeable system has not yet been demonstrated. The objectives of this study are: (1) to develop procedures using the unsteady state or steady state portions of permeable system along with the respiration model to measure and predict respiration of fresh produce; (2) to test the validity of the procedures with several different sets of literature data.

DEVELOPMENT OF PROCEDURE

Two methods — the unsteady state method and steady state method of the permeable system — are developed below to measure and predict respiration rate. The unsteady state method can generate a set of respiration data with only one permeable package, while the steady state method can generate only one datum per package. In practice a combination of the two methods may also be used.

Unsteady state method

This following procedures involve the use of a single permeable package:

Step 1. *Obtain and curve-fit data of O_2 and CO_2 concentrations versus time*
This step involves conducting an experiment similar to that for the closed system (Haggar *et al.*, 1992) except that a fresh produce is placed inside a permeable package instead of an impermeable closed jar. The O_2 and CO_2 compositions in the permeable package are monitored as a function of time with an instrument such as a gas chromatograph. A typical example of the unsteady state data obtained from this experiment is shown in Fig. 1. Before further analysis, the data must first be curve-fitted. The recommended functions for fitting the data are

$$[O_2]=21-A_1[1-e^{-(B_1+C_1t)^{D_1}}] \quad (1)$$

$$[CO_2]=A_2[1-e^{-(B_2+C_2t)^{D_2}}] \quad (2)$$

where A_1, A_2, B_1, B_2, C_1, C_2, D_1 and D_2 are the fitting coefficients. The validity of eqns (1) and (2) should always be confirmed by comparing the fitted values with the experimental data. If the comparison is poor, other forms of functions should be attempted.

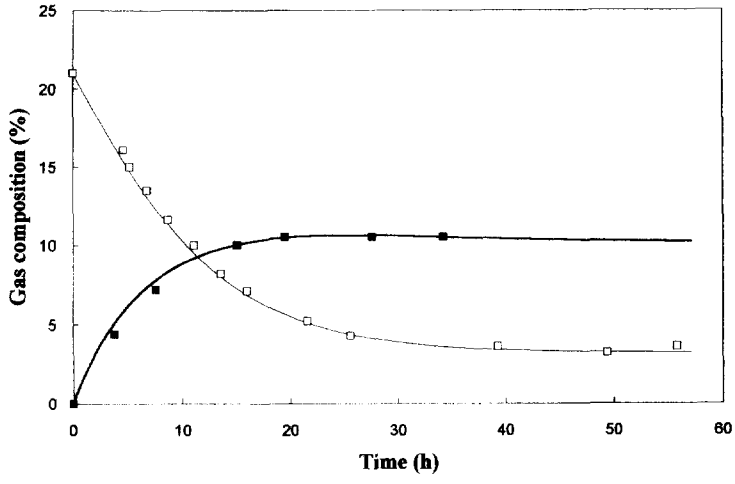

Fig. 1. Permeable system data and predicted gas compositions as a function of time inside VF-71 tomato package of Henig and Gilbert (1975). O_2 and CO_2 permeabilities (P_{O_2}/L and P_{CO_2}/L) of VF-71 film are 0·291 and 1·499 ml/h in^2 atm, respectively. For other package conditions refer to Table 1 [□, O_2 (experimental); ————, O_2 (predicted); ■, CO_2 (experimental); ————, CO_2 (predicted)].

Step 2. *Determine respiration rates*
The respiration rates at those changing O_2 and CO_2 concentrations can be calculated using the equations

$$r_{O_2} = -\frac{d[O_2]}{100\,dt}\left(\frac{V}{W}\right) + \frac{SP_{O_2}(0{\cdot}21 - [O_2]/100)p}{WL} \tag{3}$$

$$r_{CO_2} = \frac{d[CO_2]}{100\,dt}\left(\frac{V}{W}\right) + \frac{SP_{CO_2}([CO_2]/100 - 0{\cdot}00)p}{WL} \tag{4}$$

The first derivatives can be obtained from eqns (1) and (2).

$$\frac{d[O_2]}{dt} = -A_1C_1D_1[(B_1 + C_1t)^{(D_1 - 1)}]\,e^{-(B_1 + C_1t)^{D_1}} \tag{5}$$

$$\frac{d[CO_2]}{dt} = A_2C_2D_2[(B_2 + C_2t)^{(D_2 - 1)}]\,e^{-(B_2 + C_2t)^{D_2}} \tag{6}$$

The negative sign in eqn (5) signifies that the O_2 concentration in the permeable package decreases with time.

Step 3. *Estimate respiration model parameters*
The respiration data obtained from Step 2 are used to estimate the parameters of the enzyme kinetics type respiration model of Lee *et al.* (1991).

$$r=\frac{V_m[O_2]}{K_m+(1+[CO_2]/K_i)\,[O_2]} \tag{7}$$

Two sets of parameters (V_m, K_m and K_i) are obtained: one for O_2 consumption and the other for CO_2 evolution. The parameters can be estimated from the linearized form of eqn (7)

$$\frac{1}{r}=\frac{1}{V_m}+\frac{K_m}{V_m}\frac{1}{[O_2]}+\frac{1}{K_iV_m}[CO_2] \tag{8}$$

using multiple linear regression analysis. The parameter estimates must be verified by comparing the model predictions with the experimental data.

Steady state method

This method requires several (at least three) permeable packages, and the procedure are described as follows:

Step 1. *Obtain steady state O_2 and CO_2 compositions*
The experiments involve storing several permeable packages in a temperature controlled environment and allowing the O_2 and CO_2 compositions inside the packages to reach steady states. The steady state condition is unique for each package, which can be manipulated by the design variables of produce weight, package dimension, package permeability, temperature, etc. For example, Fig. 4 (later) shows a set of steady state gas compositions obtained by using temperature and sample weight as design variables.

Step 2. *Determine respiration rates*
The respiration rates of O_2 consumption and CO_2 evolution can be obtained from the following equations:

$$r_{O_2}=\frac{SP_{O_2}(0{\cdot}21-[O_2]/100)p}{WL} \tag{9}$$

$$r_{CO_2}=\frac{SP_{CO_2}([CO_2]/100-0{\cdot}00)p}{WL} \tag{10}$$

The above equations imply that the rate of respiration of the produce is equal to the rate of permeation of the package when steady state is reached.

Step 3. *Estimate the respiration model parameters*
This step is the same as Step 3 of the unsteady state method.

RESULTS AND DISCUSSION

Verification of unsteady state method

Two sets of experimental data, that of Henig and Gilbert (1975) and that of Lee *et al.* (1991), were used to verify the unsteady state method.

1. *Data of Henig and Gilbert (1975)*

The experiments were conducted by storing tomatoes inside two permeable packages in which the changes in O_2 and CO_2 concentrations were monitored as a function of time. The two permeable packages had the same dimension but different permeabilities. To demonstrate the applicability of the procedures, model parameter values were obtained from the data of the first package (Fig. 1), and they were used to predict and compare the experimental gas compositions of the second package (Fig. 2).

The data of the first package up to 34 h were curve-fitted using eqns (1) and (2), and the estimated values of the coefficients along with other pertinent information are shown in Table 1. The coefficients of determination r^2 were 1·000 and the fitted curves virtually coincided with experimental data (not shown). Equations (1) and (2) were utilized for supplying both O_2 and CO_2 concentrations at the same sampling times in Fig. 1. Respiration rates were obtained using eqns (3)–(6). Multiple linear regression analysis was performed using eqn (8) to obtain estimates of respiration model parameters (Table 2). The respiration rate data at both initial and final sampling times were excluded in the regression because the derivatives of eqns (5) and (6) were obtained from extrapolation and magnified the errors in the estimation. The r^2 of regression was greater than 0·99.

Based on the authors' experience, the estimated values for V_m are always positive, and in most cases, the values for K_m and K_i are also positive (Lee *et al.*, 1991; Haggar *et al.*, 1992; Song *et al.*, 1992). However, oxygen consumption shows a negative value of −14·7 for K_i in Table 2. It is noted

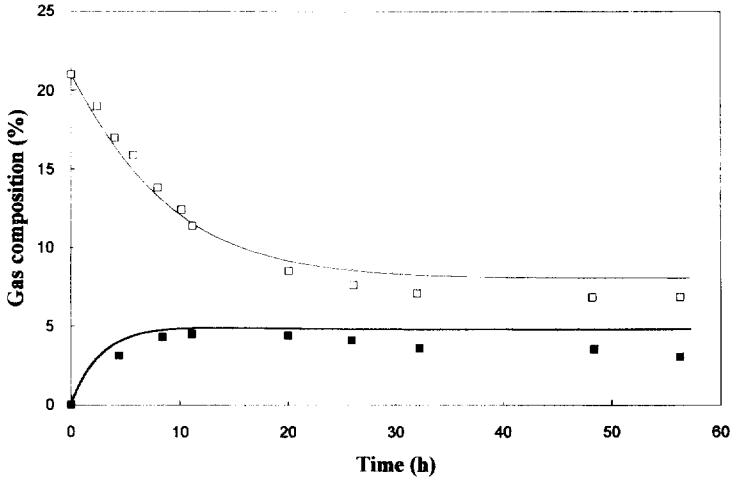

Fig. 2. Experimental versus predicted gas compositions as a function of time inside RMF-61 package of Henig and Gilbert (1975) containing 0·47 kg tomatoes. O_2 and CO_2 permeabilities (P_{O_2}/L and P_{CO_2}/L) of RMF-61 film are 0·875 and 4·505 ml/h in^2 atm, respectively. Film area and free volume are 53 in^2 and 843 ml, respectively [□, O_2 (experimental); ———, O_2 (predicted); ■, CO_2 (experimental); ———, CO_2 (predicted)].

TABLE 1
Estimated Coefficients for Eqns (1) and (2) Describing the O_2 and CO_2 Change Curves for Permeable Packages Containing Produces

Commodity and source of experimental data	*Sample weight (kg)*	*Film area*	*Free volume (ml)*	*Coefficients*			
				A_1 and (A_2)	*B_1 and (B_2)*	*C_1 and (C_2)*	*D_1 and (D_2)*
Tomato, Fig. 1 (Henig & Gilbert, 1975)	0·47	53 in^2	843	17·634 (10·603)	0·0013016 (0·010259)	0·090314 (0·15043)	1·2234 (1·2132)
Cut broccoli, Fig. 3 (Lee *et al.*, 1991)	0·1373	0·072 m^2	659	24·386 (17·392)	0·0081422 (0·0023806)	0·25064 (0·25349)	1·2744 (1·1843)

TABLE 2
Respiration Model Parameters Obtained in Eqn (7). For Cut Broccoli, the Parameters were Compared between the Open System of Lee *et al.* (1991) and the Permeable System Method

Commodity and and source of experimental data	*Respiration expression*	*V_m (ml/kg h)*	*K_m (% O_2)*	*K_i (% CO_2)*	*Method*
Tomato, Fig. 1 (Henig & Gilbert, 1975)	O_2 consumption	53·1	27·9	−14·7	Permeable system
	CO_2 evolution	36·1	1·5	16·0	
Cut broccoli, Table 3 (Lee *et al.*, 1991)	O_2 consumption	219·4	1·4	114·7	Open system
	CO_2 evolution	191·1	1·0	42·3	
Cut broccoli, Fig. 3 (Lee *et al.*, 1991)	O_2 consumption	276·1	2·5	26·8	Permeable system
	CO_2 evolution	206·7	1·6	21·2	

that oxygen consumption has a relatively high value of 27·9 for K_m. This set of parameters, K_m and K_i, means that CO_2 concentration has little effect on oxygen consumption rate, especially at low O_2 or CO_2 concentrations. Hayakawa *et al.* (1975) assumed negligible dependence of O_2 consumption rate on CO_2 concentration for tomato. Even though the respiration model is based on principles of enzyme kinetics, the parameters in Table 2 should be interpreted as empirical ones describing respiration phenomenally rather than theoretical ones having physical meaning for the enzymatic reaction mechanism.

The respiration values obtained from eqn (7) and Table 2 were compared

to the calculated data obtained from eqns (3)–(6), and good agreement was found between the two (not shown). The parameters obtained from the first permeable package data (Table 2) could simulate the gas concentration in the same permeable package of tomatoes, when incorporated into the solutions of differential eqns (11) and (12) proposed by Hayakawa *et al.* (1975) (Fig. 1).

$$\frac{d[O_2]}{dt} = 100\left\{\frac{SP_{O_2}(0{\cdot}21 - [O_2]/100)p}{VL} - \frac{Wr_{O_2}}{V}\right\} \tag{11}$$

$$\frac{d[CO_2]}{dt} = 100\left\{\frac{SP_{CO_2}(0{\cdot}00 - [CO_2]/100)p}{VL} + \frac{Wr_{CO_2}}{V}\right\} \tag{12}$$

where r_{O_2} and r_{CO_2} were calculated by using eqn (7). This cyclic process of obtaining the parameters from a permeable package, predicting respiration rates and simulating the gas compositional change in the same package satisfies a necessary requirement for verifying the model parameters.

To provide a sufficient condition for verification, the estimated parameter values obtained above should allow the prediction of the gas composition history for the second package. The gas composition history for the second package was simulated using the estimated parameter values and the differential eqns (11) and (12). The good agreement between the predicted and the experimental data (Fig. 2) verifies the parameter estimation.

2. *Data of Lee* et al. (1991)

The above procedures were also applied to the data of Lee *et al.* (1991) for a permeable package containing cut broccoli (Fig. 3 and Table 1). The estimated parameter values (Table 2) were used to simulate gas composition changes in the same package, resulting in excellent agreement between calculated and experimental data (Fig. 3).

Lee *et al.* (1991) also estimated the model parameter values for broccoli using the open system. In general, the V_m and K_m values for broccoli obtained from the two methods were in good agreement (Table 2). The larger difference in K_i values might be attributed to experimental errors, errors in regression analysis, and difference in the two methods.

The parameter values from the two methods were used to calculate the respiration rates of broccoli as a function of O_2 and CO_2, and the results were compared to the experimental values of the open system (Table 3). The permeable system had average deviations of 17·6 and 13·8 ml/kg h for O_2 consumption and CO_2 evolution, respectively, and the open system had average deviations of 15·6 and 10·4 ml/kg h. Although the deviations for the open system appeared to be smaller, it should be noted that the parameters for the open system were calculated from the same source of experimental data. Despite experimental and regression analysis errors, the permeable system was able to provide reasonably good results.

Verification of steady state method

The data of Beaudry *et al.* (1992) were used to verify the steady state

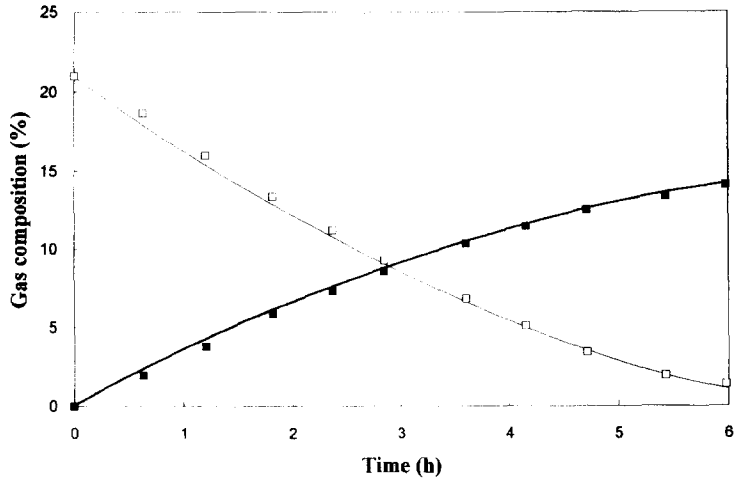

Fig. 3. Permeable system data and predicted gas composition as a function of time inside 2 mil low density polyethylene package of cut broccoli of Lee *et al.* (1991). O_2 and CO_2 permeabilities (P_{O_2} and P_{CO_2}) of the film are 198·5 and 696·0 ml mil/h m^2 atm, respectively. For other package conditions refer to Table 1 [□, O_2 (experimental); ————, O_2 (predicted); ■, CO_2 (experimental); ————, CO_2 (predicted)].

method. Their experiments involved measuring the steady state O_2 and CO_2 concentrations inside 2 mil thick permeable packages (400 cm^2 surface area) containing blueberries. The design variables were weight of the blueberry and temperature, and three sets of their data are shown in Fig. 4. The data were analyzed to obtain respiration rates and model parameter values according to the procedures described earlier. Only aerobic respiration data with a respiratory quotient below 1·3 were used in the analysis because eqn (7) is valid only for aerobic respiration (Lee *et al.*, 1991).

As shown in Table 4, all the V_m values and most of K_m and K_i are positive, as observed earlier. The negative K_i values at 5°C and 10°C cannot be explained from enzyme kinetics theory. The problem is likely due to errors introduced by curve-fitting of eqn (8). A slope value of $1/K_iV_m$ close to zero (positive or negative) will be obtained with inclusion of curve-fitting error when CO_2 concentration has little effect on the respiration. In this case large negative or positive values of K_i will be obtained. According to eqn (7), the respiration rates are not affected by CO_2 concentration if the absolute value of K_i is large. This phenomenon was observed for the data between 0 and 10°C. The small negative value of K_m for CO_2 production at 20°C is understood as a value close to zero (Song *et al.*, 1992).

To test the validity of the parameters obtained, the enzyme kinetics respiration model of eqn (7) was substituted into steady state equations (9) and (10), and equilibrium in-package gas compositions were obtained by solving numerically these two simultaneous equations. Figure 4 shows that the calculated and the experimental data are in good agreement.

TABLE 3
Comparison between Calculated Respiration Rates and Experimental Data for Cut Broccoli of Lee *et al.* (1991)

Gas composition		*Respiration rates*					
		O_2 consumption rate (ml/kg h)			*CO_2 evolution rate (ml/kg h)*		
O_2 (%)	*CO_2 (%)*	*Calculated, permeable system*	*Calculated, open system*	*Experimental, open system*	*Calculated, permeable system*	*Calculated, open system*	*Experimental, open system*
3·4	0·9	155·5	155·5	140·6	137·1	145·3	141·1
3·4	4·6	144·3	151·1	148·2	122·9	136·2	121·4
3·5	7·7	137·5	149·5	155·3	113·9	130·2	132·7
2·6	10·3	117·3	134·8	141·0	98·8	117·4	128·7
8·9	1·1	208·5	188·0	214·3	168·1	167·9	182·4
9·6	4·4	193·5	185·3	181·7	150·7	158·2	151·0
9·6	7·9	177·3	180·6	173·6	134·5	148·0	137·0
9·4	12·1	160·6	174·9	151·4	118·9	137·2	131·2
16·0	1·0	231·1	200·1	191·0	180·4	175·9	199·1
16·5	4·2	210·8	195·6	232·4	159·8	164·8	178·5
16·2	7·3	193·3	190·8	222·8	143·4	154·8	158·0
16·5	11·2	175·8	185·5	205·7	127·3	144·2	157·6
Average deviation from experimental data		17·6	15·6	0	13·8	10·4	0

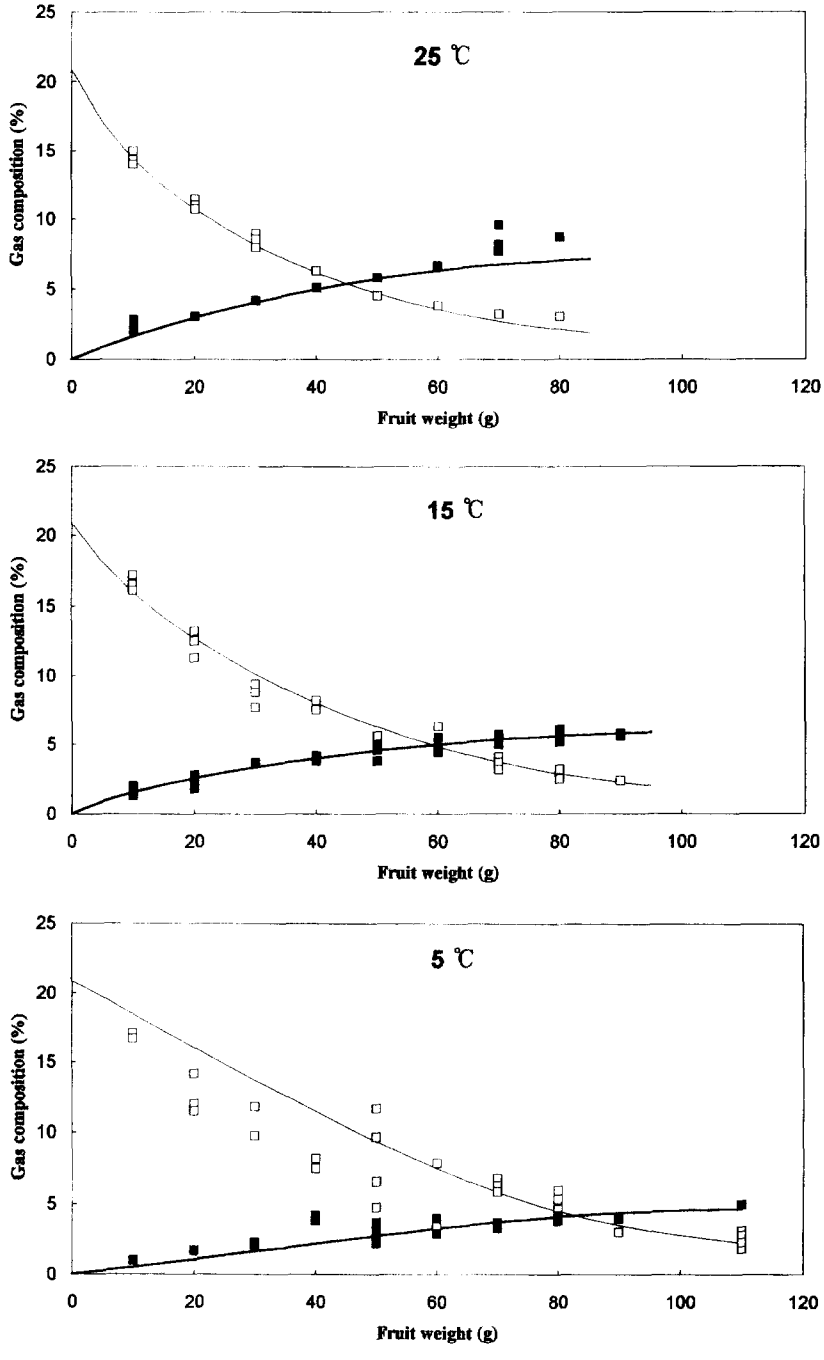

Fig. 4. Steady state O_2 and CO_2 concentrations of 400 cm^2 LDPE permeable packages of Beaudry *et al.* (1992) compared with calculated values. Thickness of the film is 2 mil [□, O_2 (experimental); ————, O_2 (predicted); ■, CO_2 (experimental); ————, CO_2 (predicted)].

TABLE 4
Respiration Model Parameters in Eqn (7) for Blueberry, Obtained from Equilibrated Gas Compositions of Permeable Packages (Beaudry *et al.*, 1992)

Temperature (°C)	*Respiration expression*	V_m *(mmol/kg h)*	K_m *(% O_2)*	K_i *(% CO_2)*	r^2
0	O_2 consumption	0·121	0·390	17·042	0·968
	CO_2 evolution	0·131	0·029	96·682	0·468
5	O_2 consumption	0·171	1·197	117·84	0·584
	CO_2 evolution	0·173	1·064	−21·913	0·678
10	O_2 consumption	0·320	3·662	−11·593	0·803
	CO_2 evolution	0·462	2·191	−174·799	0·886
15	O_2 consumption	0·950	2·875	3·457	0·866
	CO_2 evolution	1·425	2·848	3·244	0·931
20	O_2 consumption	1·521	0·979	2·502	0·992
	CO_2 evolution	1·524	−0·720	3·971	0·867
25	O_2 consumption	2·364	3·664	3·198	0·985
	CO_2 evolution	1·817	1·676	14·250	0·728

Other advantages and disadvantages of permeable system methods

The permeable system has at least two advantages. First, it can provide both unsteady and steady state data, while the closed system can provide only unsteady state data and the open system can provide only steady state data. Second, the unsteady state method of the permeable system can more closely simulate the real package than the closed system. Haggar *et al.* (1992) have mentioned that it is usually difficult for the researcher to manipulate the transit gas composition history of a closed system experiment to closely match that of the real package. This situation will create the problem that the model parameter values obtained from the closed system may not be good estimates for the real package. This problem can be alleviated somewhat with the use of the unsteady state method of the permeable system.

The permeable system also has disadvantages. Since each permeable package could have different permeability, surface area and free volume, there are more chances for errors due to measurements being introduced in the calculations. Compared to the open system, the steady state method of the permeable system requires longer time to reach a steady state condition. Sometimes wide variations in the data such as those of Beaudry *et al.* (1992) and Joles *et al.* (1994) are obtained because of the difficulty of accurately determining the steady state gas concentrations.

The permeable system is a better choice than the open system or the closed system, if the researcher has a good guess of what permeable packages are suitable for a product of interest.

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