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Respiration Rate Modelling of Royal Delicious Apple at Different Storage Temperatures

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ABSTRACT

Temperature is the most important factor that controls the rate of respiration of fruits and vegetables. Several experiments were conducted at different storage temperatures to generate respiration data using a closed system method for respiration measurement. Two different models based on regression analysis and enzyme kinetics were developed. In the model based on enzyme kinetics the Arrhenius equation was proposed for predicting the respiration rates of Royal Delicious apple as a function of O_2 and CO_2 concentrations and storage temperature. In the enzyme kinetics model, the dependence of respiration rate on O_2 and CO_2 was found to follow an uncompetitive inhibition. The regression coefficient values were used for the prediction of respiration rates using regression model. The enzyme kinetic model parameters, calculated from the respiration rate at different O_2 and CO_2 concentrations were used to fit the Arrhenius equation against different storage temperatures. The activation energy and respiration pre-exponential factor were used to predict the model parameters of enzyme kinetics at any storage temperature between 0-30°C. The developed models were verified for goodness of fit at 12°C and found to be in close agreement with the experimentally estimated respiration rates.

Keywords: enzyme kinetics, fruit maturity, fruit ripening, gas analysis, regression analysis
Abbreviations: CA, controlled atmosphere; **MA**, modified atmosphere

INTRODUCTION

Apple (*Malus pumila* Mill.) is native of eastern Europe and Western Asia and has been cultivated from pre-historic times. It is a highly remunerative deciduous fruit grown in temperate region (Kaushal and Sharma 1995). Scandinavians considered apple as the food of gods. It is a fleshy fruit with tough skin, ranging in color from greenish yellow to red (Prasad 1995; Mahajan 2001). The world production of apple is presently about 45 million tones, 71% of which is consumed as fresh apple while about 20% is processed into value added products of which 65% are processed into Apple Juice Concentrate (AJC) and the balance quantity into other products which include packed natural RTS apple juice, apple wine and cider, apple purees and jams, dried apple products, etc. (http://www.geocities.com/perfectapple/prod.html; http://mofpi.nic.in/apple.pdf). The leading apple growing country is China, producing about 41% of the world's apples, followed by the United States, Turkey, France, Poland, Italy, the Russian Federation, Germany, Argentina, India, Japan and Chile. Even warmer countries like Iraq and Mexico are able to grow apples in their cooler upland regions (Kaushal and Sharma 1995; http://www.geocities.com/perfectapple/prod.html). The production of apple in China is 20 million tonnes which is equivalent to the combined production of 9 top producing countries of the world. China and Poland are the two largest apple exporting countries with each of them exporting about 180,000 MT per year while USA and Germany are the two largest importers of AJC with an import of 300,000 tonnes of each, besides being very large producers themselves which indicate that their per capita consumption of apple juice is very large (http://mofpi.nic.in/apple.pdf).

Apple was introduced to India by the British in Kullu Valley of the Himalayan State of Himachal Pradesh (H.P.) as far back as 1865, while the colored delicious cultivars of apple were introduced to the Shimla Hills of the same state in 1917 (Ghosh 1999). India is the ninth largest producer of fresh apple in the world with a production quantity of about 1.30 million tonnes with the cultivated area of 0.24 million ha, but produces only 4500 tonnes per annum of AJC which is equivalent to about 0.64% of the total world production (http://mofpi.nic.in/apple.pdf; http://www.applejournal.com/India001.htm). It is the 7th most widely grown fruit crop in India (http://agricoop.nic.in/hort/hortrevo5.htm). The apple fruits are grown in the North-Western Indian States of Jammu and Kashmir (J&K), Himachal Pradesh (H.P.), Uttar Pradesh (U.P.) hills and in the north eastern hill regions in the states of Arunachal Pradesh, Nagaland, Meghalaya and Manipur (Chadha 1978; Ghosh 1999). J&K is leading in the production and productivity of apple in India followed by H.P. and U.P. (Ghosh 1999).

The delicious group of cultivars predominates the apple market. The areas covered under the delicious cultivars of apple in India are 83% of the area under apple in H.P., 45% in J&K and 30% in U.P. hills (Ghosh 1999). The most popular varieties of apple grown in India are Royal Delicious, Red Delicious, Golden Delicious, Rich Red, Red Gold, Granny Smith, Macintosh, Benoni, Irish Peach, Cox's Orange Pipin, Ambri, American Apirouge, Tydemans's Early, Mollies Delicious, Red Spur, Top Red, Red Chief, Early Shanburry, Chaubattia Princes, Rymer and Buckingham etc. (Ghosh 1999; Mangaraj and Varshney 2006). The apples are graded into seven grades on the basis of size and color. The size parameter is equatorial diameter (E.D.) of fruit. The seven grades includes super large (E.D. = 8.5 cm), extra large (8 cm), large (7.5 cm), medium (7 cm), small (6.5 cm), extra small (6 cm) and pitto (5.5 cm), etc. (Kaushal and Anand 1986; Varshney *et al*. 2002).

Royal Delicious apple (50-60%) and Red Delicious apples (20-30%) account for 70-80% of the total production of apple in India. Both the varieties of apple have delicious

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flavors, goodness, attractive color, sweet delicious taste and great market demand. The distinguish features of these varieties are: (i) the red delicious apple are long, conical shape and light red color whereas the Royal Delicious are large and conical; (ii) the red delicious apple have yellow skin covered with red strip but not all over the surface, however, the Royal Delicious have yellow skin covered with red strips all over the surface; (iii) the red delicious apple have determined calyx lobes but the Royal Delicious have not prominent calyx bobes (Varshney *et al*. 2002); (iv) the red delicious apple can be stored at room temperature for 25-30 days but the Royal Delicious apple's storage life is shorter, i.e. around 20 days; (v) the keeping quality of red delicious apple in ambient storage is better than the Royal Delicious apple etc. (Singh *et al*. 1978; Ingale and D'Souza 1989). India presently exports 7000 tons of apples, which is 6% of total fresh fruit export, mostly to Bangladesh and Sri Lanka. Apple like royal and red delicious, Ambri and other new colored cultivar are suitable for the export market (Ghosh 1999). The Royal Delicious apples contribute maximum percentage in total production, high quality and export oriented. However the storage period and keeping quality is not of that standard. Hence it prompted us for the respiration kinetic study of Royal Delicious apple at different temperatures for its suitability for design of modified and control atmosphere storage system.

Storage of agricultural commodities such as Royal Delicious apple has many aims, but the main objective is to prolong their availability on the market throughout the year. Certain post harvest constraints like shelf life; susceptibility to many diseases and pest; faster fruit ripening at warmer temperatures etc. limits its storage condition (Kaul and Gupta 1987; Kaushal and Sharma 1995). Apple Scab is considered the most dreaded diseases of apple and is reported from almost all the apple growing regions of the world (Gupta 1987). Decay and disorders such as Jonathan spot and bitter pit are worst at storage temperatures higher than –1 to 0°C (Kaushal and Sharma 1995). For most apple cultivars, the optimum storage temperature is –1 to 0°C with 90-95% relative humidity. For a cultivar such as delicious, storage at –1°C will give approximately 25% longer storage life than at 0°C (Kaushal and Sharma 1999). Some apples often develop soft scald in regular cold storage at 0°C, and hence, they should be stored at 2-30°C. Yellow Newton apples grown in California often develop internal browning when stored at 30°C; their optimum storage period is 3-4°C. Storage of Jonathan apples in control atmosphere storage at 0°C provides good control of soft scald and Jonathan spot (Kaushal and Sharma 1995). Many researchers have studied the influence of altered atmosphere on the commodity storage life. Two main types of altered atmosphere are modified and controlled atmosphere (MA and CA). The use of these methods provides some control of fruit and vegetable ripening, retardation of senescence, or browning in cut produce (Stiles 1991) and ultimately prolongs the shelf life. Depending on the specific commodity needs, MA and CA can prolong market availability of the produce up to the next harvest.

Fruit transpiration and respiration are affected by atmospheric composition and temperature (Fonseca *et al*. 2002). Temperature has been identified as the most important external factor influencing respiration. Biological reactions generally increase two to three times for every 10°C rise in temperature within the range of temperature normally encountered in distribution and marketing chain (Burzo 1980; Zagory and Kader 1988; Kader *et al*. 1989; Irtwange 2006). At higher temperatures, enzymatic denaturation may occur and reduce respiration rates. If temperatures are too low, physiological injury may occur, which may lead to an increase in respiration rates (Fidler and North 1967). At lower temperature the respiration rates is considerably less and the shelf life can be increased. Hence for prolonging the shelf-life of agricultural commodity, appropriate temperature control is the key to the design of any storage system. Also, accurate measurement and modeling of respiration rates is central to the design of MA/CA for agricultural commodity (Kader *et al*. 1989; Mahajan 2001; Fonseca *et al*. 2002).

Respiration is a metabolic process that provides the energy for plant biochemical processes. Various substrates used in important synthetic metabolic pathways in the plant are formed during respiration (Meyer *et al*. 1973). Aerobic respiration consists of oxidative breakdown of organic reserves to simpler molecules, including carbon dioxide (CO_2) and water, with release of energy. The organic substrates broken down in this process may include carbohydrates, lipids, and organic acids. The process consumes oxygen (O_2) in a series of enzymatic reactions. Due to these metabolic processes in fruits, which continue even after harvest, the shelf life of the product is reduced. However, the rates of these metabolic processes are generally directly proportional to the storage temperature, thus increasing the shelf life to a certain level at lower temperatures.

Measurement of respiration rate of produce at a particular storage temperature and gas composition is time consuming and needs special equipments for gas analysis. Various mathematical models have been developed to correlate the respiration rate with different storage parameters such as gas composition i.e. O_2 and CO_2 and temperature. Yang and Chinnan (1988) studied the influence of O_2 and CO_2 concentrations and storage time on the respiration rates of tomato. The effect of temperature on respiration rate was not considered in their models. The models adequately predicted the respiration rate of tomatoes stored in a controlled atmosphere. Lee *et al*. (1991) suggested an enzyme kinetics theory for modeling of respiration rate. Cameron *et al*. (1989) used an empirical approach to measure the respiration rate as a function of O_2 concentration by observing the rate of O_2 depletion in a closed system. Talasila *et al*. (1992) developed a non-linear empirical model for predicting the respiration rate of strawberry as a function of temperature, O_2 and CO_2 concentration. However, due to the specificity of the model parameters, the same need to be identified and quantified for each fruit and vegetable product. Keeping in view the aforesaid information, the objective of the present work was to determine the respiration rate of Royal Delicious apple at different temperatures by close system respirometer study and to develop and test suitable mathematical models to predict the respiration rate of apple as a function of O_2 and CO_2 concentrations and the storage temperature.

MATERIALS AND METHODS

Fruit material

Fresh mature apple fruit of local popular variety Royal Delicious was obtained from an orchard at Kulu (Himachal Pradesh), India. Fruits were washed to remove adhering dirt, and used for the study. Care was taken to ensure that the fruits were of uniform size and weight.

Physico-chemical properties of fruits

As practiced in most of the orchards in developing countries like India, fruit maturity is assessed by an experienced orchard person, subjectively (Mahajan 2001). Nevertheless, the physical and chemical properties were also determined objectively in the laboratory prior to the respiration rate study. Initial apple fruit color and firmness was determined using Hunter Lab Colorimeter and Texture analyzer respectively (Mahajan 2001). The weight of apple fruits was measured using a precision balance (Essae Teraoka, Japan) having an accuracy of 0.01 g. True volume, ascorbic acid, total soluble solids and titrable acidity of apple fruits were determined as per the methods described by Ranganna (1995). All the physical and chemical properties were determined for 10 representative samples and the average values were presented.

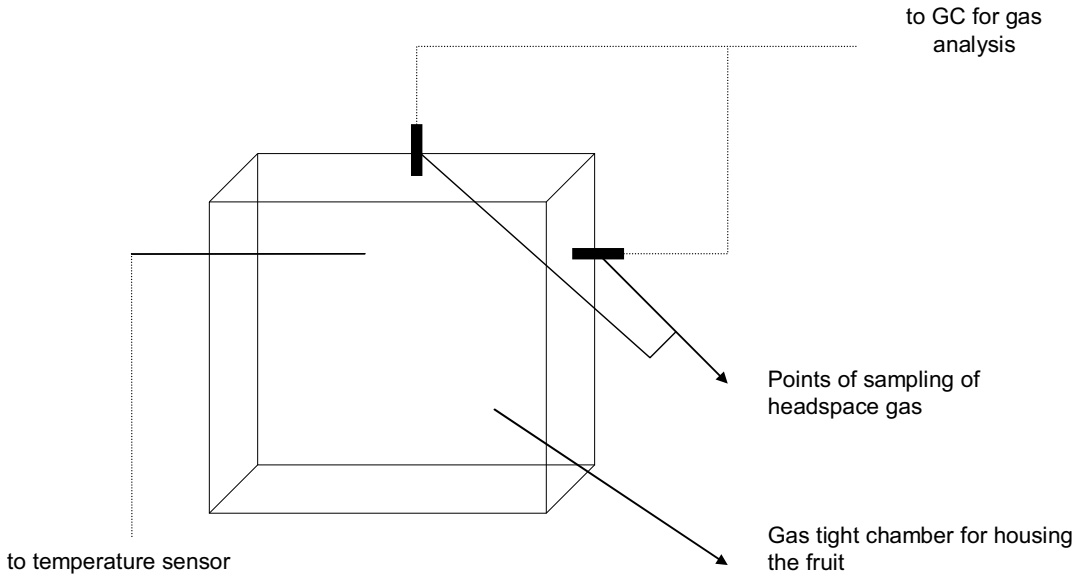

Fig. 1 Schematic sketch for the closed system method for generation of respiration data.

Gas exchange measurement

Respiration rates can be measured by observing the concentration of O_2 consumption or CO_2 evolution per unit time per unit weight of the produce. Various external factors such as O_2 concentration, CO_2 concentration, temperature and time affect respiration (Kays 1991; Lee *et al*. 1991; Hagger *et al*. 1992; Prasad 1995; Mahajan 2001). Measurement of O_2 consumption by the fruit tissue as a function of O_2 and CO_2 concentration in a flow through system is technically difficult, since it requires highly accurate analytical equipment (Cameron *et al*. 1989). Hence the respiration rate data for apple fruits was experimentally generated for different temperatures using the closed system method (Andrich *et al.* 1991; Song *et al*. 1992; Peppelenbos and Leven 1996; Dash 2007; Menon Bhande *et al*. 2008; Rekha and Goswami 2008) as described in **Fig. 1**. Closed systems can provide a convenient way of characterizing respiration of fresh produce in a single set of experiment (Kang *et al*. 1998; Hagger *et al*. 1992; Song *et al*. 1992; Hong and Kim 2001; Fonseca *et al*. 2002). In this process, the changes in O_2 and CO_2 concentrations resulting from respiration within a sealed container could be measured directly. Air-tight respirometer chambers of size 0.125 m × 0.175 m × 0.23 m made up of acrylic (Perspex) sheet was used. A Ni-Cr thermocouple was inserted into one side of the respirometer and used to measure the storage temperature. Fruits were kept in respirometer from the open topside and were closed with the lid while inserting neoprene gasket in between. Lid was closed with nuts and bolts provided on the respirometer to make it airtight. The system was kept in a humidity control chamber (Digitek System, Kolkata, India) which was maintained at the desired temperature with a tolerance limit of ± 0.2°C and relative humidity of 92 ± 2%. Gas composition of the respirometer was analyzed at regular intervals depending on the storage temperature of apple. Typically the intervals chosen were 2 h for the temperatures at 30 and 25°C, 4 h at 20 and 15°C, and 8 h at 10, 5 and 0°C, respectively. Gas analysis was done until the CO_2 concentration reached 18%, or until aerobic respiration persisted (Hagger *et al*. 1992). Changes in the concentration of O_2 and CO_2 over a certain period of time were measured and used to estimate respiration rates. After an interval of time, gas samples of the container were analyzed for O_2 and CO_2 concentration. Weight of fruits taken during the experiment and its corresponding free volume is shown in **Table 1**. Free volume of the respirometer was the total volume of the respirometer minus volume occupied by its content. The free volume of the respiration chamber (V_f) was determined by nitrogen injection method (Prasad 1995). For this a predetermined quantity (Q_N) of N_2 which could cause a measurable change in N_2 level of container's atmosphere was injected. The increased in N_2 level was determined by analyzing the gas sample on gas chromatograph. By incorporating these values in the following equation, the free volume of the respiration container was calculated.

Table 1 Physico-chemical properties of apple cv. Royal Delicious.

Parameter	Value*
Physical properties	
Fruit weight (g)	135.94 ± 10.15
Fruit volume (ml)	157.61 ± 12.44
True density (kg/m^3)	862.46 ± 22.32
Physical dimensions	
Major diameter (mm)	69.35 ± 6.12
Intermediate diameter (mm)	68.55 ± 2.25
Minor diameter (mm)	61.10 ± 2.45
Sphericity	0.96 ± 0.03
Firmness (g)	4600.20 ± 562.36
Puncture strength (g)	297.47 ± 34.65
Hunter Color parameters:	
L	33.93 ± 4.14
a	35.12 ± 3.25
b	10.95 ± 3.47
Hue	17.31 ± 1.74
Chroma	36.78 ± 1.82
Chemical properties	
Titrable acidity (% malic acid)	0.25 ± 0.02
Ascorbic acid (mg/100 ml of juice)	7.87 ± 0.75
Total soluble solids (°Brix)	12.26 ± 0.65
Starch-iodine index (1-9 scale)	4.50 ± 0.58

* Values presented are average of 10 representative apple fruits and ± indicates the standard deviation.

$$V_f = \frac{Q_N \times 100}{(N_f - N_i)} \quad (1)$$

where, V_f is the free volume of the respiration chamber in cm^3, Q_N is the volume of N_2 injected into the respiration chamber in cm^3, N_i and N_f are the initial and final N_2 concentration in %.

Gas analysis

Storage gas sample of about 1 ml was taken periodically with an airtight syringe and was analyzed quantitatively for O_2 and CO_2 concentrations using gas chromatograph (GC model 100 Knaur, Germany). A column packed with Spherocarb (18-100 mesh, 1.83 m long) was used for separation of CO_2, O_2 and nitrogen. Hydrogen was used as carrier gas at a flow rate of 25 ml/min. Oven temperature was kept at 50°C whereas injector and detector were set at 125 and 150°C, respectively with the detector voltage of 5 V. The sampling intervals were different for different storage temperatures as mentioned in the previous section. Each time three replications were taken for the gas analysis and the average gas composition was recorded. The respiration study was replicated in triplicate for accurate measurement and analysis.

Modelling and data analysis

The experimental respiration rate was calculated from the concentration difference, weight of fruit and free volume of the chamber. The respiration rates in terms of O_2 and CO_2 at a given temperature were calculated using the following equations (2) and (3) as given by Kays (1991).

$$R_{O2} = \left[\frac{(Y_{O2})_t - (Y_{O2})_{t+1}}{\Delta t}\right]\frac{V_f}{W} \quad (2)$$

$$R_{CO2} = \left[\frac{(Z_{CO2})_{t+1} - (Z_{CO2})_t}{\Delta t}\right]\frac{V_f}{W} \quad (3)$$

where: R_{O2} is the respiration rate, ml $[O_2]$ $kg^{-1}h^{-1}$, R_{CO2} is the respiration rate, ml $[CO_2]$ $kg^{-1}h^{-1}$, Y_{O2} and Z_{CO2} are the gas concentrations for O_2 and CO_2 respectively, t is the storage time in h, Δt is the time difference between two gas measurements, V_f is the free volume of the respiration chamber in ml and W is the weight of the fruit in kg.

Two different approaches were attempted to model the respiration rate based on the experimental data as outlined below.

Model 1

A regression function is often used to fit the data of gas concentration versus time, and the respiration rate at given time is determined from the first derivative of the regression function (Cameron *et al*. 1989; Hagger *et al*. 1992; Mahajan and Goswami 2001). By using the generated experimental respiration data, a nonlinear regression analysis was done using SYSTAT 8.0 to fit O_2 concentration and CO_2 concentration at different storage periods. The resultant regression equations for O_2 consumption and CO_2 evolution are shown in equations (4) and (5) to determine the values of the coefficients 'a' and 'b'.

$$Y_{O2} = 0.21 - \left[\frac{t}{a \times t + b}\right] \quad (4)$$

$$Z_{CO2} = \left[\frac{t}{a \times t + b}\right] \quad (5)$$

where: a and b are the regression coefficients; a is unit less and b is in h, t is storage period in h, Y_{O2} and Z_{CO2} are the gas concentrations for O_2 and CO_2 respectively in decimal.

The first derivative of the regression functions were used to determine the rate of change of gas concentration as outlined in equations (6) and (7).

$$\frac{d(Y_{O2})}{dt} = a \times t(a \times t + b)^{-2} - (a \times t + b)^{-1} \quad (6)$$

$$\frac{d(Z_{CO2})}{dt} = -a \times t(a \times t + b)^{-2} + (a \times t + b)^{-1} \quad (7)$$

The respiration rate of the sample at any given time was then calculated by substituting the values of dY_{O2}/dt and dZ_{CO2}/dt obtained from equations (6) and (7) in equation (8) and (9) respectively.

$$R_{O2} = -\frac{d[(Y_{O2})]}{dt}\frac{V_f}{W} \quad (8)$$

$$R_{CO2} = -\frac{d[Z_{CO2}]}{dt}\frac{V_f}{W} \quad (9)$$

The temperature dependence of the model coefficients 'a' and 'b' were estimated by linear interpolation between the two temperatures.

Model 2

The principles of enzyme kinetics have been suggested as being applicable to the respiration rate of fresh produce and Michaelies-Menten equation has been fitted to respiration rates (Lee *et al.* 1991). Considering that CO_2 acts as a respiration inhibitor, the effect of CO_2 on the product respiration can be described by the un-competitive inhibition (Pepelenbos and Leven 1996; Maneerat *et al*. 1997; McLaughlin and O'Beirne 1999). The uncompetitive type of inhibition occurs where the inhibitor (CO_2) does not bind with the enzyme, but bind reversely with the enzyme-substrate complex, reducing the rate of product formation as in the case of Michaels-Menten type equation. In this case the increase of substrate concentration (O_2 concentration) at high CO_2 concentrations has almost no influence on the O_2 consumption rate i.e. increasing the substrate concentration cannot reverse it (Cornish-Bowden 1979). Thus the maximum respiration rate is not much influenced at high CO_2 concentration. At high levels of CO_2 concentration (17-18%), however the respiration mechanism changes form aerobic to anaerobic pathway (Mahajan 2001). Hence principles of enzyme kinetics with uncompetitive inhibition were used to develop a model (Hager *et al*. 1992; Lee *et al*. 1991; Song *et al*. 1992; Lee *et al*. 1996; Pepelenbos and Leven 1996; Mahajan and Goswami 2001; Menon Rekha and Goswami 2008) for predicting the rate of respiration of Royal Delicious apple fruits. The model has three parameters viz., v_m, k_m, and k_i for both O_2 consumption and CO_2 evolution as shown in equation (10) and (11). The model parameters were determined using the experimental respiration data.

$$R_{O2} = \frac{v_{m(O2)} \times Y_{O2}}{k_{m(O2)} + \{1 + ([Z_{CO2}]/k_{i(O2)})\}Y_{O2}} \quad (10)$$

$$R_{CO2} = \frac{v_{m(CO2)} \times Y_{O2}}{k_{m(CO2)} + \{1 + ([Z_{CO2}]/k_{i(CO2)})\}Y_{O2}} \quad (11)$$

where: R_{O2} is the respiration rate, ml $[O_2]$ kg^{-1} h^{-1}, R_{CO2} is the respiration rate, ml $[CO_2]$ kg^{-1} h^{-1}, Y_{O2} and Z_{CO2} are the gas concentrations for O_2 and CO_2 respectively, $V_{m(O2)}$ and $V_{m(CO2)}$ are the maximum respiration rate for O_2 consumption and CO_2 evolution respectively, $K_{m(O2)}$ and $K_{m(CO2)}$ are the Michaels-Menten constant for O_2 consumption and CO_2 evolution, % O_2 respectively, $K_{i(O2)}$ and $K_{i(CO2)}$ are the inhibition constants for O_2 consumption, CO_2 evolution, % CO_2 respectively. In the above expression, the O_2 and CO_2 concentration are expressed in percentage. The effect of O_2 and CO_2 on respiration rate in terms of ml $[O_2]$ $kg^{-1}h^{-1}$ and ml $[CO_2]$ $kg^{-1}h^{-1}$ can be obtained from the above equations (10) and (11) respectively.

The parameters of the above equations (10) and (11) may be estimated by linearization of the equation and subsequent linear regression analysis (Andrich *et al*. 1991; Lee *et al*. 1991; Hagger *et al*. 1992; Song *et al*. 1992; Lee *et al*. 1996; Andrich *et al*. 1998; Mclaughlin and O'Beirne 1999; Mahajan and Goswami 2001; Das 2007; Bhande *et al*. 2008) or directly by non-linear regression analysis (Cameron *et al*. 1994; Joles *et al*. 1994; Dadzie *et al*. 1996; Peppelenbos and Leven 1996; Ratti *et al*. 1996; Smyth *et al*. 1998). However, linearising the equations is equivalent to changing the weight given to the data in the estimation procedure and thus should be avoided (Peppelenbos and Leven 1996; Fonseca *et al*. 2002).

Temperature is the most important environmental factor in the post harvest life of fresh produce because of its dramatic effect on rates of biological reaction including respiration. Within the physiological range (0 to 30°C), the respiration rate of fresh fruits and vegetables generally increases two to three folds for every 10°C rise in temperature (Wills *et al*. 1989). The Arrhenius relationship describes the dependence of the biological reactions, including respiration of fresh produce, on temperature. The activated complex theory for chemical rates is the basis for the Arrhenius equation, which relates reaction rates to the absolute temperature (Toledo 1991). Temperature has also major effect on the respiration rate, which has not been considered in the equation of the uncompetitive inhibition of enzyme kinetics (equations 10 and 11). As per the studies conducted by Bhande *et al*. (2008), Das (2007),

Hong *et al*. (2001) and Lakakul *et al*. (1999) on banana, sapota fruit, fresh-cut green onion and sapota slices respectively, a model parameter V_m of Michaels-Menten type enzyme kinetics was found to vary with the storage temperature. They used Arrhenius plot to describe the relationship between V_m and storage temperature. The dependence of model parameters of uncompetitive inhibition kinetics on storage temperature can be expressed by equation (12) (Lakakul *et al*. 1999). The model parameters obtained for apple were then correlated at different temperatures using Arrhenius plot as shown in equation (12).

$$R_m = R_p \exp\left[\frac{-E_a}{R \times T_{abs}}\right] \quad (12)$$

where R_m is the model parameter of enzyme kinetics, R_p is the respiration pre-exponential factor, E_a is the activation energy, kJ/g-mole, T_{abs} is the storage temperature, K, and R is the Universal gas constant (8.314 kJ/kg-mole-K). Equation (12) can be expressed in a linearised form (Forward 1960) as shown in equation (13).

$$\ln R_m = -\frac{-E_a}{R}\left[\frac{1}{T_{abs}}\right] + \ln R_p \quad (13).$$

Verification of the model

Respiration rates of Royal Delicious apple predicted by model were verified with experimental respiration rates at 12 °C storage temperature. The respiration data for O_2 and CO_2 were obtained by the closed system respirometer kept at 12°C. Free volume of respirometer and weight of apple taken for verification of the model were 4451.27 ml and 1.367 kg, respectively. The experimental respiration rates for apple at different combinations of O_2 and CO_2 at 12°C temperature were determined using equations (2) and (3), respectively.

Statistical analysis

The SYSTAT 8.0 software was used to determine the values of the regression coefficients (a and b) of equations (4) and (5) along with their coefficients of determination. Similarly the model parameters of enzyme kinetic model (equations 10 and 11) were estimated along with their coefficients of determination. The one-way ANOVA was carried out to ascertain the effect of temperature on the model parameters and effect of temperature on regression coefficients using SYSTAT 8.0 with a completely randomized block design.

Goodness of fit

The mean relative percentage deviation modulus is widely adopted throughout the literature to evaluate the goodness of fit of mathematical expression. Moduli below 10% are indicative of reasonably good fit, 10–20% fairly good fit and 20–30% not satisfactory fit for all practical purposes. The goodness of fit between predicted and experimental respiration rates was obtained by calculating the mean relative percentage deviation modulus (McLaughlin and O'Beirne 1999) as given in equation 14. In general, lower the modulus better is the agreement between experimental and predicted values.

$$E = \left[\frac{100}{N}\sum_{i=1}^{n}\frac{(R_{exp} - R_{pre})}{R_{exp}}\right] \quad (14)$$

where, E is the mean relative deviation modulus in %; N is the number of respiration data points; R_{exp} is the experimental respiration rate in ml kg^{-1} $h^{-1}$and R_{pre} is the predicted respiration rate in ml kg^{-1} h^{-1}.

RESULTS AND DISCUSSION

The physico-chemical properties of Royal Delicious apple as obtained experimentally are shown in **Table 2**. The physico-chemical properties obtained were found to be in agreement with those reported by Kaushal and Sharma (1995), Ghosh (1999) and Mahajan (2001).

Table 2 Free volume of respirometer and weight of apple taken for generating the respiration data.

Storage temperature (°C)	Weight of fruits (W), kg	Free volume of respirometer (V_f), ml
0	1.378	4440.84
5	1.352	4466.25
10	1.382	4436.78
15	1.309	4515.32
20	1.371	4446.57
25	1.327	4493.82
30	1.332	4485.36

Fig. 2 shows the periodical changes in O_2 and CO_2 concentrations inside the closed respirometer containing apple at different storage temperatures. The O_2 concentration of the respirometer decreased in proportion to increase in CO_2 concentration with the storage period, the rate being higher at the higher storage temperature.

Estimation of parameter for different models

Model 1

The regression coefficients a, and b of equations (4) and (5), and coefficients of determination (r^2) at different storage temperatures are shown in **Table 3**. The above regression functions described the experimental data very well ($r^2 \geq 0.996$) for apple.

From the values of the regression coefficients a and b in **Table 3**, it can be inferred that both parameters were influenced by the storage temperature and that coefficient b was more influenced by temperature than coefficient a. The analysis of variance of temperature with regression coefficients a and b of Model 1 is given in **Table 4**. It can be inferred that temperature has a significant effect (1% level of significance) on regression coefficients. For any unknown temperature, the values of a and b can be determined by linear interpolation of the known values. The corresponding values of a and b, when substituted in equation (6) and (7), allow to estimate dY_{O2}/dt and dZ_{CO2}/dt, respectively. The respiration rates for the given temperature can then be determined using equations (8) and (9).

Model 2

The model parameters in equations (10) and (11) were determined by fitting the experimental data at each temperature using non-linear regression analysis (SYSTAT 8.0, SPSS). According to equation (10) and (11), respiration rate was considered as the dependent variable whereas the O_2 concentration (Y_{O2}) and CO_2 concentration (Z_{CO2}) were considered as the independent variables for the regression analysis. The model parameters were calculated from the constants of non-linear regression analysis, which are given in **Table 5** along with their corresponding coefficients of determination (r^2) values. The values in **Table 5** show that the model parameters were indeed dependent on the storage temperature. The coefficients of determinations was found to be higher or equal to 0.970 indicating that the relationship between respiration rate and O_2 and CO_2 concentrations fitted well with the uncompetitive inhibition enzyme kinetics model.

The analysis of variance of temperature with model parameters of enzyme kinetics model is given in **Table 6**. It can be inferred that the temperature has a significant effect (1% level of significance) on model parameters $V_{m(O2)}$, $V_{m(CO2)}$, $K_{m(O2)}$, $K_{m(CO2)}$, $K_{i(O2)}$ and $K_{i(CO2)}$ of enzyme kinetics Model 2.

Since the model parameters such as $V_{m,}$, K_m and K_i for O_2 and CO_2 concentrations were found to vary with tempe-

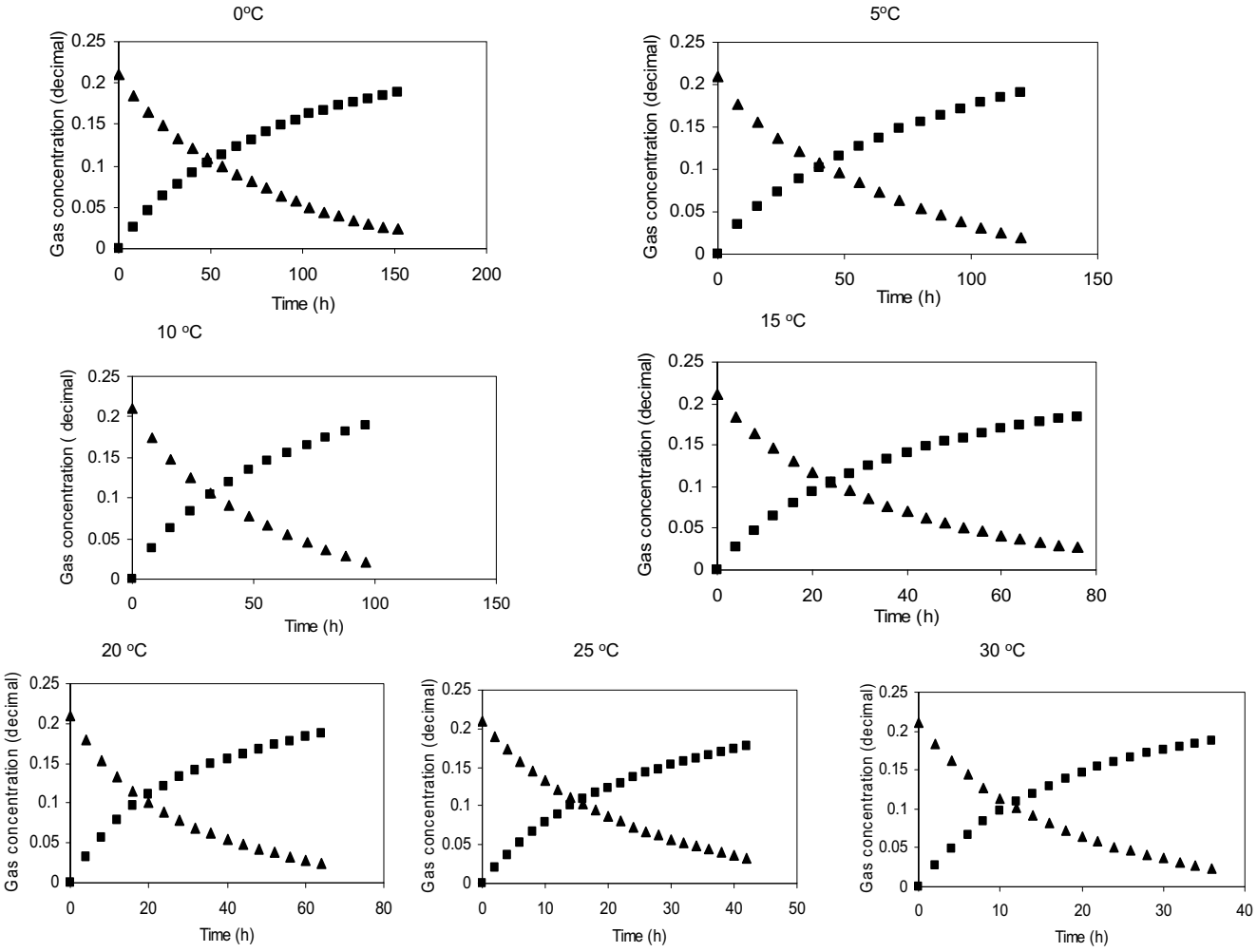

Fig. 2 Experimental data for O_2 consumption and CO_2 evolution at different temperatures. ▲is Y_{O2}, in decimal; ■ is Z_{CO2}, in decimal.

Table 3 Values of Regression Coefficients for Model 1.

Storage temperature of apple (°C)	Respiration expression in terms of	Regression coefficients		r^2
		a	b	
0	O_2 consumption	3.192	321.888	0.998
	CO_2 evolution	3.242	309.27	0.999
5	O_2 consumption	3.109	262.883	0.997
	CO_2 evolution	3.115	258.511	0.996
10	O_2 consumption	3.158	209.180	0.997
	CO_2 evolution	3.169	206.409	0.998
15	O_2 consumption	3.510	145.413	0.997
	CO_2 evolution	3.542	142.157	0.996
20	O_2 consumption	3.665	110.360	0.998
	CO_2 evolution	3.679	108.835	0.997
25	O_2 consumption	3.392	94.393	0.997
	CO_2 evolution	3.475	92.217	0.996
30	O_2 consumption	3.418	69.887	0.998
	CO_2 evolution	3.379	69.313	0.997

Table 4 Analysis of variance of effect of temperature on regression coefficients of Model 1.

Factor	DF	a_{O2}		a_{CO2}		b_{O2}		b_{CO2}	
		MSS	F value	MSS	F value	MSS	F value	MSS	F value
Replication	2	0.00	0.01	0.00	0.01	471.76	0.99	0.14	0.12
Temperature	6	0.12	30.51**	0.13	30.64**	34345.24	72.69**	24647.10	21439.06**
Error	12	0.004	---	0.004	---	472.48	---	1.15	---

** Significant at 1% level

rature and hence, Arrhenius equation was used to correlate the model parameters at different storage temperatures. As per equation (13) the model parameters were plotted at different storage temperatures; by plotting the log values of the model parameters against the inverse of corresponding temperature in absolute units. The resulting linear plot is shown as **Fig. 3**. The slope and the Y-axis intercept of equation (13) for model parameters $V_{m,}$, K_m and K_i are shown in **Table 7**. The activation energy was calculated from the slope of the straight line and the pre-exponential factor was calculated from the Y-axis intercept. **Table 8** shows the activation energy and pre-exponential factor for different model parameters of enzyme kinetics. Activation energy was found to be in negative side for $K_{i(O2)}$ and $K_{i(CO2)}$ valued at -20.23 and -16.84 kJ/g-mole respectively. The negative values of activation energies for K_i could be attributed to the

Table 5 Model parameters for uncompetitive inhibition enzyme kinetics for different storage temperatures (Model 2).

Storage temperature (°C)	Respiration expressed in terms of	V_m (ml/kg h)	K_m (% O_2)	K_i (% CO_2)	r^2
0	O_2	12.69	4.20	13.73	0.994
	CO_2	15.44	9.43	15.14	0.992
5	O_2	14.78	5.43	10.65	0.982
	CO_2	17.63	10.22	13.58	0.990
10	O_2	17.25	7.13	9.36	0.972
	CO_2	21.15	11.37	11.92	0.983
15	O_2	22.74	8.16	7.88	0.980
	CO_2	25.27	12.26	10.66	0.970
20	O_2	28.25	9.23	7.17	0.975
	CO_2	32.16	13.87	9.20	0.985
25	O_2	33.21	9.84	6.22	0.991
	CO_2	36.20	14.65	8.30	0.985
30	O_2	43.57	10.26	5.46	0.990
	CO_2	45.62	15.53	7.28	0.991

Table 6 Analysis of variance of effect of temperature on model parameters of Enzyme kinetics model (Model 2).

Factor	DF	$V_{m(O2)}$		$V_{m(CO2}$		$K_{m(O2)}$		$K_{m(CO2)}$		$K_{i(O2}$		$K_{i(CO2)}$	
		MSS	F value	MSS	F value	MSS	F value	MSS	F value	MSS	F value	MSS	F value
Replication	2	0.18	0.14	0.13	0.11	0.14	0.12	0.13	0.11	0.14	0.12	0.13	0.11
Temperature	6	371.53	390.10**	357.10	311.38**	15.70	13.77**	15.84	14.00**	24.58	21.48**	24.51	25.76**
Error	12	1.12	---	1.14	---	1.14	---	1.13	---	1.14	---	1.12	---

** Significant at 1% level
* Significant at 5% level

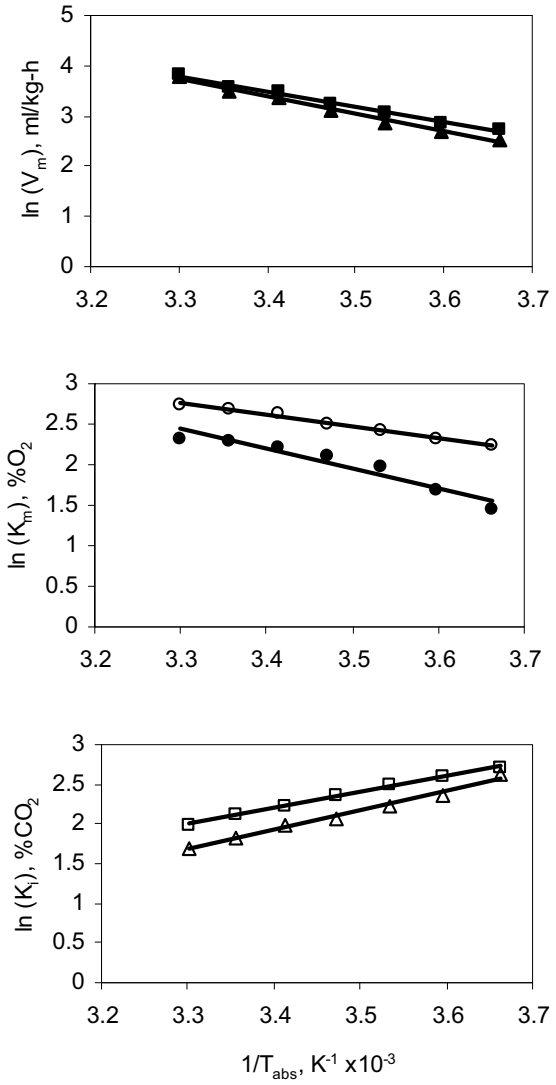

Fig. 3 Arrhenius relation for different model parameters of enzyme kinetics. ▲is $V_{m(O2)}$; ■ $V_{m(CO2)}$; ♦ is $K_{m(O2)}$; o is $K_{m(CO2)}$; Δ is $K_{i(O2)}$; □ is $K_{i(CO2)}$.

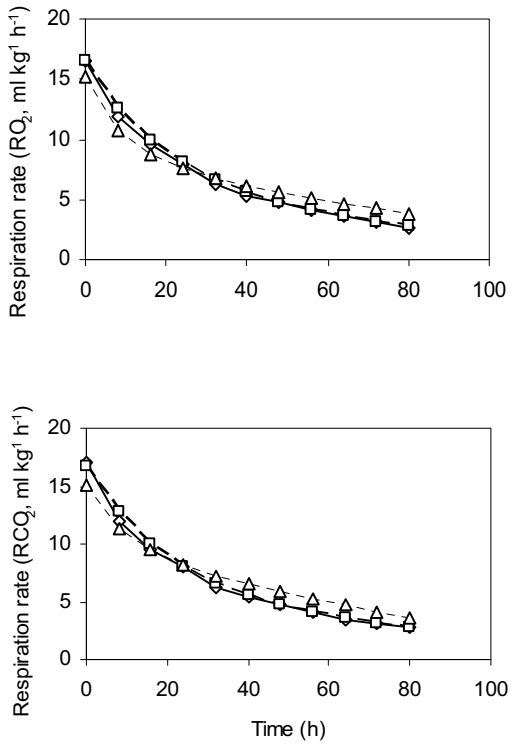

Fig. 4 Experimentally estimated and predicted respiration rates for Royal Delicious apple at 12°C. —◊— is for experimental respiration rate; --□-- is for respiration rate predicted by Model 1; --Δ-- is for respiration rate predicted by Model 2.

inhibitory effect of CO_2 concentration on respiration rate. By using these constants, the model parameters at any temperatures can be predicted by using equation (12) and then, the respiration rate at the given temperature can be estimated by employing equation (10) and (11) for respiration rates in terms of O_2 consumption and CO_2 evolution, respectively.

Verification of the models

Since the respiration rates were generated at temperature between 0 to 30°C with a 5°C step it was necessary to verify whether the developed models were capable of predicting the respiration rates at any temperature within this domain of experimental temperatures. Accordingly, the respiration rates predicted by the models were verified with the experimental respiration rate at 12°C storage temperature.

For Model 1, the values of the regression coefficient a

Table 7 Slope ($-E_a/R$) and Y axis intercept ($\ln R_p$) of Arrhenius relation for different model parameters of enzyme kinetics.

	V_m		K_m		K_i	
	O_2	CO_2	O_2	CO_2	O_2	CO_2
Slope	–3428.3	–3014.4	–2460.3	–1429.6	2434.2	2025.9
Y axis intercept	15.036	13.734	10.557	7.478	–6.350	-4.686
r^2	0.992	0.989	0.932	0.993	0.989	0.997

Table 8 Activation energy and pre-exponential factor of Arrhenius-type equation for different model parameters of uncompetitive inhibition.

Parameters for Arrhenius equation	V_m		K_m		K_i	
	O_2	CO_2	O_2	CO_2	O_2	CO_2
E_a (kJ/g-mole)	28.50	25.06	20.45	11.88	-20.23	-16.84
R_p	3.3×10^6	1.0×10^6	3.8×10^4	1.8×10^3	1.7×10^{-3}	1.0×10^{-2}

and b at 12°C were estimated by the linear interpolation of the values of the same coefficients at 10 and 15°C as shown in **Table 3**. By using activation energy and pre exponential factor from **Table 6**, the model parameters for enzyme kinetics at 12°C for both O_2 consumption and CO_2 evolution were calculated and the respiration rate at 12°C was predicted using equations (10) and (11).

The comparison of regression analysis (Model 1) and enzyme kinetics model (Model 2) with temperature dependence based on Arrhenius law against the experimentally determined respiration rate at 12°C is depicted in **Fig. 4.**

The mean relative deviation moduli (equation (14) between the respiration rates of apple at 12°C predicted by regression analysis (Model 1) and that obtained through experiments were 1.97 and 2.10% for O_2 consumption and CO_2 evolution, respectively. Similarly the mean relative deviation moduli between the respiration rates predicted by model based on enzyme kinetics (Model 2) and obtained through experiments were found to be 12.82 and 15% for O_2 consumption and CO_2 evolution, respectively. The results indicate that both the models have good agreements for predicting the respiration rates for O_2 consumption and CO_2 evolution.

The respiration rates measured for Royal Delicious apple was found to be lower than that found for the other fruits such as mango (Menon Rekha and Goswami 2008), sapota (Das 2007), and banana (Bhande *et al*. 2008). The respiration rates and the parameters of enzyme kinetics model were found to be in agreement with those found by Mahajan (2001). The goodness of fit obtained for Model 1 and Model 2 were found to be within the range reported for mango (Menon Rekha and Goswami 2008), sapota (Das 2007), and banana (Bhande *et al*. 2008).

CONCLUSIONS

Respiration rates for apple at different temperatures form 0–30 °C in steps of 5 °C were estimated using the closed system method. The respiration rates were found to decrease with storage time due to diminishing concentrations of O_2 and proportional increase in CO_2 concentrations in the respirometer. In the enzyme kinetics model, the dependence of respiration rate on O_2 and CO_2 was found to follow the uncompetitive inhibition. The activation energy and pre-exponential factors of the Arrhenius equations were used for predicting the model parameters of enzyme kinetics at any temperature between 0-30 °C.

The mean relative deviation moduli between the respiration rates of apple at 12°C predicted by both models were within 15% for O_2 consumption and CO_2 evolution. This indicates that both models have good agreements for predicting the respiration rates for O_2 consumption and CO_2 evolution. However the Enzyme kinetics model (Model 2) is preferred because principles of enzyme kinetics have been suggested as being applicable to the respiration rate of fresh produce.

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Notation used in this manuscript

a	Regression coefficient
b	Regression coefficient, h
E	Mean relative deviation modulus, %
E_a	Activation energy, kJ $g^{-1}mol^{-1}$
$K_{m(O2)}$	Michaelis-Menten constant for O_2 consumption, % O_2
$K_{m(CO2)}$	Michaelis-Menten constant for CO_2 evolution, % O_2
$K_{i(O2)}$	Inhibition constants for O_2 consumption, % CO_2
$K_{i(CO2)}$	Inhibition constants for CO_2 evolution, % CO_2
N	Number of respiration data points
N_i	Initial N_2 concentration of the respirometer, %.
N_f	Final N_2 concentration of the respirometer, %.
Q_N	Volume of N_2 injected into the respiration chamber, cm^3
R	Universal gas constant, 8.314 kJ $kg^{-1}mol^{-1}K^{-1}$
R_{CO2}	Respiration rate, ml $[CO_2]$ $kg^{-1}h^{-1}$
R_{exp}	Experimental respiration rate, ml kg^{-1} h^{-1}
R_m	Model parameter of enzyme kinetic
R_{pre}	Predicted respiration rate, ml kg^{-1} h^{-1}
R_{O2}	Respiration rate, ml $[O_2]$ $kg^{-1}h^{-1}$
R_p	Respiration pre-exponential factor
T	Storage temperature, °C
T_{abs}	Storage temperature, K
t	Storage time, h
Δt	Time difference between two gas measurements
V_f	Free volume of the respiration chamber, ml
$V_{m(CO2)}$	Maximum respiration rate for CO_2 evolution, ml/kg-h
$V_{m(O2)}$	Maximum respiration rate for O_2 consumption, ml/kg-h
W	Weight of Royal Delicious apple fruit, kg
Y_{O2}	Oxygen concentration, decimal
Z_{CO2}	Carbon dioxide concentration, decimal