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Respiration kinetic of mango (*Mangifera indica* L.) as function of storage temperature

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Cinética de respiración de mango (*Mangifera indica* L.) como función de la temperatura de almacenamiento

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Camilo Agudelo[1], Caludia Restrepo[2] and José Edgar Zapata[1*]

ABSTRACT

Key words:
Shelf life
Ripeness
Respiratory process

Respiration of cut mango (*Mangifera Indica* L.) cv. Tommy Atkins was studied using the closed system method at three temperatures (4, 20 and 35 °C). Two models were used to estimate the gas concentration, which were adjusted through non-lineal regression algorisms using Matlab R2011a software. Three mathematic models, a model based on Michaelis-Menten's enzymatic kinetics, and two models based on regression analysis, in one of which a saturation equation was included as a new proposal in this field, were set to predict the substrate respiration rate. Results made evident the positive effect of temperature on mango respiration rate. The model with the best adjustment to mango respiration rate was Michaelis-Menten's with an adjusted correlation coefficient of 0.9811 and 0.9747 for CO_2 and O_2 respectively, with a relative mean error lower than 10%.

RESUMEN

Palabras claves:
Vida útil
Madurez
Proceso respiratorio

Se estudió la respiración del mango cortado (*Mangifera Indica* L.), cv. Tommy Atkins, utilizando el método de sistema cerrado, a tres temperaturas (4, 20 y 35 °C). Se usaron dos modelos para estimar la concentración de gas, los cuales se ajustaron a través de algoritmos de regresión no lineal usando el software Matlab R2011a. Se ajustaron tres modelos matemáticos para predecir la tasa de respiración del sustrato. Un modelo basado en la cinética enzimática de Michaelis-Menten, y dos modelos basados en análisis de regresión, en uno de los cuales se incluyó una ecuación de saturación como una nueva propuesta en este campo. Los resultados evidenciaron el efecto positivo que tiene la temperatura sobre la tasa de respiración del mango. El modelo que mejor ajuste entregó para la velocidad de respiración del mango fue el modelo de Michaelis-Menten con un coeficiente de correlación ajustado de 0.9811 y 0.9747 para CO_2 y O_2, respectivamente, con un error medio relativo inferior al 10%.

[1] Facultad de Ciencias Farmacéuticas y Alimentarias. Universidad de Antioquia. Calle 67 No. 53-108. Box Air 1226, Medellín, Colombia.
[2] Instituto de Ciencia y Tecnología Alimentaria (Foundation INTAL). Carrera 50G No. 12 Sur-91, Itagüí, Colombia.
* Corresponding author <jedgar_4@yahoo.es>

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Mango (*M. indica L.*) is one of the most popular subtropical fruits (Souza *et al*., 2015), because of its high economical value (Sellamuthu *et al*., 2013), attractive color, delicious taste, rich aroma, exotic flavor, and high nutritional value. Furthermore mango is a rich source of carotenoids and provides high contents of ascorbic acid and phenolic compounds (Shahnawaz *et al*., 2012; Liu *et al*., 2013).

In general, fruits and vegetables stay metabolically active after harvest, period during which anabolic processes such as photosynthesis, flavor synthesis, fermentation and other cell wall degraders happen (Heydari *et al*., 2010). Respiration is of particular interest in postharvest technologies because it is associated to fruit and vegetable quality (Nicolaï *et al*., 2005). Respiration rate is an indicator of the metabolic level, as high rate respiration is associated with short shelf life (Lurie and Crisosto, 2005), and its control may be an effective mean to regulate the metabolism in general, this is why it is important to extend these products shelf life (Kan *et al., 2010*). This process changes according to species, variety, storage temperature and vegetable physiological state (Nicolaï *et al*., 2009). All chemical reactions during ripeness are delayed by low temperatures, these are some of the factors affecting quality and deterioration of fruits and vegetables during storage (Nicolaï *et al*., 2009) besides the effect on fungi, bacteria and insect growing (Nicolaï *et al*., 2005).

On the other hand, respiration rate may be reduced by changing the atmosphere around the food, which may also delay associated deterioration reactions, extending fruit and vegetable product shelf life (Luo *et al.,* 2011; Selcuk and Erkan, 2015). For instance, a drop on O_2 concentration results in a reduction of the metabolism of broccoli, carrot, pear, tomato (Nicolaï *et al*., 2005) and papaya (Zapata *et al*., 2004) among others. CO_2 may act as respiration suppressor in vegetables such as apples, broccoli (Nicolaï *et al*., 2005), mango (Ravindra and Goswami, 2008) and banana (Bhande *et al*., 2008), while it does not present any inhibitory effect in onions, lettuce and spinach (Nicolaï *et al*., 2005). Oxygen negative effects occur because reactive oxygen accumulation damages the integrity of mitochondrial membrane, resulting at the end in an irreversible mitochondrial dysfunction, which is believed to be the main cause of ageing in different kinds of organisms among which postharvest fruits are (Qin *et al*., 2009).

Respiratory process modeling is an important step in designing and selecting packaging and storing systems of fruit and vegetable products as it is the case of modified atmosphere packaging (Ravindra and Goswami, 2008). Then again, works oriented to assess this kind of parameters in fruit and vegetables, and in particular cut products, are rare. Accepting respiratory process modeling with all implicated factors in enzyme reaction would be highly complex and little practical, the usual strategy has been to develop empiric models for each product with controlled variable functions such as temperature or gas concentration (Guevara *et al*., 2006; Rai and Paul, 2007; Bhande *et al*., 2008).

The shelf life of minimally processed (cut) products is, then again, particularly important because it is one of the major growing sectors in food retail market (Robles-Sánchez *et al*., 2013), although the freshly cut mango still has a very limited offer in the world market (Souza *et al*., 2015). Nevertheless, the popularity of tropical fruits in the most important world markets, is an excellent opportunity for the introduction of fresh-cut mango (Siddiq *et al*., 2013). Yet, fresh-cut processing induces chemical and biochemical changes as well as increases product respiration rate leading to a reduction of shelf life (Azarakhsh *et al*., 2014). Therefore, the current work goal is to assess different models to predict respiration rate and gas concentrations of cut mango cv. Tommy Atkins variety at three different temperatures in a closed system.

MATERIALS AND METHODS

Raw material preparation

Mango (*Mangifera indica* L.) fruits cv. Tommy Atkins in physiological ripe state determined by Colombian technical norm NTC 5210 (ICONTEC, 2003) were used. These were bought in Central Minorista in the city of Medellín, to which they were brought from the southwest of the department of Antioquia. They were washed with alkaline detergent and disinfected with a solution at 100 ppm of sodium hypochlorite (Ngarmsak *et al*., 2005). They were cut in 0.5 cm edge cubes, leaving seed and epidermis aside, samples were taken to determine pH (ICONTEC, 1991), soluble solids (ICONTEC, 1999), and acidity contents expressed as citric acid percentage (ICONTEC, 1991).

Respiration experimental calculation

Respiration rate was determined to the mango in a closed system (Ravindra and Goswami, 2008), which consisted in subjecting 150 g of the product deposited in a 4,000 mL capacity airtight recipient to atmospheric conditions of 20.94 % O_2, 78.08 % N_2, and 0.03 % CO_2.

Three temperatures were set out in 4, 20 and 35 °C using an oven with temperature control (Binder, Germany). O_2 consumption and CO_2 production were measured by a PBI Dansensor Check Point O_2/CO_2 analyzer (Dansensor, Denmark). Each assay was performed twice.

Expressions (*1*) and (*2*) were used to calculate respiration rate, these had already been used in products such as pepper (Artés-Hernández *et al.*, 2010), mango (Ravindra and Goswami, 2008), and banana (Bhande *et al.*, 2008).

$$R_{O_2} = \left\{ \frac{[O_2]_t - [O_2]t+1}{\Delta t} \right\} \frac{V}{W} \qquad (1)$$

$$R_{CO_2} = \left\{ \frac{[CO_2]t+1 - [CO_2]t}{\Delta t} \right\} \frac{V}{W} \qquad (2)$$

Where R_{O_2} and R_{CO_2} are the respiration rates (mL kg^{-1} h^{-1}), $[O_2]$ and $[CO_2]$ are oxygen and carbon dioxide concentrations, *t* is time (h), *Δt* is the time between the two measures of the respective gas, *V* is the free volume in the respiration chamber (mL), and *W* is the cut fruit mass (kg).

Models for the gas behavior

The following models were used to estimate the gas concentration by a regression made with Matlab R2011a software, finding the model coefficients for all treatments.

Model 1. A model is proposed to estimate the experimental data of the O_2 and CO_2 concentrations by a growing reason expression or saturation equation (Chapra and Canale, 2007), as indicated in equations (*3*) and (*4*), adjusting them to CO_2 kinetic production and O_2 consumption, respectively.

$$[CO_2] = a\frac{t}{b+t} \qquad (3)$$

$$[O_2] = 0{,}21 - a\frac{t}{b+t} \qquad (4)$$

Where *a* and *b* are model parameters, and t is time (h).

Model 2. The model used by Bhande *et al.* (2008) in banana, and Ravindra and Goswami (2008) in mango cv. Amrapali, was also assessed. Equations (*5*) and (*6*) are used in this one adjusting O_2 and CO_2 concentrations in time function.

$$[CO_2] = \frac{t}{(a't+b')} \qquad (5)$$

$$[O_2] = 0{,}21 - \frac{t}{(a't+b')} \qquad (6)$$

Where *a'* and *b'* are model parameters and t is time (h).

Respiration models

Respiration rate for CO_2 (R_{CO_2}) and O_2 (R_{O_2}) was calculated by three different methodologies:

Equations (*3*), (4), (*5*) and (6) were derived, as derivatives of model 1and model 2, respectively.

Respiration rate was calculated with model 1 and model 2 derivatives, replacing them in equations (7) and (8), as follows:

$$R_{CO_2} = \frac{d[CO_2]}{dt}\frac{V}{W} \qquad (7)$$

$$R_{O_2} = -\frac{d[O_2]}{dt}\frac{V}{W} \qquad (8)$$

To obtain the cut mango respiration rate in the described conditions, a model based on Michaelis-Menten's kinetic equation was used, using enzymatic kinetic noncompetitive inhibition principles, where CO_2 is supposed to react to an enzyme-substrate complex (Artés-Hernández *et al.*, 2010; Ravindra and Goswami, 2008; Bhande *et al.*, 2008). Equation (9) expresses this mechanism for the respiration process in terms of O_2 consumption and CO_2 production rate. This kind of model presented a significant adjustment in the calculation of mango (Ravindra and Goswami, 2008), banana (Bhande *et al.*, 2008), pepper (Artés-Hernández *et al.*, 2010) and other fruit (Fonseca *et al.*, 2002) respiratory activity.

$$R_{Gas} = \frac{V_{mGas}\left[O_2\right]}{K_{mGas} + \left[1 + \frac{\left[CO_2\right]}{K_{iGas}}\right][O_2} \quad (9)$$

$$\frac{1}{R_{Gas}} = \frac{1}{V_{mGas}} + \frac{k_{mGas}}{V_{mGas}}\frac{1}{\left[O_2\right]} + \frac{1}{k_{iGas}V_{mGas}}\left[CO_2\right] \quad (10)$$

Arrhenius's equation

Temperature effect on O_2 consumption and CO_2 production rate was assessed by Arrhenius's equation (Iqbal *et al.*, 2009; Waghmare *et al.*, 2013).

$$R_{Gas} = R_p \exp\left[-\frac{E_a}{RT_{abs}}\right] \quad (11)$$

Where R_{Gas} is O_2 consumption and CO_2 production rate (mL $kg^{-1}h^{-1}$), R_p is respiration pre-exponential factor, E_a is the activation energy (J mol^{-1}), R is the gas universal constant (8,314 J mol^{-1} K^{-1}), and T_{abs} is the absolute temperature in K.

Relative standard deviation

Respiration rates predicted from the different models were contrasted with the experimental respiration rate, equations (*1*) and (*2*) by relative standard deviation, equation (*12*).

$$E = \frac{100}{N}\sum_{1}^{N}\frac{\left|R_{\exp} - R_{pred}\right|}{R_{\exp}} \quad (12)$$

Where E is the relative standard deviation module (%), N is the respiration data point number, R_{exp} is the experimental respiration rate (mL kg^{-1} h^{-1}), and R_{pred} is the predicted respiration rate (mL kg^{-1} h^{-1}). A good adjustment is defined with E<10 % (Ravindra and Goswami, 2008).

RESULTS AND DISCUSION

The physicochemical parameters to fresh mango were pH 3.4, °Bx 9.5 and acidity 1.05% as citric acid. Experimental data of the O_2 consumption and CO_2 production at different temperatures are shown in figure 1. The nonlinear drop on O_2 concentration in the conditions of the current work matches the pattern expected from other climacteric products kept in sealed containers (Ravindra and Goswami, 2008; Hagger *et al.*, 1992; Jacxsens *et al.*, 1999; Guevara *et al.*, 2006) and agrees with the hypothesis of saturation equation (model 1), as this type of equations start assuming that the system is saturated with CO_2 and O_2 consumed until exhaustion (Chapra and Canale, 2007). It is observed that a rise in temperature also raises the gradient of the graph for the gas behavior, consequently the respiration rate increases as it happens with the papaya (Zapata *et al.*, 2004), banana (Bhande *et al.*, 2008), and mango cv. Amrapali (Ravindra and Goswami, 2008). This is due to that the rate of the enzymatic reactions grows exponentially with the rise of the temperature (Lee *et al.*, 1996). Low temperatures reduce respiration rate, O_2 consumption, CO_2 and ethylene production. As the tissues react to the latter (Mendoza *et al.*, 2016), in order for maturity to occur, the needs of ethylene and the time of exposure are higher at low temperatures (Kader, 1994).

Models 1 and 2 parameters

Table 1 shows the values of the coefficients *a* and *b* for equations (*3*), (*4*), (*5*) and (*6*) with their respective correlation coefficients (R^2), obtained for the three temperatures assessed in the closed system. Model 1 presented an excellent adjustment to the data in the three assessed temperatures, while model 2 had a very poor adjustment to the data at 4 °C. Model 2 coefficients presented a high variability with temperature compared with model 1; it can be seen that parameter *b* is more influenced with temperature than coefficient *a* in both.

Respiration kinetics

Table 2 presents Michaelis-Menten's model parameters, with their corresponding adjusted correlation coefficient (R^2). A good adjustment of the experimental data with such model for R_{CO_2} and R_{O_2} is observed.

Table 2 data analysis based on the form of equation (*9*), allows to observe the effect gases have on the rate of CO_2 production and O_2 consumption. These effects are more

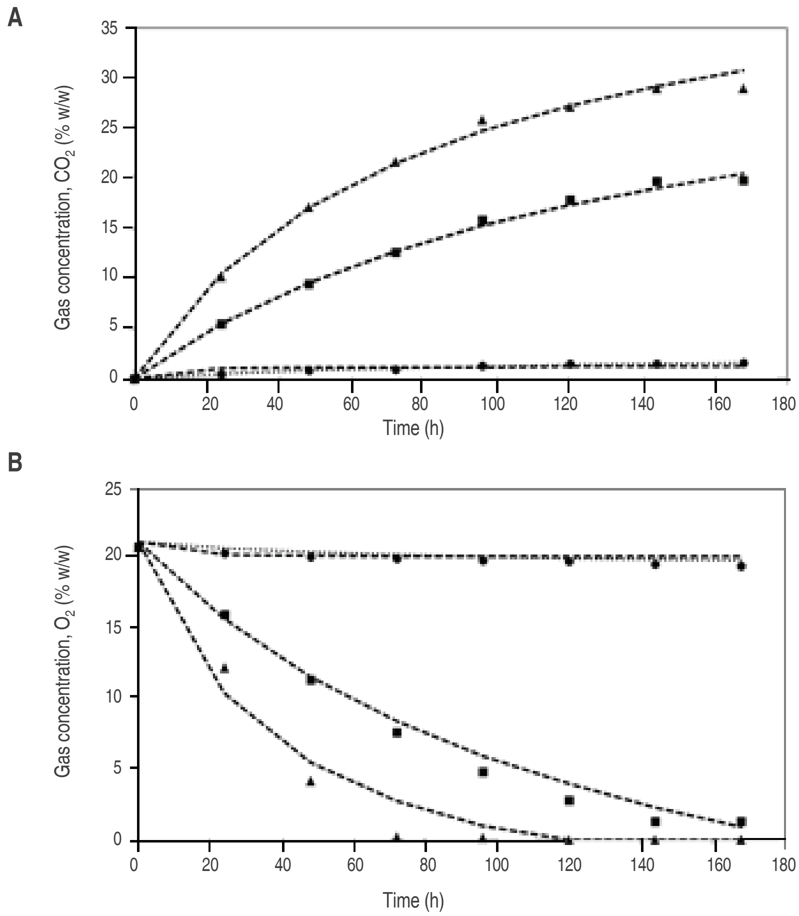

Figure 1. Experimental data of CO_2 consumption (**A**) and O_2 production (**B**) at different temperatures: ● 4 °C ■ 20 °C y ▲ 35 °C and using the Model 1: ·· and Model 2: - - - to predict the gas concentration.

Table 1. Values of adjusted coefficients to predict gas concentration.

Temperature (°C)	Gas type	Model 1			Model 2		
		a	b	R^2	a	b	R^2
4	CO_2	25.60 x10^{-3}	101.60	0.9940	81.17	392.50	0.5660
	O_2	19.30 x10^{-3}	75.58	0.9960	90.28	345.70	0.5814
20	CO_2	37.45 x10^{-2}	139.70	0.9959	2.67	372.90	0.9959
	O_2	36.15 x10^{-2}	133.70	0.9936	2.77	369.80	0.9936
35	CO_2	45.21 x10^{-2}	79.18	0.9996	2.21	175.10	0.9996
	O_2	28.22 x10^{-2}	39.08	0.9463	3.55	138.20	0.9463

noticeable at low CO_2 and high O_2 concentrations, at 20 and 35 °C. K_m and K_i values indicate CO_2 production rate is more sensible to the concentration of both gases than O_2 consumption rate, due to both constants are lower for the CO_2 production than O_2 consumption expressions. On the other hand, low K_m values indicate the significant effect of O_2 concentration on respiration rates in terms of CO_2 as well as O_2. While relatively high K_i values indicate that CO_2 concentration does not have such as significant effect on respiration rates. Nevertheless, the effect this gas has on respiration rate, given the adjust the model shows, cannot be denied. Besides, it is known that very high CO_2 and very low O_2 concentrations may cause respiration to change from aerobic to anaerobic, causing the creation of fermentation products such as acetaldehyde and ethanol (Angós *et al.*, 2008).

Table 2. Parameters for the expression of the respiration rate in the noncompetitive inhibition model of Michaelis-Menten, at different temperatures.

Temperature (°C)	Respiration rate expressed as	V_m (mL kg^{-1} h^{-1})	K_m (% O_2)	K_i (% CO_2)	R^2
4	CO_2	7.13	6.89	1.17	0.8814
	O_2	7.73	8.70	0.74	0.9747
20	CO_2	61.07	0.29	24.81	0.9811
	O_2	54.80	0.56	44.82	0.9463
35	CO_2	112.105	0.14	47.24	0.8040
	O_2	95.20	0.27	102.21	0.9749

Figure 2 shows that CO_2 experimental consumption rate (R_{CO_2}) has a starting value at 4 °C of 5.09 mL kg^{-1} h^{-1}, compared to the same gas experimental rate at 20 °C of 59.21 mL kg^{-1} h^{-1}, and at 35 °C it is of 110.76 mL kg^{-1} h^{-1}. This difference is due to reduction of temperature becomes into a reduction of the rate at which parameter such as respiration, texture and microbial growth change; this is why the organoleptic and physiological characteristics of mango stored at 4 °C did not present significant change in odor, texture and flavor.

Reduction of temperature not only reduces ethylene production, but also the response rate of tissues to such gas; therefore, the more the temperature drops, the higher exposure time required by the metabolic cycle to start at a certain ethylene concentration is (Mendoza *et al.*, 2016). Associated to this the temperature affect R_{O_2}, as can be seen in figure 3.

Table 3 shows the R_{CO_2} and R_{O_2} average value predicted for the three temperatures with Michaelis-Menten's equation, models 1 and 2, being noncompetitive inhibition Michaelis-Menten's equation the best model. Figures 2 and 3 show graphic behavior of experimental respiration and the one predicted by the models in terms of R_{CO_2} and R_{O_2}.

The model proposed (Model 1) is the second that best adjusts to O_2 consumption and CO_2 production, and also to the prediction of R_{CO_2} and R_{O_2}, being relevant

Table 3. Average respiration rate of the different models for the three temperatures.

Temperature (°C)	Average respiration rate (mL kg^{-1} h^{-1})				
	Respiration rate expressed as	Experimental	Model 1	Model 2	Michaelis-Menten's
4	CO_2	3.74	2.33	0.56	3.75
	O_2	3.41	1.90	0.42	3.42
20	CO_2	44.51	30.18	30.18	44.54
	O_2	42.76	29.66	29.66	42.77
35	CO_2	83.81	51.17	51.18	83.81
	O_2	73.20	35.47	35.44	73.18

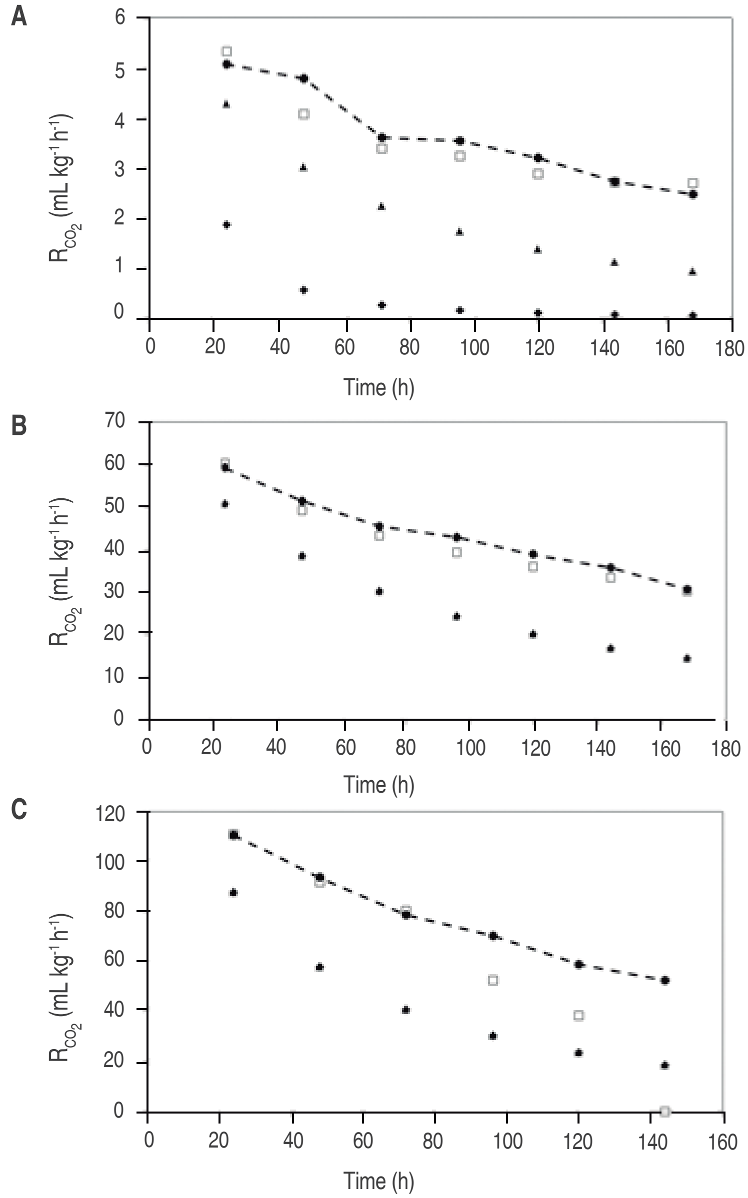

Figure 2. CO_2 production rate (R_{CO_2}) for cut mango stored at 4 °C **(A)** 20 °C **(B)** and 30 °C **(C)**; -●- Experimental consumption rate; ▲ consumption rate calculated with Model 1 (proposed); ♦ consumption rate calculated with Model 2; □ consumption rate predicted with Michaelis-Menten's equation.

at 4 °C (Figure 2 (A) for CO_2 and Figure 3 (A) for O_2); while at 20 °C and 35 °C the proposed model and the one in the literature present the same values predicted of R_{CO_2} and R_{O_2}. A cell change due to the incapacity of the enzymes associated to mitochondrial membranes to metabolize glycolysis products may happen at these low temperatures (Kader, 1994) therefore, model 2 may not give a good adjustment.

Relative standard deviation values of each one of the models are shown in table 4. Michaelis-Menten's kinetic model obtained an excellent adjustment for the experimental value with E<10% in the different temperatures, while the model reported in literature had the least fitting, except for R_{CO_2} and R_{O_2} in refrigeration temperature; this is due to this model adjustment expressed in the R^2 in table 1 is not adequate for this

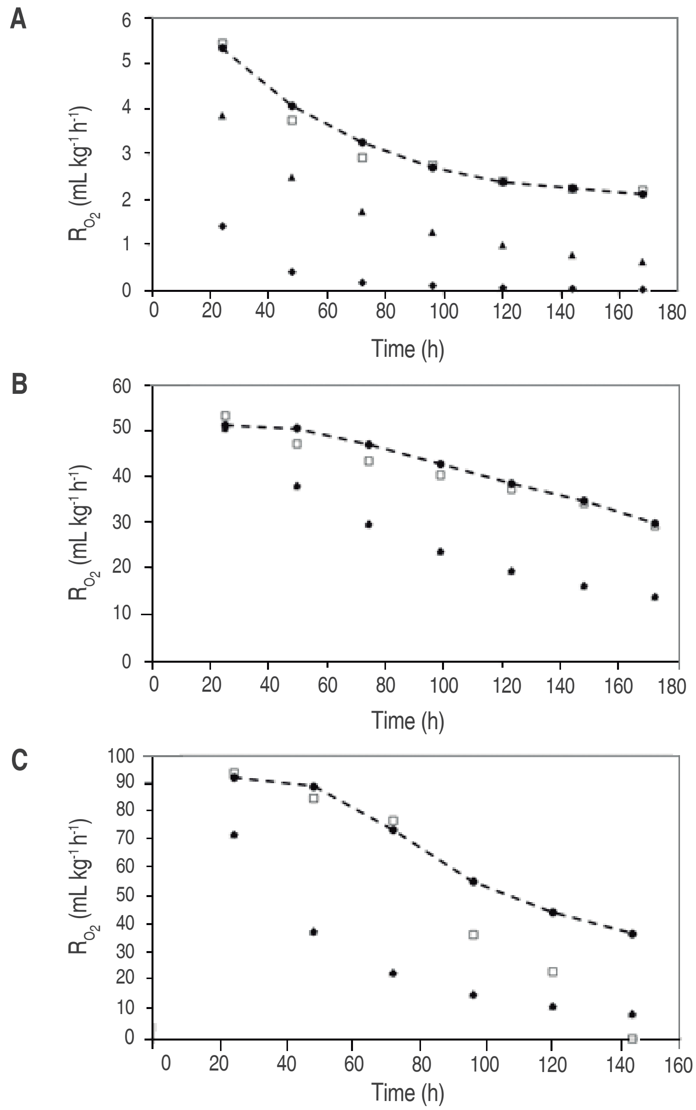

Figure 3. O_2 consumption rate (R_{O_2}) for cut mango stored at 4 °C **(A)**. 20 °C **(B)** and 30 °C **(C)**; -●- Experimental consumption rate; ▲ consumption rate calculated with Model 1 (proposed); ♦ consumption rate calculated with Model 2; □ consumption rate predicted with Michaelis-Menten's equation.

Table 4. Relative standard deviation for the models developed for the three storage temperatures.

Temperature (°C)	Relative standard deviation (%)			
	Respiration rate expressed as	Model 1	Model 2	Michaelis-Menten's
4	CO_2	45.68	90.35	7.69
	O_2	51.25	51.25	3.94
20	CO_2	38.52	38.51	4.79
	O_2	37.79	37.79	4.39
35	CO_2	32.01	32.01	9.18
	O_2	42.04	42.04	13.08

condition. Model 2, reported by Bhande *et al*. (2008) and Ravindra and Goswami (2008), could be useful in temperatures higher to 4 °C, but does not present good adjustment.

Table 5 shows Arrhenius type equation parameters, activation energy and pre-exponential factor for O_2 consumption and CO_2 production rates of the experimental data, proposed model 1 and Michaelis-Menten's equation. Activation energy values of each gas are very alike between experimental data and Michaelis-Menten's, evidencing a good adjustment of this equation to the sensitivity of the reaction to temperature; besides, activation energy values are between normal values, from 29 to 93 kJ mol^{-1} for fruit (Benítez *et al*., 2012). Certain effects could be observed in some cut mango pieces due to cold sensitive reactions generating a brown color, beginning around the vascular bundles, because of the polyphenol-oxidase action on the phenolics released out of the vacuole after freezing (Lee *et al*., 1996; Blanco-Díaz *et al*., 2016).

Table 5. Activation energy and pre-exponential factor of Arrhenius type equation for the experimental data, Model 1 and Michaelis-Menten's equation.

Gas	Arrhenius' equation parameters, Ea kJ/ mol					
	Experimental		Model 1		Michaelis-Menten's	
	Ea	Rp	Ea	Rp	Ea	Rp
CO_2	72.03	18.06×10^{13}	71.96	11.12×10^{13}	72.19	19.24×10^{13}
O_2	71.36	12.49×10^{13}	68.52	22.21×10^{12}	71.30	12.18×10^{13}

The descending form of the gradient in figures 2 and 3 indicates an inverse relationship between respiration rate and shelf life, which is due to O_2 reduces and CO_2 increases in an airtight container, consequently with what is observed in figure 1. It is also known that respiration rate decreases with CO_2 rising and O_2 dropping due to the first is a product of the reaction and the second is a reactive in it, as can be seen in the general reaction for respiration (Devanesan *et al*., 2011):

$$C_6H_{12}O_6 + 6O_2 \longrightarrow 6CO_2 + 6H_2O + 673\ \text{kcal}$$

Consequently and accordingly to Le Chatelier's principle, when increasing the concentration of a product or reducing the concentration of a reactive, the chemical reaction rate reduces.

CONCLUSIONS

Noncompetitive inhibition Michaelis-Menten's kinetic equation presented better results in the prediction of the respiration rate in contrast with others two models.

Models 1 and 2 are useful to predict gas concentration but do not predict the respiration rates.

Temperature was an influencing factor in O_2 consumption and CO_2 production of the cut mango determining a better preservation at 4 °C.

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