## The Development of a Model to Describe the Influence of Temperature and Relative Humidity on Respiration Rate of Prickly Pear Cactus Stems in Reduced O<sub>2</sub> Conditions

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#### Abstract

Respiration rate  $(R_{02})$  of prickly pear cactus stems (*Opuntia* spp.) was measured as a function of 4 temperature (T) and 6 relative humidity (RH) combinations for O<sub>2</sub> partial pressures between 15 and 0.8 kPa, which were considered to support aerobic respiration. The rate of respiration  $(R_{02})$  was determined based on O<sub>2</sub> depletion of the atmosphere in sealed containers containing 1 kg of stems. The O<sub>2</sub> partial pressure declined linearly over time and the slopes of the fitted lines were used to calculate the rate of O<sub>2</sub> uptake. The rate of O<sub>2</sub> uptake increased with increasing temperature and decreased with increasing RH. The respiratory rate at 25°C was approximately 30 to 40 times higher than at 5°C. The respiratory rate at 65% RH was between 30 and 90% greater than at 90% RH, depending on the temperature. Data for  $\ln(R_{02})$  for each RH level were regressed against the inverse of the T (K<sup>-1</sup>) to determine Arrhenius constants and calculate the apparent Ea of respiration for the six RH conditions. The Ea was similar for each RH level, varying between a low of 113 to a high of 131 kJ·mol<sup>-1</sup>. An equation having an R<sup>2</sup> of 0.95 was developed describing respiration as a function of RH and T (°C) using only four constants.

#### **INTRODUCTION**

Microbial contamination and physiological changes limit post-harvest shelf life of prickly pear cactus stems in refrigeration to approximately 6 days for stems with thorns and 2 days for stems without thorns (Guevara et al., 2001, 2003b). Modified atmosphere packaging (MAP) can inhibit post-harvest spoilage and has been shown to extend the cactus stem shelf-life for up to 30 days at 5°C (Guevara et al., 2001, 2003b). Optimum ranges for gas concentrations can be determined from physicochemical analysis and/or sensory tests, once appropriate limiting quality features are defined. Once optimum storage conditions are defined, needed package and film characteristics or features may be derived. Designing MAP systems, especially those that compensate for temperature changes during the postharvest holding period, requires knowledge of product (respiration rate, optimum gases concentration during storage), package (surface area and film thickness and permeability), and storage conditions (temperature and relative humidity).

Proc. IX<sup>th</sup> Intl. Contr. Atmos. Res. Conf. Ed.: R.M. Beaudry Acta Hort. 857, ISHS 2010 It is well recognized that the respiratory demand of fresh, harvested products is a function of temperature,  $O_2$  availability, and in some cases,  $CO_2$  concentration. Typically, low temperature, low  $O_2$  levels, and high  $CO_2$  levels cause a decrease in enzymatic activity, thus, reducing the uptake of  $O_2$  and the production of  $CO_2$ . However, although water is liberated in the process of respiration and water vapor loss is associated with the dissipation of respiratory and sensible heat, especially in leafy tissues, the impact of water vapor in the external atmosphere on respiratory activity has been little studied. Different equations have been developed to correlate respiration rate with different storage parameters such as  $O_2$  and  $CO_2$  concentrations (Salvador et al., 2002), but to our knowledge, the impact of the humidity of the storage environment on respiration has not been described mathematically.

A convenient way to measure respiration is to seal the plant product in containers and monitor gas concentrations in that container. Yang and Chinnan (1988), Cameron et al. (1989) and Talasila et al. (1992) used closed systems to measure and model respiratory activity under various temperature and atmospheric conditions. The closed system provides the additional benefit of permitting humidity modification using salt solutions within the sealed respiratory chamber. To date, no study has been performed to describe the aerobic respiratory activity of prickly pear cactus stems as a function of humidity and temperature.

The objectives of this work are to measure the aerobic respiration rate of prickly pear cactus stems as a function of temperature and RH within a range of non-limiting  $O_2$  levels and to develop a mathematical function describing that relationship for prickly pear cactus stems.

### **MATERIALS AND METHODS**

Prickly pear cactus (*Opuntia* spp.) stems (approx. 40 days from budding) were obtained from a local market in Querétaro, Mexico and were immediately brought to the laboratory and selected by size, uniformity and freedom from defects.

Oxygen uptake was monitored using a closed system (Guevara et al., 2003a; Haggar et al., 1992; Yahia et al., 2005) at 65–90% RH (at 5% intervals) and at 5, 14, 20 and 25°C. Sealed 4-L glass jars equipped with sampling ports (one per condition), each one with approximately 1 kg of cactus stems and 25 ml of different saturated saline solutions. The saline solutions were used to produce different RH; chambers were equilibrated for 12 h before each assay in temperature-controlled rooms. Changes in O<sub>2</sub> and CO<sub>2</sub> levels were monitored periodically using a NITEC portable O<sub>2</sub>/CO<sub>2</sub> gas analyzer, model GA-20 in a configuration that recirculated headspace gases. The data for the decline in the headspace oxygen as a function of holding period was fitted to a linear function to determine the rate of O<sub>2</sub> uptake for each of the 24 T/RH combinations. Only data considered to be a result of aerobic respiration were used; some initial data points, apparently associated with the adjustment of the measuring system were not used in the analysis. Data for CO<sub>2</sub> accumulation in the chamber headspace were not used for calculating respiratory activity because of concerns regarding the solubility of CO<sub>2</sub> in the cladodes, and to a lesser extent, in the salt solutions.

Once  $O_2$  uptake rates were determined, the data were transformed using the natural logarithm to normalize variance and to more evenly weight the data across the range of temperatures and thereby improve fit at the lower temperature. The natural logarithm of respiration was regressed against RH using linear curves for each of the four holding temperature. Intercept and slope constants for all four curves were regressed against temperature and fitted lines were used to create a model for all the data as a function of temperature (°C) and RH (%). Respiratory data were un-transformed and reported as ml·kg<sup>-1</sup>·hr<sup>-1</sup>. The natural logarithm of the respiratory data was also regressed against the inverse of temperature (K<sup>-1</sup>) for each of the six RH to determine Arrhenius constants including the apparent energy of activation, Ea. Statistical analysis was done using statistical analysis software for PC (Excel, Microsoft Corp.).

#### **RESULTS AND DISCUSSION**

The rate of  $O_2$  uptake declined linearly with time for each of the 24 T/RH combinations of 5, 14, 20 and 25°C and 65, 70, 75, 80, 85 and 90% RH (Fig. 1). The  $r^2$  for fitted linear lines ranged from a low of 0.98 to a high of 0.99 (Table 2). For all temperatures tested, the changes in  $O_2$  (and  $CO_2$ ) profiles for 85 and 90% RH were not different from one another, but differed markedly for depletion profiles for 65% and 70% RH. Linear changes in  $O_2$  partial pressure agree with the expected pattern of a linear decline for many products held in closed containers under aerobic conditions including tomato (*Lycopersicum esculentum* Mill.) by Cameron et al. (1989), fresh green onions (*Allium fistulosum* L.) by Hong and Kim (2001), and for different horticultural products by Jacxsens et al. (2000). Non-linearity in the decline of the atmospheric  $O_2$  level can be indicative of developmentally-dependent changes in the aerobic respiratory rate or the suppression of respiratory activity due to limiting  $O_2$  partial pressures. Several models have described  $O_2$  uptake as a function of  $O_2$  including partial pressures in the range capable of inhibiting the terminal oxidases in the electron transport pathway (Cameron et al., 1994; Hayakawa et al., 1975; Mannapperuma et al., 1989).

Even as the  $O_2$  partial pressures in this study did not apparently impact  $O_2$  depletion rate, the  $CO_2$  levels, which accumulated to partial pressures between 15 and 18 kPa by the conclusion of the study, also seemed to have had no impact on the  $O_2$  content of the containers. Data for  $CO_2$  inhibition of respiration in prickly pear cactus stems are not in the literature, and in fact, there is little to support a universal inhibition of  $CO_2$  on  $O_2$  uptake. Beaudry (1993) found that  $CO_2$  did not reduce  $O_2$  uptake of blueberry (*Vaccinium corymbosum* L.) fruit even at partial pressures from 20 to 60 kPa  $CO_2$ . Peppelenbos et al. (1993) noted little effect of  $CO_2$  on  $O_2$  uptake by mushrooms (*Agaricus bisporus* L.), and no effect of <20 kPa  $CO_2$  on  $O_2$  uptake was also noted by Joles et al. (1993) for several fruits. Kubo et al. (1990) measured the impact of  $CO_2$  on  $O_2$  uptake for 18 fruit and vegetables, several of which had no response or experienced an increase in  $O_2$  consumption. They suggested that high  $CO_2$  levels decreased or increased the respiration rate via its impact on ethylene biology. A more thorough exploration of the response of prickly pear respiration in response to  $CO_2$  will be needed to conclusively demonstrate the impact.

The slope of the linear equations (Table 2) applied to the  $O_2$  depletion curves yielded respiratory rates for the 24 T/RH combinations.  $R_{02}$  increased with temperature, but, at each temperature, declined with an increasing RH (Table 2). O<sub>2</sub> consumption increased 7- to 10-fold between 5 to 14°C, and approximately 5-fold between 14 to 25°C, which may be a result of a shift in physiological factors limiting metabolism in cactus stems. Similar results were obtained by Jacxsens et al. (2000) for broccoli florets. The extent of change in respiration with RH differed somewhat for the four temperature regimes; the increase in respiration was approximately 30, 60, 90 and 90% for 5, 14, 20 and 25°C, respectively. Curves fitted to respiratory data revealed that the increase in respiration with declining RH was roughly linear for each temperature (Table 2). The slopes for these fitted lines declined, and the intercepts increased, with increasing temperature. The relatively regular change in intercept and slope with temperature enabled fitting these data to linear curves; one describing the change in slope with temperature, and the other describing the change in the intercept with temperature (data not shown). When these equations were combined with the equation for changing respiration with humidity, they yielded a close fit between experimental and predicted values ( $R^2 = 0.97$ ), however, the predictive curves were not accurate at the lowest temperature (data not shown). For this reason, the data were transformed using the natural log of the respiratory data and the inverse of temperature (°K) as indicated in Table 3 to provide greater statistical weighting to the lower temperature data. Equations regressing  $\ln(R_{02})$  against RH for each temperature resulted in four lines with r<sup>2</sup> values ranging between 0.78 and 0.90 (Table 4). The constants for these lines were found to vary with temperature linearly (Table 4, right portion). Weighting the respiratory rates by using the natural logarithm eliminated the problem of inaccuracy at the lowest temperature, while lowering the fit only marginally ( $R^2 = 0.95$ , Fig. 3) and resulted in the following equation:

$$\ln(R) = \left(-0.1985 + 50.96 \times \left(\frac{1}{273.15 + T}\right)\right) \times RH + 67.64 - 18430 \times \left(\frac{1}{273.15 + T}\right)$$

where *T* is temperature in °C. Using this equation, the rate of  $O_2$  uptake can be reasonably well predicted across a range of temperatures and relative humidity. To our knowledge, no previous study has integrated the effect of RH and T in a predictive model.

The Arrhenius equation is typically applied to describe the temperature dependence of different biological reactions, and, like the  $Q_{10}$ , has been used to model the temperature responsiveness of respiration of fresh horticultural crops (Cameron et al., 1995). The values of  $\ln(R)$  regressed against T<sup>-1</sup> (T in °K) yielded essentially linear relationships with similar slopes (Fig. 2). There was no indication that the slope of the Arrhenius relationship varied with RH (Table 3). The apparent activation energy (*Ea*) of the six relationships varied between 113 to 131 kJ·mole<sup>-1</sup> and is a function of the slope of the Arrhenius relationship (slope = -*Ea*/R, where R = 0.0083144 kJ·mole<sup>-1</sup>·K<sup>-1</sup>). The average Ea was approximately 120 kJ·mole<sup>-1</sup>, which is relatively high compared to published values of 95, 55, 60 and 60 kJ·mole<sup>-1</sup> for broccoli, lettuce, blueberry, and strawberry, respectively (Cameron et al., 1995).

The relation of  $\ln(R_{O2})$  and T<sup>-1</sup> (T in °K) for experimental values may not be fully linear; there may be a change in the slope at 14°C, but additional temperature points would be needed to verify changing slope (Fig. 2). Haggar et al. (1992) and Song et al. (1992) observed a similar phenomenon. Cameron, et al. (1994) also reported a higher apparent *Ea* at lower temperature; they also noted that the apparent Ea increased 1.5- to 2fold as oxygen increased from levels that limited respiration to non-limiting levels.

#### CONCLUSIONS

The natural log of the oxygen consumption rate was described by a linear model dependent on RH in which the slope and constant were linear relationships with temperature as follows:

$$\ln(R) = \left(-0.1985 + 50.96 \times \left(\frac{1}{273.15 + T}\right)\right) \times RH + 67.64 - 18430 \times \left(\frac{1}{273.15 + T}\right)$$

and by Arrhenius equations for each humidity level, which varied linearly with the inverse of temperature (K). Parameters from the respiration model and kinetic data may be useful in designing effective packaging models for perishable horticultural products such as prickly pear cactus stems.

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## Tables

Table 1. Nomenclature and abbreviations used.

Abbreviation	Description	Units
R	Respiration rate	ml·kg <sup>-1</sup> ·h <sup>-1</sup>
RH	Relative humidity	%
Т	Temperature	°C or Kelvin
W	Fruit weight	kg
V	Free volume	L
t	Time	h
Ea	Activation energy	kJ∙mol <sup>-1</sup>

Table 2. Values for slope (m), intercept (b) and r<sup>2</sup> for linear best fit curves fitted to data in Figure 1 for container oxygen as a function of holding time at 24 different temperature/relative humidity combinations; the slope (O<sub>2</sub> uptake) units are in ml·kg<sup>-1</sup>·h<sup>-1</sup>. Also, linear best fit values (slope, intercept, and r<sup>2</sup>) for O<sub>2</sub> uptake data in columns regressed against relative humidity for each of the four temperatures.

	Temperature (°C)												
RH (%)	5				14			20			25		
	m	b	$r^2$	m	b	$r^2$	m	b	r <sup>2</sup>	m	b	$r^2$	
65	1.17	12.4	0.99	11.4	12.5	0.99	27.8	14.6	0.98	51.1	13.3	0.99	
70	1.17	13.7	0.99	10.1	12.3	0.99	25.8	14.5	0.98	39.0	12.8	0.99	
75	1.14	14.4	0.99	8.29	12.0	0.98	16.7	13.2	0.99	33.9	12.7	0.99	
80	1.09	15.1	0.99	7.21	12.0	0.99	15.2	13.6	0.99	29.0	12.1	0.98	
85	0.84	14.1	0.99	6.66	11.9	0.99	15.3	13.6	0.99	28.2	12.6	0.99	
95	0.89	15.0	0.99	6.94	12.1	0.99	14.8	13.5	0.99	26.9	12.7	0.99	
	Constants for best fit lines fitted to above slope data												
Slope	-0.01409			-	-0.1939			-0.5598		-0.9040			
intercept	2.142				23.47			62.66			104.7		
$R^2$	0.79				0.88			0.79			0.85		

Table 3. To the left, natural logarithm of oxygen uptake of prickly pear stems in Table 2 for 24 different relative humidity/temperature combinations. Also, to the right, linear best fit constants for  $r^2$ , intercept, and slope for ln(R) data regressed against the inverse temperatures (Kelvin) for each of the six humidity levels, by row. Units for the apparent energy of activation (Ea) are kJ.mol<sup>-1</sup>.

1/Temperature (K <sup>-1</sup> )						Arrhenius constants				
RH (%)	0.00360 (10°C)	0.00348 (15°C)	0.00341 (20°C)	0.00335 (25°C)	Slope	Intercept	$r^2$	Ea (kJ/mol)		
65	0.159	2.44	3.33	3.93	-15743	56.9	0.98	131		
70	0.157	2.32	3.25	3.66	-14832	53.7	0.97	123		
75	0.130	2.12	2.81	3.52	-13940	50.4	0.98	116		
80	0.0845	1.98	2.72	3.37	-13573	49.0	0.99	113		
85	-0.177	1.90	2.73	3.34	-14632	52.6	0.98	122		
90	-0.118	1.94	2.69	3.29	-14163	50.9	0.98	118		

Table 4. Linear best fit constants (slope, intercept, and  $r^2$ ) for ln(R) data in Table 3 regressed against relative humidity for each temperature. Also, to the right, constants for lines fitted to the slope and intercept constants, by row.

	_	1/Tempera	ature (K <sup>-1</sup> )	Change in constants with 1/T			
	0.00360	0.00348	0.00341	0.00335	Slana	Intercent	<b>r</b> <sup>2</sup>
	(10°C)	(15°C)	(20°C)	(25°C)	Slope	Intercept	I
Slope	-0.01391	-0.0222	-0.0275	-0.0248	50.96	-18430	0.81
Intercept	1.117	3.837	5.053	5.440	0.953	67.64	0.96
$r^2$	0.78	0.89	0.80	0.90			

# **Figures**



Fig. 1. Changes in headspace oxygen partial pressure (kPa) as a function of different temperatures and relative humidity. (●) 65% RH, (♥) 70% RH, (■) 75% RH, (♦) 80% RH, (♥) 85% RH and (○) 90% RH.



Fig. 2. The Arrhenius model applied to the Vm for oxygen, obtained from the power function. (●) 65% RH, (▽) 70% RH, (■) 75% RH, (◇) 80% RH, (▼) 85% RH and (○) 90% RH.



Fig. 3. Actual versus predicted oxygen uptake for prickly pear stems held at 24 combinations of temperature and relative humidity. Equation for modeled respiration: EXP((-0.1985+50.96\*(1/(273.15+T)))\*RH+67.638-18430\*(1/(273.15+T))).