Modelling respiration rate of shredded Galega kale for development of modified atmosphere packaging

Susana C. Fonseca ^a, Fernanda A.R. Oliveira ^{b,*}, Jesus M. Frias ^b, Jeffrey K. Brecht ^c, Khe V. Chau ^d

^a Escola Superior de Biotecnologia, Universidade Católica Portuguesa, Rua Dr. António Bernardino de Almeida, 4200-072 Porto, Portugal ^b Department of Process Engineering, University College Cork, Cork, Ireland

^c Horticultural Sciences Department, University of Florida, 1217 Fifield Hall, Gainesville, FL 32611-0690, USA

^d Agricultural and Biological Engineering Department, University of Florida, 37 Frazier Rogers Hall, Gainesville, FL 32611-0570, USA

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Abstract

The design of modified atmosphere packaging (MAP) for fresh-cut produce requires an adequate model for prediction of respiration rate as a function of both temperature and gas composition. In this work, the O_2 consumption and CO_2 production rates of shredded Galega kale were studied. The storage temperatures used were 1, 5, 10, 15 and 20 °C. The atmospheres tested were all combinations of 1, 5 and 10% v/v O_2 plus 0, 10 and 20% v/v CO_2 with the balance being N_2 , as well as ambient air. Temperature was the variable with the greatest influence on respiration rate and the effect of gas composition increased with temperature. The dependence of respiratory quotient (RQ) was found to be constant for the range of temperatures and gas compositions tested and was equal to 0.93 ± 0.01 . The constants of the Michaelis–Menten equation increased exponentially with temperature. The change over time of respiration rate of leaves exposed to air at 20 °C was also analysed. It was observed that respiration rate decreased with time and that the ratio between the respiration rate of shredded and intact leaves was approximately constant in the period tested and equal to 2.8. © 2002 Elsevier Science Ltd. All rights reserved.

1. Introduction

In Portugal shredded Galega kale is a traditional fresh-cut vegetable. It is thinly shredded and consumed in a soup. Galega kale (*Brassica oleracea var. acephala* DC) is a headless leafy cabbage with long petioles and large midribbed leaves. It represents an important contribution to the total production and consumption of vegetables in Portugal (Almeida & Rosa, 1996). This vegetable is very well adapted and grows all year round due to the mild winters and cool summers in Portugal (Monteiro & Dias, 1996). Galega kale was found to have higher levels of protein, calcium and magnesium than other Brassica crops (Rosa & Almeida, 1996). The potential market of shredded Galega kale is still unexploited by the food industry. The preparation and

preservation of shredded Galega kale is a challenging example in the fresh-cut produce's technology.

Due to damaged cells, fresh-cut products have shorter shelf life than intact products (Bolin & Huxsoll, 1991). Thus, techniques for extending fresh-cut product shelf life may have a major impact on the fresh-cut market. Refrigeration is essential for the preservation of these products and modified atmosphere packaging (MAP) is an important complementary technique. MAP is an atmosphere modification that relies on the interplay between the natural process of produce respiration and gas exchange through the package, leading to a build-up of CO_2 and depletion of O_2 . MAP retards respiration, ageing and oxidative reactions, and may suppress microbial growth (Gorris & Tauscher, 1999, Chap. 19).

The success of MAP greatly depends upon the choice of packing materials and on the package design. The higher respiration rates of fresh-cut products, as well as their higher tolerance to CO_2 in general, require the use of packaging materials with a high O_2 transmission

^{*}Corresponding author. Tel.: +353-21-490-2383; fax: +353-21-427-0249.

E-mail address: faroliveira@ucc.ie (F.A.R. Oliveira).

Nomenclature

F	flow rate (ml h^{-1})	ϕ,γ	Michaelis-Menten equation constants
f	model constant (Eq. (7)) (dimensionless)		(Eq. (8)) (% v/v)
M	product weight (kg)	ϕ_1, γ_1	Michaelis-Menten equation constants (Eq.
R	respiration rate (ml kg ^{-1} h ^{-1})		(10)) (% v/v)
RQ	respiratory quotient (dimensionless)	ho	product density (kg ml ^{-1})
SD	standard deviation	τ	Weibull model time constant (Eq. (5)) (h)
Т	temperature (°C)	$ au_{ m r}$	residence time of the air in the flow through
t	time (h)		system (h)
V	volume (ml)	~	
y	volumetric concentration (% v/v)	Supers	cripts
Caral		exp	experimental
Greek	Symbols	int	intact leaves
β	weibull model scale constant (Eq. (5)) (di-	pred	predicted by the model
	mensionless)	shr	shredded leaves
$ \Delta y $	absolute volumetric concentration change	in	at jar inlet
	during Δt (% v/v)	out	at jar outlet
Δt	time interval (h)	0	at time zero in the flow through system
α	Michaelis–Menten equation constant (Eq. (8)) (ml kg ⁻¹ h ⁻¹)	∞	at steady state in the flow through system
α_1	Michaelis–Menten equation constant	Subscr	<i>ipts</i>
	(Eq. (10)) (ml kg ⁻¹ h ⁻¹)	O_2	oxygen
α_2, ϕ_2, γ	y ₂ Michaelis–Menten equation constants	CO_2	carbon dioxide
-//2/	$(Eq. (10)) (°C^{-1})$	f	free

rate and alternative materials and packaging systems are being investigated, such as laser microperforated films and microporous membranes (Mannapperuma & Singh, 1994; Zagory, 1997) or perforation-mediated MAP (Fonseca, Oliveira, Lino, Brecht, & Chau, 2000). The design of a MA package requires a mathematical model relating respiration rate (both O₂ consumption and CO₂ production rates) to storage temperature and gas composition. Respiration involves a very complex set of biochemical reactions, which impairs the development of mechanistic models. The Michaelis-Menten equation has been thoroughly reported in the literature as giving good fits to experimental data on respiration rate of different products (Andrich, Fiorentini, Tuci, Zinnai, & Sommovigo, 1991; Cameron, Beaudry, Banks, & Yelanich, 1994; Hertog, Peppelenbos, Evelo, & Tijkens, 1998; Joles, Cameron, Shirazi, Petracek, & Beaudry, 1994; Lee, Haggar, Lee, & Yam, 1991; Lee, Song, & Yam, 1996; McLaughlin & Berine, 1999; Peppelenbos & van't Leven, 1996; Peppelenbos, van't Leven, van Zwol, & Tijskens, 1993; Ratti, Raghavan, & Gariépy, 1996; Solomos & Kanellis, 1989). CO₂ is often assumed to have an inhibitory effect on respiration, either uncompetitive, non-competitive or uncompetitive/competitive (Lee et al., 1991, 1996; McLaughlin & Berine, 1999; Peppelenbos & van't Leven, 1996; Renault, Souty, & Chambroy, 1994). The parameters of the model are however not true Michaelis-Menten constants describing a simple quasi-equilibrium enzymatic reaction, and therefore the choice of the inhibition mechanism is simply based on the quality of the fit and/or on the simplicity of the model.

The objectives of this work were: (i) to analyse the change over time of respiration rate of Galega kale after shredding, (ii) to analyse the influence of O_2 and CO_2 concentrations and temperature on the respiration rate, and (iii) to develop a predictive model relating respiration rate to O_2 and CO_2 concentrations and temperature that may be used in the design of MAP for this product.

2. Materials and methods

2.1. Produce and sample preparation

Galega kale plants were grown in the horticultural fields of the University of Florida, Gainesville, USA. Leaves at full maturity were picked early in the morning and transported immediately to the experimental site. The leaves were selected on the basis of uniform colour and absence of defects. The midrib was excised with a sharp knife and discarded. The leaves with midribs removed were washed to remove dirt and insects, shredded in a hand shredder machine (1.5 mm wide), washed with chlorinated water (100 ppm) for 30 s, and centrifuged in

a salad spinner to remove excess water. Intact leaves, not subjected to any treatment, were used as control.

2.2. Change of respiration rate after shredding

Shredded or intact leaves were placed in 1.7 l glass jars and weighed (approximately 150 g). The jar lids had stoppers for gas sampling and rubber tubes for gas flow. The inlet tube was inserted down to the bottom of the jar to ensure uniform flushing of the gas mixture. The jars were stored in a cold room at 20 ± 0.5 °C and a flow through system was used to allow to quantify the CO₂ production rate over time. A humidified stream of air at 20 °C was fed to the jars at a flow rate of 1.5 1 h^{-1} . The gas stream was humidified by bubbling in deionised water to avoid water loss that might influence the respiration rate. Weight variations were monitored and found to be less than 0.74% of the initial weight. Gas samples of 0.5 ml were taken at the jar inlet and outlet at selected sampling times with a 1.0 ml BD (Benton Dickinson, Rutherford, NJ, USA) plastic syringe with 23G1 BD needles. CO₂ production rate (R_{CO_2}) at a given time was calculated from a mass balance:

$$F \times y_{\rm CO_2}^{\rm out} = F \times y_{\rm CO_2}^{\rm in} + 100 \times R_{\rm CO_2} \times M - V_{\rm f} \times \frac{\mathrm{d}y_{\rm CO_2}^{\rm out}}{\mathrm{d}t},$$
(1)

where *F* is the gas flow rate, $y_{CO_2}^{in}$ and $y_{CO_2}^{out}$ are the CO₂ concentrations in the gas stream at the jar inlet and outlet, respectively, M is the weight of product in the jar and $V_{\rm f}$ is the free volume inside the jar. $V_{\rm f}$ can be calculated as

$$V_{\rm f} = V - M/\rho, \tag{2}$$

where V is the volume of the jar and ρ is the true density of the kale $(1.0 \times 10^{-3} \text{ kg ml}^{-1})$.

Two replicates were performed both for shredded and intact leaves. In order to assess jar seal effectiveness, a gas mixture of known composition was flushed into a jar with no produce, the jar sealed, and the gas composition measured over time; no variations of concentration were noticed.

2.3. Influence of gas composition and temperature on respiration rate

The respiration rate was analysed at 1, 5, 10, 15, and 20 °C. The atmosphere tested were: (i) all combinations of approximately 1, 5, and 10% v/v O₂ and 0, 10, and 20% v/v CO₂ with the balance N₂ and (ii) ambient air. The exact composition of the atmospheres used is shown in Table 1. These gas concentrations are within normal values recommended for MAP of fresh-cut products and the range of temperatures covers normal distribution and retail conditions. Three replicates were performed for each set of conditions.

1 °C			5 °C			10 °C			15 °C			20 °C		
у _{О2} (% v/v)	усо ₂ (% v/v)	$\mathbf{RQ}\pm\mathbf{S.D.}$	у _{О2} (% v/v)	yco ₂ (% v/v)	$RQ \pm S.D.$	УО ₂ (% v/v)	усо ₂ (% v/v)	$RQ \pm S.D.$	y _{O2} (% v/v)	усо ₂ (% v/v)	$RQ\pm S.D.$	УО ₂ (% v/v)	усо ₂ (% v/v)	$RQ\pm S.D.$
1.85	0.63	0.85 ± 0.12	1.39	0.58	0.97 ± 0.08	1.10	0.64	1.09 ± 0.26	1.58	0.60	1.27 ± 0.54	1.42	0.73	0.96 ± 0.19
1.03	9.53	0.68	1.59	10.25	1.14 ± 0.31	0.84	8.39	1.38 ± 0.50	1.47	11.02	0.98 ± 0.32	1.71	11.65	0.95 ± 0.21
1.07	19.80	1.27 ± 0.33	1.82	20.06	0.84 ± 0.19	1.00	18.53	1.05 ± 0.30	1.45	21.50	0.99 ± 0.25	1.14	19.11	0.79 ± 0.20
5.00	0.00	na	5.59	0.33	1.15 ± 0.18	3.88	0.63	0.90 ± 0.09	4.48	0.61	1.00 ± 0.12	4.80	0.93	0.92 ± 0.05
5.00	10.00	na	4.87	9.51	0.96 ± 0.26	4.05	11.10	0.82 ± 0.12	4.71	10.17	0.85 ± 0.09	4.67	10.22	0.92 ± 0.10
5.00	20.00	na	5.61	20.88	0.85 ± 0.26	3.86	21.79	0.97 ± 0.17	5.25	20.56	0.88 ± 0.12	4.98	17.66	0.78 ± 0.08
9.26	0.48	0.87 ± 0.07	10.38	0.55	1.01 ± 0.18	11.57	0.44	0.94 ± 0.07	10.00	1.04	1.02 ± 0.14	11.01	1.07	0.93 ± 0.04
9.22	10.01	0.85 ± 0.13	11.25	9.94	1.01 ± 0.22	10.26	10.15	0.91 ± 0.12	10.06	11.27	1.04 ± 0.14	11.60	16.99	0.84 ± 0.16
10.41	18.12	1.42 ± 0.45	8.73	17.41	0.85 ± 0.10	11.01	18.89	0.96 ± 0.11	10.67	19.82	0.96 ± 0.11	9.31	20.54	0.82 ± 0.08
20.16	0.34	0.81 ± 0.20	21.97	0.18	0.90 ± 0.12	21.51	0.40	0.90 ± 0.13	21.32	0.34	1.08 ± 0.08	20.22	1.24	0.95 ± 0.13

not available

The closed system was chosen to measure the respiration rate. Shredded kale was placed in 1.7 l glass jars and weighed (approximately 150 g). The jar lids had stoppers for gas sampling and rubber tubes for gas flow as described before. The jars were stored at different temperatures in cold rooms equipped with a gas mixing board and flushed with the humidified selected mixtures of O₂, CO₂, and N₂ for 24 h before measurements, to equilibrate the samples (the flow rate inside each jar was constant and equal to $6 \ l \ h^{-1}$). The gas flow was then halted, the gas stream inlet and outlet closed, and gas samples of 0.5 ml were withdrawn from the jars. Other samples were withdrawn at given times up to 560 min, depending on temperature conditions. Changes in gas composition during these periods were smaller than 4%v/v. At least two samples were taken from each jar at different times.

 O_2 consumption and CO_2 production rates were determined as:

$$R = \frac{V_{\rm f} \times |\Delta y|}{100 \times M \times \Delta t},\tag{3}$$

where $|\Delta y|$ is the absolute value of concentration changes during the time interval Δt .

2.4. Gas concentration analysis

The gas samples were analysed with a Gow-Mac series 580 gas chromatograph (Gow-Mac Instrument, Bridgewater, NJ, USA) and a HP 3396 series II integrator (Hewlett Packard, Avondale, PA, USA). The gas chromatograph was equipped with two columns in series and a thermal conductivity detector. One column was a porous polymer column (80–100 mesh Columpak PQ) and the other was a molecular sieve 5A column (60–80 mesh). The gas carrier was helium at a pressure of 275 kPa and a flow rate of $1.8 \ l h^{-1}$. Temperature of both columns was set at 44 °C and temperature of both columns was set at 44 °C and temperature of injector and detector was performed using a single calibration mixture of 7.04% v/v O₂ and 7.03% v/v CO₂.

2.5. Model parameter estimation

The model constants were estimated by fitting the model to the experimental data by non-linear regression using the Statistica software (release 5.1, 97 edition, Statsoft, Tulsa, OK, USA).

3. Results and discussion

3.1. Modelling the change of CO_2 production rate after shredding

A preliminary analysis of the change of CO_2 production rate with time was done by calculating its value at every experimental time, using Eq. (1) after a simple manipulation

$$R_{\rm CO_2} = \frac{F}{100 \times M} \times \left(y_{\rm CO_2}^{\rm out} - y_{\rm CO_2}^{\rm in} \right) + \frac{V_{\rm f}}{100 \times M} \times \frac{\mathrm{d}y_{\rm CO_2}^{\rm out}}{\mathrm{d}t}.$$
(4)

This analysis showed that: (i) the ratio between the CO_2 production rate of shredded and intact kale was constant with time and equal to 2.8, (ii) CO_2 production rate decreased with time, and (iii) this dependence could be well described by the Weibull model levelling off to an equilibrium value (Seber & Wild, 1989, Chap. 7; Weibull, 1951):

$$\frac{R_{\rm CO_2} - R_{\rm CO_2}^{\infty}}{R_{\rm CO_2}^0 - R_{\rm CO_2}^{\infty}} = e^{-(t/\tau)^{\beta}},\tag{5}$$

where R_{CO_2} , $R_{CO_2}^0$ and $R_{CO_2}^\infty$ are the CO₂ production rates at time *t*, at the beginning of the experiment and when the system becomes stable, respectively, and τ and β are the model constants, respectively, a time and a scale parameter.

Substituting Eq. (5) in Eq. (1) and integrating the resulting equation with respect to time, we get

$$y_{\rm CO_2}^{\rm out} = y_{\rm CO_2}^{\rm in} + \frac{100 \times M}{V_{\rm f}} \times \left[\tau_{\rm r} \times R_{\rm CO_2}^{\infty} \times \left(1 - e^{-t/\tau_{\rm r}} \right) + \frac{R_{\rm CO_2}^0 - R_{\rm CO_2}^{\infty}}{1/\tau_{\rm r} - (\beta \times t^{\beta-1})/\tau^{\beta}} \times \left(e^{-(t/\tau)^{\beta}} - e^{-t/\tau_{\rm r}} \right) \right],$$
(6)

where $\tau_{\rm r}$ is the residence time of the air in the jar $(=V_{\rm f}/F)$.

Because the ratio between the CO_2 production rate of shredded and intact kale is constant with time, Eq. (6) may be re-written as

$$y_{CO_{2}}^{out} = y_{CO_{2}}^{in} + \frac{100 \times M}{f \times V_{f}} \left[\tau_{r} \times R_{CO_{2}}^{\infty, shr} \left(1 - e^{-t/\tau_{r}} \right) + \frac{R_{CO_{2}}^{0, shr} - R_{CO_{2}}^{\infty, shr}}{1/\tau_{r} - (\beta \times t^{\beta-1})/\tau^{\beta}} \left(e^{-(t/\tau)^{\beta}} - e^{-t/\tau_{r}} \right) \right], \quad (7)$$

where $R_{CO_2}^{0,\text{shr}}$ and $R_{CO_2}^{\infty,\text{shr}}$ are the CO₂ production rates of shredded kale at the beginning of the experiment and at steady-state, respectively, and *f* is 1 for shredded kale and 2.8 for intact kale.

Fig. 1 shows the change of CO_2 production rate with time both for shredded and intact Galega kale leaves at 20 °C under ambient air. This dependence was estimated by fitting Eq. (7) to the CO_2 concentration measured in the gas stream at the jar outlet with the flow through system. Fig. 2 shows the good relationship between the experimental data and the model and Table 2 summarises the estimates of the constants and relevant statistical data. The model fits the data well, as shown by the statistical data: all the parameters are significant, the



Fig. 1. Change of CO₂ production rate with time at ambient air and T = 20 °C, using Eq. (5) and the model constants in Table 2 (— shredded leaves, — intact leaves).



Fig. 2. Relationship between CO_2 concentration measured in the gas stream at the jar outlet in the experiments for determination of CO_2 production rate with the flow through system, and the values predicted using Eq. (7) and the model constants in Table 2.

correlation between parameters is small, and the variance explained by the model is high.

As shown in Fig. 1, CO₂ production rates decreased with time and levelled off at 161.7 and 57.8 ml kg⁻¹ h⁻¹, respectively, for shredded and intact leaves, after 24 h. Smyth, Song, & Cameron (1998) have also reported a decrease of CO₂ production rate over time for cut Iceberg lettuce at 5 °C under CO₂-scrubbed air. In terms of packaging design, this pattern of respiration rate change with time would only affect the time needed to achieve steady state concentrations. Thus, it would be advantageous to pack the product as soon as possible, as the initial respiration rate (405.5 and 144.8 ml kg⁻¹ h⁻¹, respectively, for shredded and intact leaves) is approximately threefold that at steady state.

The greater respiration rates of shredded leaves (approximately threefold that of intact leaves) most probably are due both to the physiological response to wounding (Brecht, 1995) and to increased surface area (Bastrash, Makhlouf, Castaigne, & Willemot, 1993). Respiration rate increases from 2 to 3 times that of intact fruit were reported for apple slices (Lakakul, Beaudry, & Hernandez, 1999).

3.2. Influence of gas composition and temperature on respiration rate

The O₂ consumption (R_{O_2}) and CO₂ (R_{CO_2}) production rates of Galega kale ranged from 5.6 ± 1.6 to 161 ± 22 ml kg⁻¹ h⁻¹ and from 7.9 ± 1.1 to $153 \pm$ 4 ml kg⁻¹ h⁻¹, respectively, over all the combinations of O_2 levels, CO_2 levels, and storage temperatures tested (Fig. 3). Respiration rate decreased with a decrease in O_2 concentration and temperature, and increased with a decrease in CO₂ concentration. Temperature was the variable with the greatest effect on respiration rate: lowering the temperature of samples stored in air from 20 to 1 °C decreased R_{CO_2} and R_{O_2} by 90% and 88%, respectively, whereas changing the atmosphere composition from air to 1% v/v O₂ and 20% v/v CO₂ at 20 °C decreased R_{CO_2} and R_{O_2} by 80 and 76%, respectively. At 1 °C, changing the atmospheric composition from air to 1% v/v O₂ and 20% v/v CO₂ decreased R_{CO_2} and R_{O_2} by

Table 2

Parameter estimates of the mathematical model describing the change of respiration rate with time (Eq. (5)) and relevant statistical data

Model constant Estimate \pm S.E.		Correlation coefficient between the model constants				
		$R_{ m CO_2}^{0, m shr}$	$R^{\infty,\mathrm{shr}}_{\mathrm{CO}_2}$	τ		
$ \begin{array}{c} R_{\rm CO_2}^{0,\rm shr} ~({\rm ml}~{\rm kg}^{-1}~{\rm h}^{-1}) \\ R_{\rm CO_2}^{\infty,\rm shr} ~({\rm ml}~{\rm kg}^{-1}~{\rm h}^{-1}) \\ \tau ~({\rm h}) \\ \beta ~({\rm dimensionless}) \end{array} $	$\begin{array}{c} 405.5 \pm 23.5 \\ 161.7 \pm 4.9 \\ 5.2 \pm 1.1 \\ 0.72 \pm 0.06 \end{array}$	0.074 -0.815 -0.727	-0.555 0.450	0.262		
<i>N</i> = 129		$R^2 = 96.3\%$				

N – number of experimental points, R^2 – variance explained by the model.



Fig. 3. O₂ consumption rates for the different gas concentrations and temperatures tested: (a) $y_{CO_2} = 0\%$; (b) $y_{CO_2} = 10\%$; (c) $y_{CO_2} = 20\%$ ($\Diamond T = 1 \degree C$, $\Delta T = 5 \degree C$, $\bigcirc T = 10 \degree C$, $\Box T = 15 \degree C$, $\Diamond T = 20 \degree C$, — individual model, — overall model). The bars represent the standard deviation.

only 50% and 68%, respectively. These results stress the importance of cooling in the extension of fresh-cut produce shelf life and show that the effect of gas composition is more important at above optimum temperatures, as previously reported by Kader (1987) and Emond, Chau, and Brecht (1993).

The respiratory quotient (RQ), ratio of CO₂ production and O₂ consumption rates, ranged from 0.81 ± 0.20 to 1.4 ± 0.5 and did not show any dependence on temperature or gas composition (Table 1). These values are within the range of those reported in the literature regarding aerobic respiration (Kader, Zagory, & Kerbel, 1989). Thus, there was no evidence of anaerobic respiration for the conditions of temperature and gas composition tested and one can assume that the RQ breakpoint of shredded kale (lowest O₂ concentration that does not induce anaerobic respiration) is lower than 1% v/v O₂ in the range of temperatures tested. The RQ value estimated by linear regression of R_{CO_2} vs. R_{O_2} was equal to 0.93 ± 0.01 (R^2 adj = 99.2%).

3.3. Modelling the influence of gas composition and temperature on respiration rate

The dependence of respiration on gas composition was modelled by a Michaelis–Menten type equation, with O_2 as substrate and CO_2 uncompetitive inhibition and constant RQ

$$R_{\rm O_2} = \frac{\alpha \times y_{\rm O_2}}{\phi + y_{\rm O_2} \times (1 + (y_{\rm CO_2})/\gamma)},\tag{8}$$

$$\mathbf{RQ} = \frac{R_{\rm CO_2}}{R_{\rm O_2}},\tag{9}$$

where R_{O_2} and R_{CO_2} are the O₂ consumption and the CO₂ production rate, respectively, y_{O_2} and y_{CO_2} are the



Fig. 4. Dependence of the constants of the Michaelis–Menten equation (Eq. (8)) on temperature. The dots represent the estimates of the individual model (the bars represent the standard error of the estimates), whereas the lines represent the fit of the overall model.

volumetric percentages of O₂ and CO₂, respectively, and α , ϕ and γ are the model constants. Fig. 3 shows the fit of this model to the experimental data (78.2% $\leq R^2 \leq$ 94.2%). Similar fits were obtained when assuming non-competitive and uncompetitive/competitive inhibition

mechanisms, yet the non-competitive model was found to be structurally indistinguishable from the selected model (Walter & Pronzato, 1997) and the uncompetitive/competitive model has a greater number of constants. The constants of the model (α , ϕ and γ) increased exponentially with temperature (Fig. 4). This dependence was included in Eq. (8), yielding global model that describes the effects of both gas composition and temperature (*T*) on respiration rate

$$R_{O_2} = \frac{\alpha_1 \times e^{(\alpha_2 \times T)} \times y_{O_2}}{\phi_1 \times e^{(\phi_2 \times T)} + y_{O_2} \times (1 + (y_{CO_2})/(\gamma_1 \times e^{(\gamma_2 \times T)}))}.$$
(10)

Eqs. (9) and (10) where then fitted to the whole set of experimental data by non-linear regression, estimating the model constants $\alpha_1, \alpha_2, \phi_1, \phi_2, \gamma_1$ and γ_2 . The model appropriately describes both the influence of gas composition and temperature, as shown by the high R^2 (96.6%). The scatter plot in Fig. 5 shows the fair agreement between predicted and experimental respiration rates, both for O₂ consumption and CO₂ production rates. Table 3 summarises the estimates of the model constants and relevant statistical data. The correlation between the model constants was generally low, except between the constants α_1 and α_2 , ϕ_1 and ϕ_2 and γ_1 and γ_2 (correlation coefficients of 0.96, 0.98 and 0.96, respectively). However, absolute values below 0.99 are considered acceptable (Bates & Watts, 1988).

The CO₂ production rate of shredded leaves under air at 20 °C predicted by the model is 158 ml kg⁻¹ h⁻¹, which is within the standard error of $R_{CO_2}^{\infty,\text{shr}}$ reported in Table 2.



Fig. 5. Relationship between experimental respiration rates and those predicted using the Michaelis–Menten equation with CO_2 uncompetitive inhibition, assuming an exponential dependence of the model constants on temperature (Eq. (10)) and constant RQ.

Table 3 Parameter estimates of the mathematical model describing the influence of gas composition and temperature on respiration rate (Eqs. (9) and (10)) and relevant statistical data

Model constant	$Estimate \pm S.E.$	Correlation coefficient between the model constants							
		α1	α2	ϕ_1	ϕ_2	γ_1			
$\alpha_1 \ (ml \ kg^{-1} \ h^{-1})$	17.6 ± 0.7								
$\alpha_2 (^{\circ}C^{-1})$	0.124 ± 0.002	-0.96							
$\phi_1 (\% v/v)$	0.30 ± 0.06	0.67	-0.62						
$\phi_2 (^{\circ} \mathrm{C}^{-1})$	0.14 ± 0.01	-0.69	0.69	-0.98					
$\gamma_1 (\% v/v)$	14.3 ± 2.3	-0.48	0.46	-0.02	0.03				
γ_2 (°C ⁻¹)	0.05 ± 0.01	0.45	-0.47	0.03	-0.04	-0.96			
N = 480		$R^2 = 96.6\%$							

4. Conclusions

Both intact and shredded Galega kale leaves stored in air at 20 °C showed a decrease of CO₂ production rate with time, the initial value being approximately threefold that at steady state. Under these conditions, the respiration rate of shredded leaves was 2.8 times that of intact leaves. Temperature was the variable with the greatest influence on respiration rate and the effect of gas composition was found to increase with temperature. This stresses the importance of product refrigeration and suggests that the use of MAP is more important when the product is handled at above optimum temperature. The RQ was independent of both temperature and gas composition within the ranges of those variables that were tested. A Michaelis-Menten type equation with O_2 as substrate, uncompetitive inhibition of CO_2 and an exponential increase of the equation constants with temperature appropriately described the effect of temperature and gas composition on respiration rate. This model may be used to design an appropriate MA package for shredded kale, although further studies are required to analyse the effect of time on respiration rate at different temperatures and gas composition.

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