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## Enzyme kinetics modelling approach to evaluate the impact of high CO<sub>2</sub> and super-atmospheric O<sub>2</sub> concentrations on respiration rate of pomegranate arils

Zinash A. Belay<sup>a</sup>, Oluwafemi J. Caleb<sup>b</sup> and Umezuruike Linus Opara<sup>a,c</sup>

<sup>a</sup>Postharvest Technology Research Laboratory, South African Research Chair in Postharvest Technology, Department of Food Science, Faculty of AgriSciences, Stellenbosch University, Stellenbosch, South Africa; <sup>b</sup>Postharvest and Wine Technology, Agricultural Research Council Infruitec/Nietvoorbij, Stellenbosch, South Africa; <sup>c</sup>Postharvest Technology Research Laboratory, South African Research Chair in Postharvest Technology, Department of Horticultural Science, Faculty of AgriSciences, Stellenbosch University, Stellenbosch, South Africa

### ABSTRACT

Super-atmospheric O<sub>2</sub> has been shown to affect respiration rate (RR), but no model describing its effect on RR for pomegranate arils has been reported. This study investigated the effects of four different gas compositions (5 kPa O<sub>2</sub>, 10 kPa CO<sub>2</sub> and 85 kPa N<sub>2</sub>; 10 kPa O<sub>2</sub>, 5 kPa CO<sub>2</sub> and 85 kPa N<sub>2</sub>; 70 kPa O<sub>2</sub>, 10 kPa CO<sub>2</sub> and 20 kPa N<sub>2</sub>; and air) on RR of pomegranate arils (cv. Wonderful) stored at 5°C. Michaelis–Menten enzyme kinetics models were used to investigate the effect of CO<sub>2</sub> inhibition on O<sub>2</sub> consumption rate. Respiratory quotient was used to determine fermentation threshold. The O<sub>2</sub> consumption rate increased from 0.87 to 2.81 mL/kg h, with increase in O<sub>2</sub> concentration from 5 kPa to 70 kPa. All enzyme kinetics model parameters adequately described the influence of gas concentration on aril RR with correlation coefficient ( $R^2_{adj} = 81\text{--}91\%$ ).

### Modelado de cinética enzimática para evaluar el impacto de altas concentraciones de CO<sub>2</sub> y de concentraciones súper-atmosféricas de O<sub>2</sub> en la frecuencia respiratoria de arilos de granada

#### RESUMEN

Se ha demostrado que el O<sub>2</sub> súper-atmosférico incide en la frecuencia respiratoria (FR); sin embargo, no existe ningún modelo que describa su efecto en la FR de los arilos de granada. El presente estudio analizó los efectos que cuatro composiciones diferentes de gas (5 kPa O<sub>2</sub>, 10 kPa CO<sub>2</sub> y 85 kPa N<sub>2</sub>; 10 kPa O<sub>2</sub>, 5 kPa CO<sub>2</sub> y 85 kPa N<sub>2</sub>; 70 kPa O<sub>2</sub>, 10 kPa CO<sub>2</sub> y 20 kPa N<sub>2</sub>; y aire) tuvieron en la FR de arilos de granada (cv. Wonderful) almacenados a 5°C. Así, para investigar el efecto de inhibición que el CO<sub>2</sub> produce en la tasa de consumo de O<sub>2</sub> se utilizaron modelos de cinética enzimática de Michaelis–Menten (MM), mientras que para determinar el límite de fermentación se empleó el cociente respiratorio (CR). Se constató que el aumento de la concentración de O<sub>2</sub> de 5 kPa a 70 kPa produjo un incremento de la tasa de consumo de O<sub>2</sub>, que se elevó de 0.87 a 2.81 mL/kg h. Todos los parámetros del modelado de cinética enzimática describen adecuadamente la influencia de las concentraciones de gas en la FR de arilos de granada, con el coeficiente de correlación ( $R^2_{adj} = 81\text{--}91\%$ ).

### ARTICLE HISTORY

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### KEYWORDS

Modified atmosphere; gas flushing; postharvest physiology; Michaelis–Menten; *Punica granatum* L

### PALABRAS CLAVE

Atmósfera modificada; inyección de gas; fisiología poscosecha; Michaelis–Menten; *Punica granatum* L.

## Introduction

After harvest, fresh produce are susceptible to increased physiological stress and deterioration in quality due to accelerated respiration rate (RR) (Aindongo, Caleb, Mahajan, Manley, & Opara, 2014; Caleb, Mahajan, Opara, & Witthuhn, 2012a). This process consumes O<sub>2</sub> and produces CO<sub>2</sub> in a series of enzymatic reaction (Iqbal, Rodrigues, Mahajan, & Kerry, 2009). Thus, higher RR could indicate a faster rate of physiological aging, senescence and deterioration of fresh produce (Cliffe-Byenes & O'Beirne, 2007). Improved postharvest handling methods which apply modified atmosphere (MA) around the produce can be used to reduce RR and slow down metabolic processes, thereby maintaining quality and extending shelf life (Belay, Caleb, & Opara, 2016; Caleb et al., 2012a; Jing-Jun et al., 2012).

Studies have reported different approaches to determine suitable gas concentration for reducing the RR of pomegranate arils. Ersan, Gunes, and Zor (2010) investigated the effect of O<sub>2</sub> (2, 10 and 21 kPa) and CO<sub>2</sub> (0, 10 and 20 kPa) concentration on

the RR of pomegranate arils (cv. Hicaznar) at 5°C. Similarly, Banda, Caleb, Jacobs, and Opara (2015) studied the effect of O<sub>2</sub> (5, 21 and 30 kPa) and CO<sub>2</sub> (0, 10 and 40 kPa) on the RR of 'Wonderful' pomegranate arils. These studies showed that low O<sub>2</sub> atmosphere significantly reduced the RR. In addition, 2–4 kPa O<sub>2</sub> was recommended for maintaining qualities of 'Mollar de Elche' pomegranate arils by López-Rubira, Conesa, Allende, and Artés (2005). Overall, the results reported in these studies demonstrated that the application of MA systems was effective in maintaining quality and extended shelf life of pomegranate arils. However, low O<sub>2</sub> concentration can cause the build-up of anaerobic atmospheres which then leads to fermentation. Recent studies have introduced the benefits of super-atmospheric O<sub>2</sub> (>70 kPa) to overcome the limitation of low O<sub>2</sub> atmosphere on postharvest physiology and quality maintenance of fresh produce (Belay, Caleb, & Opara, 2017; Maghoumi et al., 2013; Molinu et al., 2016).

In addition, predicting respiratory kinetics of different fruits and vegetables has been based on consideration of

the RR as a function of gas composition using enzyme kinetics model (Belay et al., 2016; Iqbal et al., 2009; Torrieri, Perone, Cavella, & Masi, 2010). For pomegranate fruit, Caleb, Opara, and Witthuhn (2012b) investigated the effect storage temperature (5, 10 and 15°C) and time on RR of pomegranate arils (cvs. Acco and Herskawitz) by using Arrhenius-type model. Similarly, Aindongo et al. (2014) studied the effect of temperature (5, 10, 15 and 22°C) on RR of different pomegranate fractions cv. 'Bhagwa' by using Arrhenius equation. These studies reported that temperature had significant effect on RR of pomegranate aril and Arrhenius-type model adequately predicted this effect regarding different temperatures and cultivars used.

On the other hand, the effects of gas composition (2, 10, 21 kPa O<sub>2</sub> and 0, 10, 20 kPa CO<sub>2</sub>) on the RR of pomegranate arils (cv. Hicaz) was studied by Ersan et al. (2010) using Michaelis–Menten (MM) model. The study showed that MAs affected the RR of pomegranate arils and this relationship was best expressed using the MM competitive inhibition model. However, their study did not report on the application of super-atmospheric O<sub>2</sub> condition and its effects on the physiological response of pomegranate arils cv. 'Wonderful'. In addition, pomegranate fruit undergo a long-term shipping from southern hemisphere (producers) to northern hemisphere (market) and vice versa, prior to processing into arils (Belay et al., 2017). There is limited report on the impacts of this long supply chain on the physiology of the fruit. Therefore, the aim of this study was to investigate the effects of low and super-atmospheric O<sub>2</sub> combined with low and high CO<sub>2</sub> concentrations on the RR of pomegranate arils (cv. Wonderful) after long-term storage. The goal of this study is to provide valuable information on the respiratory kinetics of pomegranate arils cv. 'Wonderful', which would guide the role players along the value chain to better design and optimize the postharvest handling and packaging of arils.

## Materials and methods

### Plant materials and sample preparation

Pomegranate fruits (cv. Wonderful) were obtained at commercially ripened stage with characteristic deep-red skin and aril with mature kernel (Mphahlele, Caleb, Fawole, & Opara, 2016), from Sonlia Packhouse, Wellington, Western Cape (33° 38' 23" S, 19° 00' 40" E), South Africa. Fruits were transported in an air-conditioned and ventilated vehicle to the Postharvest Research Laboratory at Stellenbosch University and stored in a cold room (about 5°C and 95% relative humidity (RH)) for 4 months until the experiment started. The storage was done to simulate pre-shipping storage plus long-term shipping duration from southern hemisphere production region to the northern hemisphere market and vice versa. Damaged fruits were removed and the outer skin of selected healthy fruits was surface disinfected using 70% ethanol solution (Aindongo et al., 2014). Arils were extracted manually by carefully removing the husk. Extracted arils were collected in a tray and mixed together to create a homogenous batch of all the fruits. Arils (350 g) were transferred to 3000 mL airtight glass jars, which were designed to achieve a completely hermetic seal. The glass jars were prepared in triplicate for each gas mixture and stored at 5°

**Table 1.** Experimental gas combinations investigated.

**Tabla 1.** Combinaciones experimentales de gas investigadas.

Modified atmospheres	Name	Gas composition
Low oxygen	MA-1	5 kPa O <sub>2</sub> + 10 kPa CO <sub>2</sub> + 85 kPa N <sub>2</sub>
	MA-2	10 kPa O <sub>2</sub> + 5 kPa CO <sub>2</sub> + 85 kPa N <sub>2</sub>
Super-atmospheric oxygen	MA-3	70 kPa O <sub>2</sub> + 10 kPa CO <sub>2</sub> + 20 kPa N <sub>2</sub>
Passive atmosphere	MA-4	21 kPa O <sub>2</sub> + 0.03 kPa CO <sub>2</sub> + 78 kPa N <sub>2</sub>

C. Measurement was performed using four gas mixtures as shown in Table 1.

### Experimental set-up

Rate of O<sub>2</sub> consumption ( $R_{O_2}$ ) and CO<sub>2</sub> evolution ( $R_{CO_2}$ ) of the arils were measured using a closed system as described by Iqbal et al. (2009), Mahajan and Goswami (2001) and Techavuthiporn and Boonyaritthongchai (2016). Airtight jars of size 3000 mL made up of glass fitted with lid were used. Each jar lid had three valves (inlet, outlet and gas sampling ports). A rubber ring was fixed between the bottle and the lid seal from air leakage. A plastic tube was attached to the inlet valve, which was inserted down to the bottom of the jar to ensure uniform flushing of gas mixture. Before sealing, jars were flushed with humidified air and the selected gas composition, until equilibrium archived. Three jars were flushed for each of the four MAs, resulting in 12 jars. The headspace gas (about 100 µL) was sampled at hourly intervals for the duration of 5 h at 5°C through the silicon septum provided in the jar lid. The headspace gas was analysed for O<sub>2</sub> and CO<sub>2</sub> concentrations using O<sub>2</sub>/CO<sub>2</sub> gas analyser (Checkmate 3, PBI Dansensor, Ringstead, Denmark). This measurement cycle was repeated over a period of 12 days, based on experimental design reported by Ayhan and Esturk (2009). The  $R_{O_2}$  and  $R_{CO_2}$  were calculated using Equations (1) and (2):

$$R_{O_2} = \left( \frac{Y_{O_{2t}} - Y_{O_{2i}}}{t - t_i} \right) * \frac{V_f}{W} * \frac{1}{RT} \quad (1)$$

$$R_{CO_2} = \left( \frac{Y_{CO_{2t}} - Y_{CO_{2i}}}{t - t_i} \right) * \frac{V_f}{W} * \frac{1}{RT} \quad (2)$$

where  $Y_{O_{2i}}$  and  $Y_{O_{2t}}$  are, respectively, O<sub>2</sub> concentration (kPa) at the initial time  $t_i$ (hours, h) and at time  $t$  (h) and  $Y_{CO_{2i}}$  and  $Y_{CO_{2t}}$  are, respectively, CO<sub>2</sub> concentration (kPa) at the initial time  $t_i$ (h) and at time  $t$  (h).  $R_{O_2}$  and  $R_{CO_2}$  are RR in mL/kg h,  $T$  is temperature (Kelvin),  $R$  is universal gas constant (0.008314 kJ/K mol),  $W$  is the total weight of the product (kg) and  $V_f$  is the free volume ( $V_f = V - \frac{W}{\rho}$ ), where  $V$  is the volume of the jar (m<sup>3</sup>),  $W$  is the weight of the minimally processed arils (kg) and  $\rho$  the apparent density of the arils (0.98 g/cm). The ratio of CO<sub>2</sub> produced to O<sub>2</sub> consumption is further expressed by the respiration quotient as follows:

$$RQ = \frac{R_{CO_2}}{R_{O_2}} \quad (3)$$

### Development of mathematical model of aril RR

RR was described by enzyme kinetics approach as a function of one limiting enzymatic reaction in which the substrate is O<sub>2</sub> by using a simple MM model as described in Equation (4).

$$R_{O_2} = \frac{R_{CO_2} \times Y_{O_2}}{R_{O_2} + Y_{O_2}} \quad (4)$$

In order to account for the possible inhibitory effect of  $CO_2$ ,  $R_{O_2}$  was described by an MM-type model for change in gas composition under MA storage (Guillard, Guillaume, & Destercke, 2012; Paul & Clarke, 2002). MM competitive inhibition (Equation (5)), where both the inhibitor ( $CO_2$ ) and the substrate ( $O_2$ ) compete for the same active site; uncompetitive (Equation (6)), where  $CO_2$  reacts with the substrate-enzyme complex; and non-competitive (Equation (7)), when  $CO_2$  reacts both with the enzyme and within the enzyme-substrate complex, models were fitted to the data obtained experimentally to account for the possible effect of  $Y_{CO_2}$  on  $O_2$  consumption rate (Gomes, Beaudry, Almeida, & Malcata, 2010). According to Fonseca, Oliveira, and Brecht (2002), during competitive inhibition, the maximum RR is lower in high  $CO_2$  concentration, whereas, for uncompetitive inhibition, the maximum RR is not much influenced by high  $CO_2$ ; on the other hand, for non-competitive, the maximum RR lies between competitive and uncompetitive inhibitions. The models variables were analysed and compared to select the best fit of the experimental data (Equations (5)–(7)):

$$R_{O_2} = \frac{V_{max} \times Y_{O_2}}{K_m \times \left(1 + Y_{CO_2}/K_i\right) + Y_{O_2}} \quad (5)$$

$$R_{O_2} = \frac{V_{max} \times Y_{O_2}}{K_m + \left(1 + Y_{CO_2}/K_i\right) \times Y_{O_2}} \quad (6)$$

$$R_{O_2} = \frac{V_{max} \times Y_{O_2}}{(K_m + Y_{O_2}) \times \left(1 + Y_{CO_2}/K_i\right)} \quad (7)$$

where  $V_{max}$  is the maximal value of RR for  $O_2$  consumption  $R_{O_2}$  (mL/kg h),  $Y_{O_2}$  and  $Y_{CO_2}$  are the concentrations of  $O_2$  and  $CO_2$ , respectively, inside the glass jar in kPa,  $K_m$  is MM constant for  $O_2$  consumption and  $K_i$  is inhibition constant for  $CO_2$  evolution. Estimation of model MM parameters and constants, based on the experimental data, was performed by non-linear regression based on the Levenverg–Marguardt method using STATISTICA software (vr. 13 StatSoft Inc.,

Tulsa, USA). The model estimates obtained at each MA conditions were used to describe the dependency of the model parameters on gas composition. The accuracy of the models' estimation was given by standard error (SE) value.

### Statistical analysis

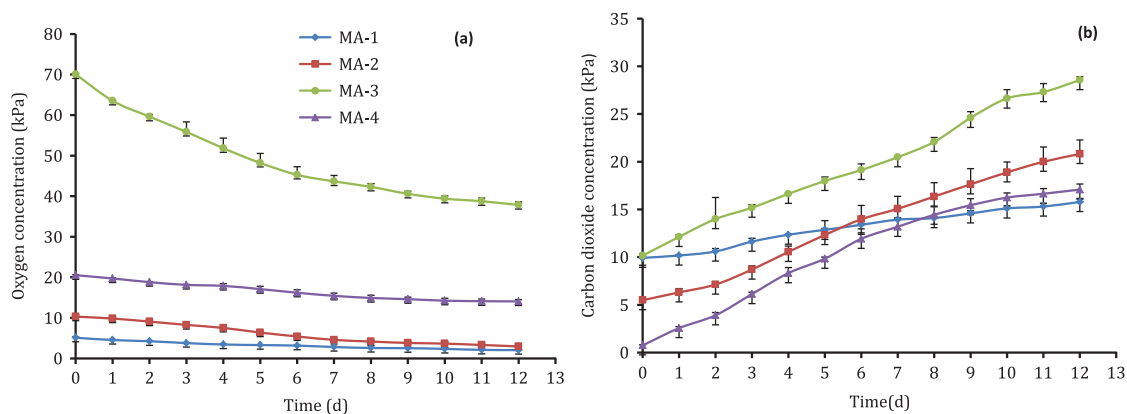
The results were presented as mean and standard deviation of three measurements. The data were analysed using STATISTICA software (vr. 13 StatSoft Inc., Tulsa, USA). Two-way ANOVA was used to investigate the effects of atmosphere modification and storage duration on RR of pomegranate arils. Fishers' least significant difference test was used to determine significant differences among the means of response variables ( $p < 0.05$ ).

## Results and discussion

### Change in head space gas composition

Concentrations of  $O_2$  and  $CO_2$  changed significantly with respect to the different initial gas concentrations and storage duration as shown in Figure 1. The decrease in  $O_2$  concentration showed similar trend. Initial  $O_2$  concentration of 5 kPa decreased to a concentration of 2.04 kPa for low  $O_2$  (MA-1) and from 10 kPa for MA-2 atmospheres at day 12. Similarly,  $O_2$  concentration decreased from 21 kPa to 14.06 kPa for air storage and from 70.05 kPa of  $O_2$  decreased to 37.83 kPa under super-atmospheric  $O_2$ . Meanwhile, the headspace  $CO_2$  concentration increased in all MA storage from the first day of storage and it reached the recommended range of <10–20 kPa  $CO_2$  for pomegranate arils except under MA-3 storage treatment where the  $CO_2$  concentration was >20 kPa on day 6. Furthermore,  $CO_2$  increased from 9.3 kPa to 15.7 kPa for low  $O_2$  atmosphere and from 10.15 kPa to 28.56 kPa under super-atmospheric  $O_2$  condition at day 12. However, the increase in  $CO_2$  was highest (17.08 kPa) for arils stored under MA-4 at day 12 compared with the initial concentration (0.03 kPa).

Increase in  $O_2$  consumption and  $CO_2$  production corresponding to different initial gas concentrations were



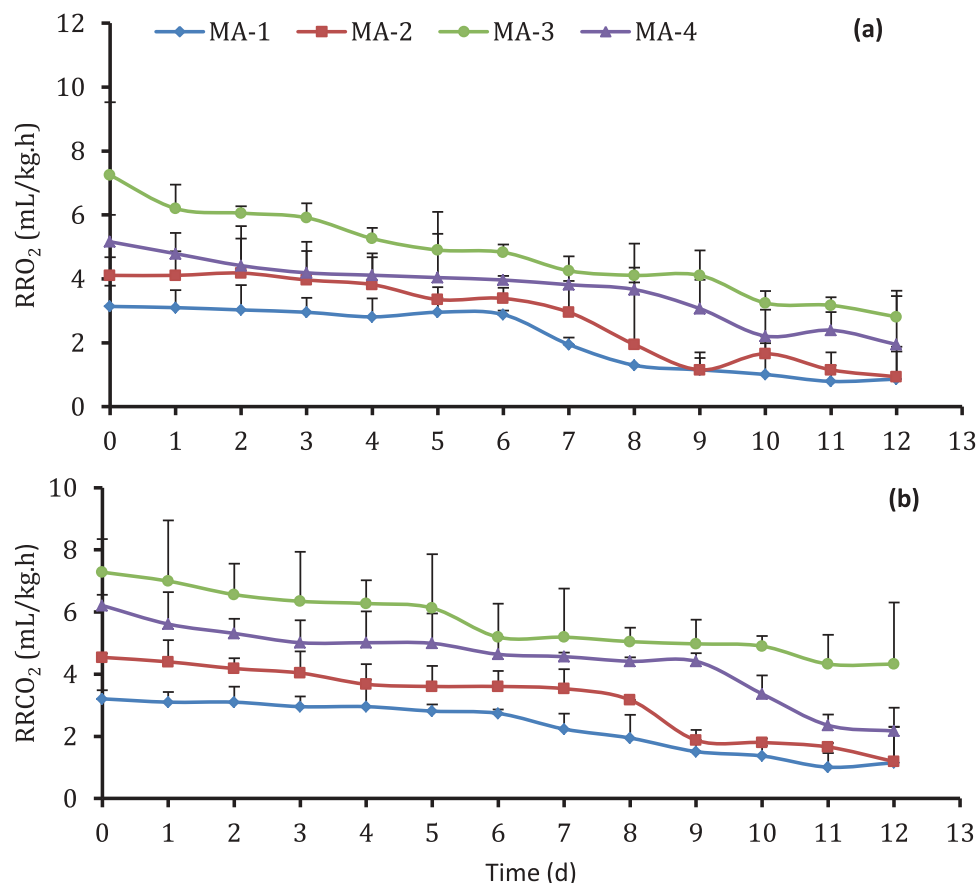
**Figure 1.** Effect of initial gas atmosphere on the change in headspace gas concentration of pomegranate arils stored at 5°C for 12 days. Error bar represents standard deviation of mean values ( $n = 3$ ) at 95% confident interval. (a)  $O_2$  consumption and (b)  $CO_2$  production. MA-1: (5 kPa  $O_2$ , 10 kPa  $CO_2$  and 85 kPa  $N_2$ ); MA-2: (10 kPa  $O_2$ , 5 kPa  $CO_2$  and 85 kPa  $N_2$ ), MA-3: (70 kPa  $O_2$ , 10 kPa  $CO_2$  and 20 kPa  $N_2$ ); and MA-4 (air).

**Figura 1.** Efecto de la atmósfera de gas inicial en el cambio de concentración de gases en el espacio de cabeza de arilos de granada almacenados a 5°C durante 12 días. La barra de error representa la desviación estándar de los valores medios ( $n = 3$ ) con un intervalo de confianza de 95%. (A) Consumo de  $O_2$  y (B) Producción de  $CO_2$ . MA-1: (5 kPa  $O_2$ , 10 kPa  $CO_2$  y 85 kPa  $N_2$ ); MA-2: (10 kPa  $O_2$ , 5 kPa  $CO_2$  y 85 kPa  $N_2$ ), MA-3: (70 kPa  $O_2$ , 10 kPa  $CO_2$  y 20 kPa  $N_2$ ); y MA-4 (aire).

observed. This phenomenon was reflected in the gradual linear reduction of slope for  $O_2$  concentration while coefficients of determination ( $R^2$ ) for fitted linear models ranged from 0.98 to 0.99 for all MA conditions. The linearity of the model could be attributed to the effect of increasing production of  $CO_2$  and decreasing  $O_2$  concentration over time. The linear changes in  $O_2$  concentration in this study were in agreement with the pattern of a linear decline under aerobic conditions whereas non-linearity can be indicative of anaerobic RR or suppression of respiratory activity due to limited  $O_2$  concentration (Guevara, Yahia, Beaudry, & Cedeño, 2006). The result showed significant ( $p < 0.05$ ) effects of storage MA condition and duration on headspace  $O_2$  consumption and  $CO_2$  evolution for arils. Similarly, Ayhan and Esturk (2009) reported high  $CO_2$  concentration (35 kPa) for  $O_2$  enriched atmosphere at 18 days of storage. Accumulation of  $CO_2$  concentration within high  $O_2$  atmosphere was reported by Maghoubi et al. (2014). This study further reported that the presence of high  $O_2$  concentration and accumulation of  $CO_2$  resulted in enhancement of the production of reactive  $O_2$  species that could cause some respiratory stress, increase in RR, induced more  $CO_2$  and depletion of antioxidants. Therefore, the increase in RR at MA-3 in the current study could be associated with the reason of highest accumulation of  $CO_2$ .

### Influence of initial gas composition on RR

The potential effect of initial gas concentration on RR of pomegranate arils stored at different MAs was analysed. The results indicated that gas concentration had significant ( $p < 0.05$ ) effect on RR of pomegranate arils during cold storage. However, the interaction of time and MA types did not significantly affect the RR of pomegranate arils for all MA conditions applied. Based on the calculated RR values obtained from  $O_2$  consumption and  $CO_2$  production, it was observed that RR decreased over storage duration across all MA conditions. Comparing the RR of pomegranate arils at different atmospheres; for low  $O_2$  atmospheres,  $RO_2$  decreased from 3.14 mL/kg h to 0.87 mL/kg h at low  $O_2$  atmosphere for MA-1 and  $RO_2$  decreased from 4.11 mL/kg h to 0.94 mL/kg h for MA-2 and from 7.25 mL/kg h to 2.81 at super-atmospheric  $O_2$  atmosphere. Whereas for arils at ambient atmosphere,  $RO_2$  decreased from 5.16 mL/kg h to 1.95 mL/kg h (Figure 2). Generally, the RR appeared to slow down as time progressed for all MA storage conditions. Furthermore, the effect of  $O_2$  concentration on RR of pomegranate arils clearly showed when the RR decreases with the decrease in  $O_2$  concentration. Similarly,  $RO_2$  pomegranate arils increased from 0.875 mL/kg h to  $2.81 \pm 0.37$  mL/kg h by increasing the  $O_2$  concentration from 5 to 70 kPa at day 12. Lowest  $CO_2$  production rate ( $R_{CO_2}$ )



**Figure 2.** Effect of gas concentration on the calculated respiration rate (RR) oxygen (a) and carbon dioxide (b) of minimally processed pomegranate arils stored at 5°C for 12 days. Error bar represents standard deviation of mean values ( $n = 3$ ) at 95% confident interval. MA-1: (5 kPa  $O_2$ , 10 kPa  $CO_2$  and 85 kPa  $N_2$ ); MA-2: (10 kPa  $O_2$ , 5 kPa  $CO_2$  and 85 kPa  $N_2$ ), MA-3: (70 kPa  $O_2$ , 10 kPa  $CO_2$  and 20 kPa  $N_2$ ); and MA-4 (air).

**Figura 2.** Efecto de la concentración de gas en la frecuencia de respiración (FR) calculada, oxígeno (A) y dióxido de carbono (B) de arilos de granada mínimamente procesados y almacenados a 5°C durante 12 días. La barra de error representa la desviación estándar de los valores medios ( $n = 3$ ) con un intervalo de confianza de 95%. MA-1: (5 kPa  $O_2$ , 10 kPa  $CO_2$  y 85 kPa  $N_2$ ); MA-2: (10 kPa  $O_2$ , 5 kPa  $CO_2$  y 85 kPa  $N_2$ ); y MA-3: (70 kPa  $O_2$ , 10 kPa  $CO_2$  y 20 kPa  $N_2$ ); y MA-4 (aire).

was 1.15 mL/kg h under MA-1 atmosphere compared to MA-2, MA-3 and MA-4. For the other MA conditions (MA-2 and MA-4), which had 10 and 21 kPa initial O<sub>2</sub> concentration, respectively, R<sub>O<sub>2</sub></sub> of arils ranging from 0.94 mL/kg h for MA-2 and 1.95 mL/kg h for MA-4 was observed at the end of storage time (day 12).

In addition, reducing the O<sub>2</sub> concentration from 70 to 5 kPa decreased the R<sub>CO<sub>2</sub></sub> at the same magnitude as R<sub>O<sub>2</sub></sub>. This finding is supported by the fact that RR decreases with decreasing availability of O<sub>2</sub> through the reduction of overall metabolic activity (Oms-Oliu, Soliva-Fortuny, & Martín-Belloso, 2008). The reduction in RR response due to low O<sub>2</sub> could be a result of decrease in activity of oxidative enzymes such polyphenoloxidase, ascorbic acid oxidase and glycolic acid oxidase (Conesa, Verlinden, Artés-Hernández, Nicolai, & Artés, 2007). MA-2 enhanced CO<sub>2</sub> production rate after 5 days of storage in comparison to MA-1 and MA-4 atmospheres. Furthermore, the rapid depletion of O<sub>2</sub> and accumulation of CO<sub>2</sub> can be related to the low barrier properties of the glass jars used for the experiment. Similarly, low RR of pomegranate arils (cv. Hicaz) stored at 4°C under a combination of low O<sub>2</sub> (2 kPa) and high CO<sub>2</sub> (10 kPa) was reported by Ersan et al. (2010). The authors reported that the minimum R<sub>O<sub>2</sub></sub> of 1.5 mL/kg h and R<sub>CO<sub>2</sub></sub> of 0.52 mL/kg h of pomegranate arils was obtained at low O<sub>2</sub> (2 kPa). Aindongo et al. (2014) reported R<sub>O<sub>2</sub></sub> and R<sub>CO<sub>2</sub></sub> of 'Bhagwa' pomegranate arils with ranges 1.9–18.6 mL/kg h and 3.2–28.9 mL/kg h, respectively, stored at 5–22°C, while Caleb, Mahajan, Opara, and Witthuhn (2012c) observed R<sub>O<sub>2</sub></sub> of 2.5–7.6 mL/kg h and R<sub>CO<sub>2</sub></sub> ranges from 2.7 to 9.0 mL/kg h for pomegranate arils (cvs. Acco and Herskawitz) stored at 5–15°C. In addition, Banda and others (2015) investigated the effects of different combination of atmospheres (5 kPa O<sub>2</sub> + 10 kPa CO<sub>2</sub> + 85 kPa N<sub>2</sub>; 30 kPa O<sub>2</sub> + 10 kPa CO<sub>2</sub> + 60 kPa N<sub>2</sub>; 100 kPa N<sub>2</sub>; and air) on post-storage RR of 'Wonderful' pomegranate arils. The authors reported that R<sub>CO<sub>2</sub></sub> significantly changed over time and the highest RR was observed for pomegranate arils stored under passive MA in high barrier films, while arils packed under 100 kPa N<sub>2</sub> maintained the lowest RR (0.58 ± 0.12 kg<sup>-1</sup> h<sup>-1</sup>) at day 12.

Increase in RR (from 1.77 to 2.30 mL/kg h) of pomegranate arils (cv. Malese-Saveh) under 90 kPa O<sub>2</sub> condition has been reported (Maghoumi et al., 2013). Furthermore, increase in RR under super-atmospheric O<sub>2</sub> concentration was reported by Jaxsens, Devlieghere, and Debevere (2002) for mushroom, grated celeriac and shredded chicory endives. Similar observation was reported by Allende, Luo, Mc-Evoy, Artés, and Wang (2004) for baby spinach leaves under super-atmospheric O<sub>2</sub> (>70 kPa). The authors observed highest R<sub>O<sub>2</sub></sub> and R<sub>CO<sub>2</sub></sub> under super-atmospheric O<sub>2</sub> MA condition. The increase in RR was associated with the production of reactive O<sub>2</sub> species and respiratory stress due to the presence of high O<sub>2</sub> concentration (Jaxsens et al., 2002). However, the effect of super-atmospheric O<sub>2</sub> depends on the commodity, maturity and ripeness stage, O<sub>2</sub> and CO<sub>2</sub> concentration, time and storage temperature (Kader & Ben-Yehoshua, 2000). Overall, the data suggested that low O<sub>2</sub> atmosphere would be important to lower the RR of pomegranate arils at 5°C compared to super-atmospheric O<sub>2</sub> and passive atmosphere.

### Respiration quotient (RQ)

The average ratio of CO<sub>2</sub> produced and O<sub>2</sub> consumed (RQ) value of pomegranate arils stored at all MA conditions was

within the range of acceptable limit (0.7–1.3) for aerobic respiration of fruits and vegetables (Kader, Zagory, Kerbel, & Wang, 1989). In contrast, arils under super-atmospheric O<sub>2</sub> (MA-3) condition after 10 days of storage had the highest RQ of 1.54. The lowest RQ was observed for pomegranate arils stored under MA-4 treatment. The results further showed that the RQ for all MA conditions increased slightly at the end of 7 d storage. Based on the assumption that the value of RQ for fresh produce is equal to 1 when the metabolic substrates oxidized during respiration are carbohydrates (Castro-Giraldez, Fito, Ortola, & Balaguer, 2013; Fonseca et al., 2002), it can be suggested that the fermentation threshold for stored pomegranate arils was not reached across all MA conditions. On the other hand, a slight increase in RQ > 1 suggests that the oxidized substrates were organic acidic (Fonseca et al., 2002). Furthermore, Conesa et al. (2007) associated the higher value of RQ with the production of high CO<sub>2</sub> for fresh-cut bell peppers. Similar to the current study, RQ values ranges from 1.14 to 1.26 for minimally processed pomegranate arils 'Acco' and 'Herskawitz' were reported by Caleb et al. (2012b) and RQ values of 0.9–1.24 for citric acid treated and untreated pomegranate arils (cv. Wonderful) by Banda, Caleb, and Opara (2014) at passive atmosphere.

The results showed that gas concentration had no influence on the RQ of pomegranate arils (as shown in Table 2), except a slight increase (38%) from 0.95 to 1.33 when the O<sub>2</sub> concentration dropped below 2.81 kPa for MA-1 at day 12. Since the lower O<sub>2</sub> limit can be determined by the immediate increase in RQ (Beaudry, 1999), and if the lowest O<sub>2</sub> limit is considered to be the O<sub>2</sub> concentration that causes a 50% increase in the RQ as stated by Hong and Kim (2001), then the lowest O<sub>2</sub> limit of 'Wonderful' pomegranate arils can be estimated to be less than 2.8 kPa. This further showed the influence of gas concentration on RQ and the slight but non-significant change in the respiratory process.

### Modelling the influence of gas composition on RR

MM model with different types of inhibition were tested using the experimental data. The predictions from the MM models as well as the inhibition models (competitive, un-

**Table 2.** Effects of modified atmosphere on respiratory quotient (RQ) of pomegranate arils stored at 5°C for 12 d.

**Tabla 2.** Efectos de la atmósfera modificada en el cociente respiratorio (CR) de arilos de granada almacenados a 5°C durante 12 días.

Number of days	Modified atmosphere conditions*			
	MA-1	MA-2	MA-3	MA-4
0	1.02 ± 0.3	1.10 ± 0.2	1.12 ± 0.6	1.20 ± 0.2
1	1.00 ± 0.3	1.07 ± 0.2	1.15 ± 0.4	1.17 ± 0.2
2	1.22 ± 0.4	1.00 ± 0.4	1.14 ± 0.2	1.20 ± 0.3
3	1.01 ± 0.3	1.02 ± 0.4	1.07 ± 0.3	1.19 ± 0.4
4	1.05 ± 0.2	0.96 ± 0.4	1.19 ± 0.1	1.22 ± 0.4
5	0.95 ± 0.1	1.07 ± 0.1	1.25 ± 0.7	1.24 ± 0.4
6	0.95 ± 0.0**	1.06 ± 0.1**	1.07 ± 0.2**	1.16 ± 0.2**
7	1.15 ± 0.2	1.19 ± 0.6	1.22 ± 0.4	1.19 ± 0.2
8	1.17 ± 0.2	1.30 ± 0.1	1.23 ± 0.2	1.20 ± 0.3
9	1.31 ± 1.0	1.19 ± 0.2	1.21 ± 0.3	1.44 ± 0.4
10	1.27 ± 0.7	1.09 ± 0.4	1.13 ± 0.1	1.52 ± 0.8
11	1.27 ± 1.1	1.05 ± 0.9	1.36 ± 0.3	0.98 ± 0.2
12	1.33 ± 0.8	1.27 ± 0.2	1.54 ± 0.4	1.12 ± 0.1

Values are means ± standard deviation (n = 3). \*MA compositions are presented in Table 1, and \*\* are significantly different.

Los valores representan medias ± la desviación estándar (n = 3). \*Las composiciones MA se presentan en la Tabla 1 y los \*\* son significativamente diferentes.

**Table 3.** Model parameter estimates obtained from non-linear regression analysis for enzyme kinetics model for O<sub>2</sub> consumption rate in closed system under various initial O<sub>2</sub> and CO<sub>2</sub> concentrations.

**Tabla 3.** Estimaciones de los parámetros del modelo obtenidas del análisis de regresión no lineal para el modelo de cinética enzimática de la tasa de consumo de O<sub>2</sub> en un sistema cerrado bajo varias concentraciones iniciales de O<sub>2</sub> y CO<sub>2</sub>.

Models	Modified atmospheres <sup>a</sup>	R <sup>2</sup> <sub>adj</sub>	V <sub>max</sub> mL/kg h	SE	K <sub>m</sub> , kPa O <sub>2</sub>	SE	K <sub>i</sub> , kPa CO <sub>2</sub>	SE
Simple Michaelis–Menten	MA-1	0.67	3.67	0.29	0.94	0.39	*	*
	MA-2	0.86	6.49	0.98	5.52	1.93	*	*
	MA-3	0.91	19.83	7.32	140.59	73.68	*	*
	MA-4	0.81	8.31	0.01	1.07	0.54	*	*
Competitive inhibition	MA-1	0.71	3.67	2.67	0.94	0.23	6.83	547.06
	MA-2	0.89	4.62	1.95	5.00	5.59	0.11	31.4
	MA-3	0.91	10.69	15.12	31.23	179.57	24.93	183.3
	MA-4	0.81	8.86	1.15	109.60	0.69	18.69	64.89
Uncompetitive inhibition	MA-1	0.68	3.64	0.31	0.85	0.00	*	*
	MA-2	0.86	6.50	1.03	5.50	2.23	58.53	0.00
	MA-3	0.91	20.00	8.45	141.00	85.09	*	*
	MA-4	0.81	8.45	0.18	*	1.90	*	*
Non-competitive inhibition	MA-1	0.68	3.64	0.45	1.31	3.02	132.9	1087
	MA-2	0.86	6.51	4.00	5.56	12.00	79.60	1157
	MA-3	0.91	20.40	52.3	147.61	644.7	207.9	1396.2
	MA-4	0.81	8.02	1.59	305.7	1.69	57.93	6.00

V<sub>max</sub> is highest respiration rate due to O<sub>2</sub> consumption, K<sub>m</sub> is Michaelis–Menten constant, K<sub>i</sub> is CO<sub>2</sub> inhibition constant, SE is standard error and R<sup>2</sup><sub>adj</sub> is percentage variance. \* were very high value(s); <sup>a</sup>MA compositions are presented in Table 1.

V<sub>max</sub> representa la frecuencia de respiración más alta atribuible al consumo de O<sub>2</sub>, K<sub>m</sub> es la constante Michaelis-Menten, K<sub>i</sub> es la constante de inhibición de CO<sub>2</sub>. SE es el error estándar y R<sup>2</sup><sub>adj</sub> es la varianza porcentual. \* son valores muy altos; <sup>a</sup> composiciones de MA figuran en la Tabla 1.

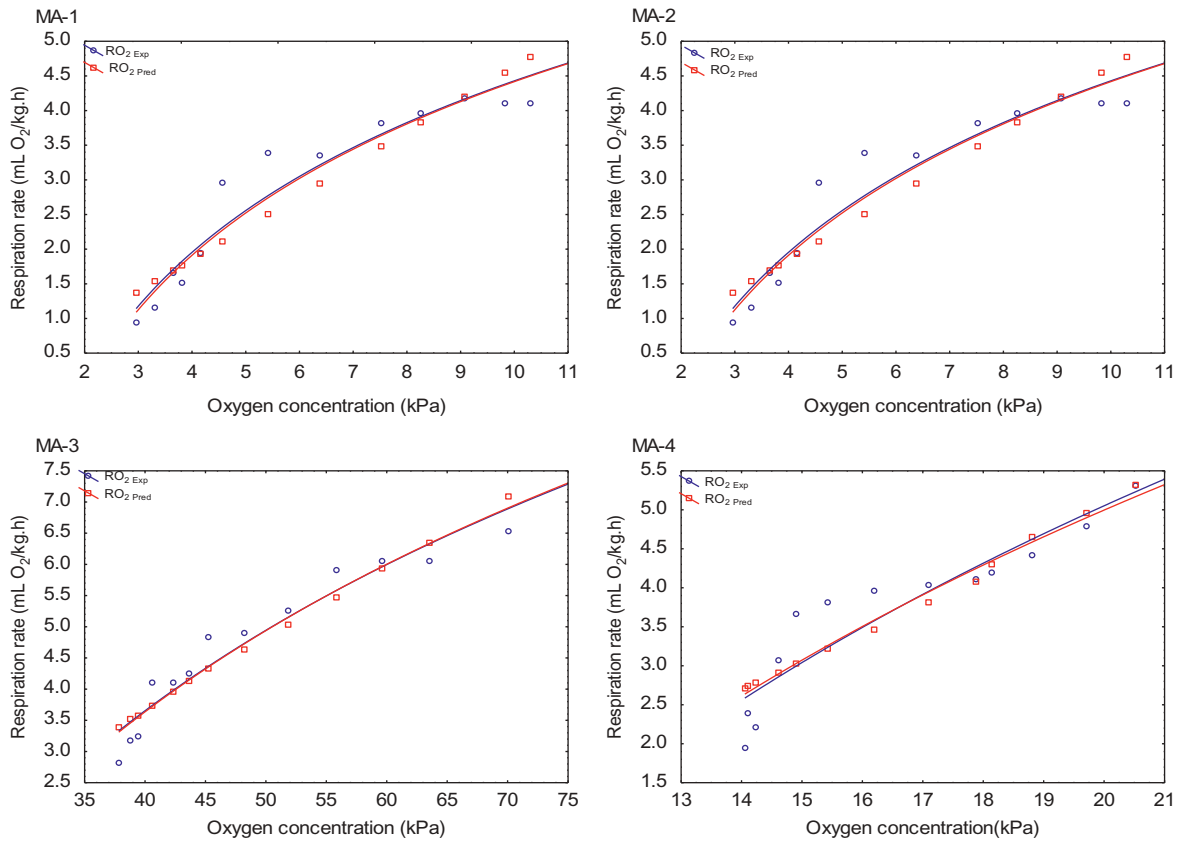
competitive and non-competitive) were compared by using the model parameter estimates as presented in Table 3. The MM model parameters for each gas concentration (V<sub>max</sub>O<sub>2</sub> and K<sub>m</sub>O<sub>2</sub>) and the CO<sub>2</sub> inhibition constant (K<sub>i</sub>CO<sub>2</sub>) were estimated individually from the experimental data obtained at each gas concentration and their MA dependence subsequently studied using Equations (5), (6) and (7). All the four types of MM enzyme kinetics models were capable of describing the O<sub>2</sub> consumption rate as function of MA (MA-2, MA-3 and MA-4 treatments) with observed percentage variance (R<sup>2</sup><sub>adj</sub> = 81–91%). Whereas, lower percentage variance of R<sup>2</sup><sub>adj</sub> > 70% were found for (MA-1) which had the lowest initial O<sub>2</sub> concentration (2 kPa), except for competitive inhibition model. This could be due to the presence of very low O<sub>2</sub>, which can influence effectiveness of MM model, since the model is effective when the reaction is in MM type (Hertog, Peppelenbos, Evelo, & Tijssens, 1998).

Both the maximum O<sub>2</sub> consumption rate and the MM model constant (V<sub>max</sub>O<sub>2</sub> and K<sub>m</sub>O<sub>2</sub>) varied between the different MAs. Highest V<sub>max</sub>O<sub>2</sub> was observed in MA-3 and lowest was for MA-1. This can be inferred that both parameters were influenced by gas composition. The increase in V<sub>max</sub> with increase in O<sub>2</sub> concentrations suggested that V<sub>max</sub> was dependent on gas composition. The gas concentration dependency of R<sub>O<sub>2</sub></sub> increased substantially when O<sub>2</sub> concentration increased from low to super-atmospheric O<sub>2</sub> (Figure 3). In addition, the highest value of K<sub>m</sub>O<sub>2</sub>, which represent the O<sub>2</sub> concentration at which half the maximum RR is reached (assuming no inhibition by CO<sub>2</sub>), was observed under super-atmospheric O<sub>2</sub> (MA-3). Given that all the arils were harvested at commercial maturity and processed under the same condition, the observed variation in the K<sub>m</sub>O<sub>2</sub> values might be attributed to the differences in the tissue resistance to diffusion commonly associated with storage MA (Gomes et al., 2010). Furthermore, this observation could suggest that the internal O<sub>2</sub> concentration in the arils increased proportional to the external O<sub>2</sub> concentration applied (Hertog et al., 1998).

Comparing simple and inhibition models for each atmosphere, there was no clear difference on effect of CO<sub>2</sub> on O<sub>2</sub> consumption rate for all MAs. However, according to the K<sub>i</sub>CO<sub>2</sub> values, which showed the extent to which RR can be inhibited by CO<sub>2</sub> in Table 3, the simultaneous existence of competitive, un-competitive and non-competitive inhibition were observed depending on the initial gas concentration. Comparing all models, relatively higher K<sub>i</sub>CO<sub>2</sub> value was observed in MA-1 during competitive inhibition model, which implies inhibition by CO<sub>2</sub> has not occurred, whereas, a slight un-competitive and non-competitive inhibition was observed. This indicated that a model which combined un-competitive and non-competitive inhibition could better explain the CO<sub>2</sub> effect on the RR (Guillard et al., 2012). On the contrary, the RR of pomegranate arils for the other atmospheres was competitive inhibition, with the lowest K<sub>i</sub>CO<sub>2</sub>. However, the influence of CO<sub>2</sub> on the metabolic rate could be due to its effect on changing the pH than its direct influence on enzymatic reaction (Torrieri et al., 2010).

The V<sub>max</sub>O<sub>2</sub> values obtained from the current experiment were similar to those reported by Ersan et al. (2010) for 'Hicaz' pomegranate arils at 5°C. At low O<sub>2</sub>, V<sub>max</sub>O<sub>2</sub> of 3.6 mL kg/h was comparable to 3.1 mL kg/h reported by Ersan et al. (2010). In contrast, the K<sub>m</sub>O<sub>2</sub> and K<sub>m</sub> values were different. The authors found relatively higher K<sub>m</sub>O<sub>2</sub> ranging from 3.8 to 5.1 for 'Hicaz' pomegranate arils, whereas the values in the current experiment were within the range of 0.29–2.67 for 'Wonderful' under-low O<sub>2</sub> atmosphere. This variation in K<sub>m</sub>O<sub>2</sub> could be associated with the different model-fitting methods used. Ersan et al. (2010) linearized the data before fitting, while for the current study a non-linear regression model has been used, since linearization is equivalent to changing the weight given to the data in the estimation procedure and it should be avoided (Fonseca et al., 2002).

Comparing the accuracy of the parameter estimates by using the estimated SEs for all models, small SE values of all V<sub>max</sub>O<sub>2</sub> and K<sub>m</sub>O<sub>2</sub> were observed. Low SE values (lower

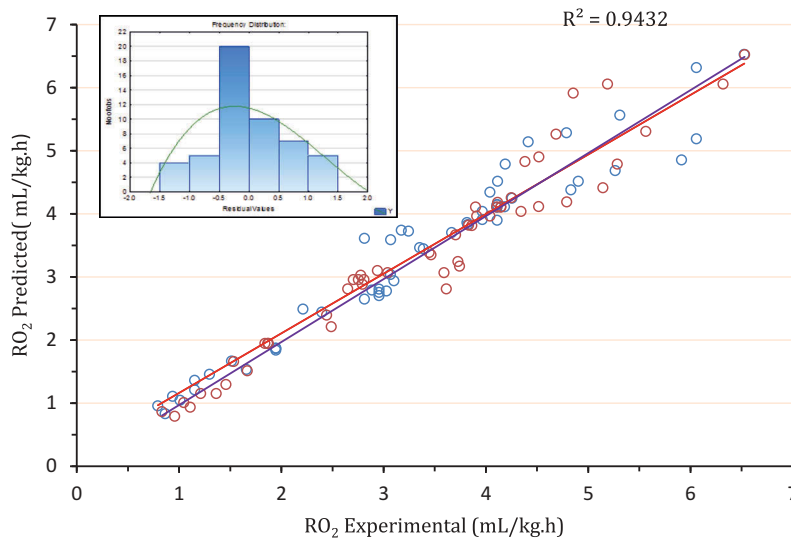


**Figure 3.** Correlation of head space O<sub>2</sub> concentration (kPa) on the O<sub>2</sub> consumption rate (mL/kg h) of minimally processed pomegranate arils stored at 5°C for 12 days in a closed system. MA-1: (5 kPa O<sub>2</sub>, 10 kPa CO<sub>2</sub> and 85 kPa N<sub>2</sub>); MA-2: (10 kPa O<sub>2</sub>, 5 kPa CO<sub>2</sub> and 85 kPa N<sub>2</sub>), MA-3: (70 kPa O<sub>2</sub>, 10 kPa CO<sub>2</sub> and 20 kPa N<sub>2</sub>); and MA-4 (air).

**Figura 3.** Correlación de espacio de cabeza, concentración de O<sub>2</sub> (kPa) en la tasa de consumo de O<sub>2</sub> (mL/kg h) de arilos de granada mínimamente procesados y almacenados a 5°C durante 12 días en un sistema cerrado. MA-1: (5 kPa O<sub>2</sub>, 10 kPa CO<sub>2</sub> y 85 kPa N<sub>2</sub>); MA-2: (10 kPa O<sub>2</sub>, 5 kPa CO<sub>2</sub> y 85 kPa N<sub>2</sub>), MA-3: (70 kPa O<sub>2</sub>, 10 kPa CO<sub>2</sub> y 20 kPa N<sub>2</sub>); y MA-4 (aire).

value than the sample mean) for the parameter estimates can be considered as well defined (Hertog, Lammertyn, Scheerlinck, & Nicolai, 2007). Thus, it can be concluded that the models accurately predicted the influence of MA

on the RR of 'Wonderful' pomegranate arils. On the other hand, the accuracy for the competitive and non-competitive inhibition model for MA-1 and for non-competitive inhibition for MA-3 could be less defined by the increase



**Figure 4.** Correlation between experimental data and predicted R<sub>02</sub> (mL/kg h) using Michaelis–Menten competitive inhibition model. Small plot inserted shows the frequency distribution of the residuals.

**Figura 4.** Correlación entre los datos experimentales y el R<sub>02</sub> (mL/kg h) previsto, utilizando el modelo de inhibición competitiva Michaelis-Menten. El pequeño trazado insertado muestra la distribución de frecuencia de los residuales.



in SE for  $K_m$ . However, the higher SE could also explain the coexistence of both competitive and uncompetitive inhibition of RR by  $\text{CO}_2$  (Hertog et al., 2007). Furthermore, the model parameters were statistically significant ( $p < 0.05$ ) and the correlation coefficient of determination was  $R^2 = 0.85\text{--}0.97\%$  for all atmospheres. This showed a good fit of the model to the experimental data. Therefore, these results demonstrated that the MM enzyme kinetics models can be used to describe the dependency of RR of pomegranate arils on the modified storage atmospheres. Since the results for MA-2, MA-3 and MA-4 showed a competitive inhibition RR and a slight combination of competitive and un-competitive inhibition for MA-1, a non-linear regression was applied to the whole set of experimental data using competitive model. The model adequately predicted the  $\text{O}_2$  consumption rate ( $R^2 = 0.94\%$ ) as shown in Figure 4, with a normal distribution of the residual values. Based on this result, it can be suggested that competitive inhibition can be able to describe the effect of  $\text{CO}_2$  to  $\text{O}_2$  consumption rate at low and super-atmospheric  $\text{O}_2$  condition.

## Conclusion

This study showed that RR of pomegranate arils (cv. Wonderful) was significantly affected by initial gas concentrations during storage. Low  $\text{O}_2$  MA conditions reduced the RR of the arils. In contrast, the use of super-atmospheric  $\text{O}_2$  concentration (70 kPa) induced physiological stress, and consequently slightly increased the RR of arils. Based on the respiratory kinetics of pomegranate arils, this study showed that super-atmospheric  $\text{O}_2$  is of limited or no benefit in slowing down the metabolic process of pomegranate arils. The MM enzyme kinetics model (using competitive-inhibition-type equation) adequately described the effect of  $\text{CO}_2$  on  $\text{O}_2$  consumption rate at low  $\text{O}_2$  (5 kPa), super-atmospheric  $\text{O}_2$  (70 kPa) and enriched  $\text{CO}_2$  concentrations. The parameter estimates obtained provide a guiding step for the management of MA systems to avoid unfavourable conditions for pomegranate arils (cv. Wonderful).

## Disclosure statement

No potential conflict of interest was reported by the authors.

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