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Modelling respiration rate of soybean seeds (*Glycine max* (L.)) in hermetic storage

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ABSTRACT

The dynamic of oxygen (O_2) and carbon dioxide (CO_2) concentration was characterized in soybean (*Glycine max* (L.)) samples hermetically stored in glass jars at 15, 25 and 35 °C and 13, 15 and 17% moisture content (m.c., wet base). Two correlations were used for smoothing gas concentration in time: linear and exponential. Then, the respiration rate at each temperature and m.c. combination was calculated as storage time progressed and oxygen was consumed and two predictive models for respiration were proposed: Model I (temperature and m.c. dependent) and Model II (temperature, m.c. and oxygen dependent). It was observed that respiration rate increased with storage m.c. and temperature. However, respiration rate was not mainly affected by O_2 until a critical concentration limit of about 2% was reached. Respiration rates were from 0.341 to 22.684 mg O_2/(kgDM d) and from 0.130 to 20.272 mg CO_2/(kgDM d) for a range of storage condition of 13–17% m.c. and 15–35 °C temperature. The respiration rate of soybean seeds obtained in this study resulted significantly lower than the rates reported in the literature for other grains at similar temperature and a_w (water activity) storage condition. For hermetic storage simulations in which O_2 concentration is not expected to drop below 2%, the simplest model (Model I) could be used, but if the O_2 concentration of the hermetic system is expected to be depleted, Model I would under estimate the time at which O_2 is consumed, and thus a model with O_2 dependency is recommended instead (Model II).

1. Introduction

Hermetic storage consists of a sealed storage system containing a modified atmosphere caused by the respiration of grain, insects and microorganisms which results in low oxygen (O_2) and high carbon dioxide (CO_2) environment (Navarro and Donahaye, 2005). This particular gaseous condition has advantages in preserving the product by controlling pests (insects and mites) (Navarro, 2006), reducing microbial activity (Samapundo et al., 2007) and prolonging seed longevity by reducing deleterious oxidative processes (Groot et al., 2015). As a general result, a better final quality of the product is obtained with hermetic storage systems compared with conventional storage systems (Bartosik, 2012; De Dios et al., 2000; Donahaye, 1999; Donahaye et al., 2000; Noor et al., 2011; Williams et al., 2014).

Modelling was extensively used for studying the effect of temperature, moisture content (m.c.), gas composition and time on quality parameters of different agricultural products during storage and conditioning (Arias Barreto et al., 2013; Bartosik and Maier, 2004; Lawrence and Maier, 2011; Thorpe, 1997). Correlations for predicting respiration rate of commodities is a key input for these storage models, since respiration was related to dry matter loss (DML) (Thompson, 1972) and to deleterious effects in various grain and seed quality parameters (Pronyk et al., 2004; White et al., 1982). Additionally, a novel technology was developed for evaluating the storage condition of the grain in hermetic plastic bags (silo bags) based on the measurement of the CO_2 concentration (Bartosik et al., 2013, 2008). CO_2 concentration is measured in the

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silo bag and compared with a referential CO_2 concentration to detect increasing biological activity due to grain spoilage. Similarly, Ileleji et al. (2006) proposed to monitor CO_2 concentration at the headspace of metal and concrete bins for early detection of grain spoilage. Thus, correlations for predicting typical CO_2 concentrations of the interstitial air composition are required as reference levels for these CO_2 monitoring systems.

Respiration rates were reported for soybean (Jian et al., 2014; Ochandio et al., 2012; Sood, 2015) and predictive correlations for CO_2 production based on temperature and m.c. were proposed for corn (Bern et al., 2002) and wheat (Lacey et al., 1994; White et al., 1982) for the typical range of storage conditions. On the other hand, Rukunudin et al. (2004) proposed predictive CO_2 release correlations for soybean stored at 21% m.c., unusually high for typical storage conditions. However, these correlations were developed for non-restrictive oxygen conditions (i.e. O_2 concentration about 21%) and could lead to overestimate respiration rate when applied to hermetic storage. Besides, using these correlations (not affected by decreasing O_2 concentration) implies that O_2 reduced atmospheres have no effect on biological activity and, hence, controlled and modified atmospheres have no storage benefit. In order to further refine the prediction of respiration (O_2 consumption and CO_2 generation) under hermetic storage conditions, a correlation that would take into account the effect of the oxygen depleting environment is required. Such a correlation is not available in the literature for soybean. Thus, the objectives the present study were: 1) characterize the respiration rate dynamics of soybean seeds hermetically stored (O_2 decreasing concentration); 2) develop correlations for predicting the respiration as a function of: a-temperature and moisture content, b-temperature, moisture content and oxygen concentration of the interstitial atmosphere; and 3) validate the correlations developed in Objective 2 with an independent set of data.

2. Materials and methods

Soybean (*Glycine max* (L.)) samples from a pool of 82 different varieties (10 maturity groups II and short maturity groups III, 24 long maturity groups III, 13 short maturity groups IV and 35 long maturity groups IV) harvested in May 2011 at 13.5% m.c. were used for the experiment. A pool of varieties was preferred as experimental material instead of a single variety in order to obtain a more representative respiration model. Soybean with mechanical or biological damage could affect the respiration rate and, hence, the experiment. Thus, a germination test was performed (ISTA, 2008), to confirm that soybean samples used in this study were not affected by any kind of damage (all samples had a germination test above 95%).

2.1. Preparation of the soybean seeds samples and experimental setup

The procedure for sample preparation and CO_2 and O_2 measuring was similar to that implemented by Dillahunty et al. (2000); Jian et al. (2014); Pronyk et al. (2004) and Weinberg et al. (2008). After harvest, samples were conditioned to 13, 15, and 17% m.c. and cool stored at 4 °C until the experiment. Samples were conditioned to different m.c. either by rewetting with distilled water or drying with natural air at laboratory ambient conditions (temperature: 22–25 °C; r.h: 60–65%). The m.c. was determined by the oven method, exposing the soybean samples at 130 °C during 19 h (ASABE, 2003).

Soybean samples at the three m.c. (13, 15 and 17%) were sealed in glass jars (660 ml), holding 450 g, and stored at 15, 25 and 35 °C (±1 °C) in temperature controlled chambers (three replicates per each m.c. and temperature combination were considered). A septum was placed in the lid for taking gas samples and to prevent air from leaking in or out during sampling. Samples were collected through the septum with a disposable syringe (1 ml for CO_2 and 0.5 ml for O_2) at different storage times until O_2 was completely depleted and CO_2 stabilized (with a maximum of 250 days).

The volume of the void space of the jars (interstitial air and headspace) containing soybean at different m.c. was determined by measuring the volume of distilled water that filled it (Weinberg et al., 2008), being of 296.2, 292.5 and 288.8 ml for soybean samples at 13, 15 and 17% m.c., respectively.

2.2. Gas measuring

Gas samples were analyzed with a gas chromatograph (Shimadzu, model GC-17A, Japan) equipped with an integrator (Shimadzu, model C-R5A, Japan). The setup for O_2 detection were: column: GS-MOLE 30 m 0.55 mm Megabore; detector: FID (flame ionization detector); gas carrier: helium; column temperature: 40 °C; injector temperature: 100 °C; detector temperature: 200 °C; run time: 200 s; compound retention time: 150 s. The setup for CO_2 detection were: column: GS-Q 30 m 0.53 mm Megabore; detector: TCD (thermal conductivity detector); gas carrier: nitrogen; column temperature: 40 °C; injector temperature: 100 °C; detector temperature: 200 °C; run time: 400 s; compound retention time: 350 s.

2.3. Correlations for O_2 and CO_2 evolution

The measured O_2 and CO_2 concentrations (%, V/V) over time present the typical variations due to experimental errors. Calculation of respiration rates from root values would exhibit unrealistic oscillation over time. Therefore, gas concentrations measurement were first smoothed by fitting different correlations of time and then used to determine the rate of respiration. Two correlations were selected to fit measured data: 1) linear correlation (Eq. (1) - which implies constant respiration rate over time); 2) exponential correlation (Eq. (2) - decreasing respiration rate over time):

$$O_2 = a + b\,t \tag{1a}$$

$$CO_2 = a + b\,t \tag{1b}$$

$$O_2 = \frac{42}{1 + e^{kt}} \tag{2a}$$

$$CO_2 = \frac{a}{1 + e^{-kt}} - \frac{a}{2} \tag{2b}$$

Where a, b and k are parameters that depend on each treatment (temperature and m.c.) and t is the storage time in days. In the linear fitting (Eq. (1)) the intercept was fixed to 21% for O_2 and 0% for CO_2.

2.4. Respiration rate

The respiration rates based on CO_2 generation were calculated using the linear and the exponential correlations. The transformation from predicted CO_2 concentration to respiration rate, R_{CO2} in mg CO_2/(kgDM d), was as follows:

$$R_{CO2} = \frac{1}{100}\,P\,V\,\frac{MCO_2}{R\,T}\,\frac{1}{DM}\,\frac{d[CO_2]}{dt} \tag{3}$$

The respiration rate based on O_2 consumption, R_{O2} in mg O_2/(kgDM d), was calculated as:

$$R_{O2} = \frac{1}{100} P V \frac{MO_2}{R T} \frac{1}{DM} \frac{d[O_2]}{dt} \quad (4)$$

Where $\frac{d[CO_2]}{dt}$ and $\frac{d[O_2]}{dt}$ in %V/V d^{-1}, are calculated according to the fitted correlations to measured concentration values (Eq. (1) and (2)), P is the atmospheric pressure in Pa (101,325 Pa); V is the volume of air in the container in m^3 (including the headspace and the interstitial air); MCO_2 and MO_2 are the molar mass of gas (MCO_2: 44010 mg mol^{-1}; MO_2: 32000 mg mol^{-1}); R is the ideal gas constant (8.314 J K^{-1} mol^{-1}); T is the experiment temperature in K; DM is the dry matter of the sample (0.391 kg, 0.382 kg and 0.373 kg for 13, 15 and 17% m.c., respectively).

2.5. Respiration rate models

Once the rate of CO_2 production and O_2 consumption as function of time for each treatment was calculated with Eqs. (3) and (4), different models were proposed to predict respiration rates. The fitting was carried out with the statistical software SIGMA PLOT 12.0.

Model I - Oxygen concentration independent

$$\log|R| = a_1 + a_2 T + a_3 M \quad (5)$$

Model II - Oxygen concentration dependent

Within this group, two different models were considered. First, for each m.c. (13, 15 and 17%) the following correlations was considered:

Model IIA:

$$R = a_1 + a_2 T + a_3 O_2 + a_4 T O_2 \quad (6)$$

Second, a general expression which included m.c. was obtained:

Model IIB:

$$log|R| = a_1 + a_2 T + a_3 O_2 + a_4 M \quad (7)$$

In the above models, R is the respiration rate (mg/(kgDM d)) for O_2 or CO_2, T is the temperature (°C), M is the m.c. (% w.b) and O_2 is the oxygen concentration (% V/V).

2.6. Experimental data for validation of models

A data set different from that used to derive the respiration rate was used to validate the proposed model. Arias Barreto (2016) measured the change in gas concentration in sealed glass jars (1000 ml) holding 400 g of soybean with 17% m.c. at 35 °C. Six jars were placed in a temperature controlled chamber (±1 °C). The procedure for sample preparation, m.c. and volume of the void space of the jars determination was the same as described in section 2.1.

Gas concentration was measured with a Pack Check O_2 and CO_2 portable analyzer (Model 325, MOCON, USA). The analyzer measures the O_2 and CO_2 concentration of a gas mixture by drawing a sample through an internal infrared and electro-chemical cell. The electro-chemical sensor generates an electrical current that is converted to an O_2 percentage on an easy-to-read LCD display. The IR sensor generates a voltage that is inversely proportional to the CO_2 concentration. Gas samples (3 ml) were taken through a septum placed in the lid of the jars to prevent any leakage at different storage times until O_2 was completely depleted and CO_2 stabilized.

2.7. Validation of models

To validate the proposed correlations, the previous experimental set up was modeled by a coupled set ODE (Ordinary Differential Equations) and CO_2 and O_2 gas concentrations were predicted applying the rate of respiration according to Model I, Model IIA and Model IIB and compared to the measured data at 17% and 35 °C (Arias Barreto, 2016).

To determine the accuracy of the three models, mean relative difference (MRD), mean absolute difference (MAD) and standard error of the estimate (SE) were calculated:

$$MRD = \frac{1}{n_s} \sum_{i=1}^{n_s} \frac{|(X_m - X_p)|}{X_m} \quad (9)$$

$$MAD = \frac{1}{n_s} \sum_{i=1}^{n_s} |(X_m - X_p)| \quad (10)$$

$$SE = \sqrt{\sum_{i=1}^{n_s} \frac{(X_m - X_p)^2}{n_s}} \quad (11)$$

Where X is the O_2 or the CO_2 concentration (% V/V), m indicates the measured value, p indicates the predicted value, n_s is sample size.

3. Results

3.1. Evolution of O_2 and CO_2 during hermetic storage

Fig. 1 shows the evolution of O_2 and CO_2 concentrations during hermetic storage of soybeans at different temperatures and m.c. In all treatments O_2 was depleted during the experimental time, but at 13% and 15 °C. In this treatment, respiration was so low that the experiment was concluded after 250 days, when O_2 concentration was still at 12.7%. O_2 concentration decreased more rapidly in time with the increase in m.c. and temperature. At 35 °C, O_2 was depleted in 70 days for 13% m.c. and in 11 days for 17% m.c. At 15% m.c., O_2 was depleted in 168 days for 15 °C and in 20 days for 35 °C. The maximum CO_2 concentration increased with m.c. (when temperature was fixed in 35 °C, CO_2 maximum concentration increased from 12 to 18% when m.c. increased from 13 to 17%), temperature (when m.c. was fixed in 15%, CO_2 maximum concentration increased from 8% to 15% when temperature increased from 15 °C to 35 °C) and time (in general, CO_2 maximum concentration was observed when O_2 was depleted).

3.2. Modelling O_2 and CO_2 evolution with a linear function of time

Eq. (1a) and (1b) were fitted to experimental O_2 and CO_2 concentration data. Only O_2 concentration values above 2% V/V (and the corresponding CO_2 values) were considered in the fitting process shown in Fig. 1(a). Intercept was fixed to 0% for CO_2 and 21% for O_2. Table 1 shows the estimated parameters (slope *b*), and the adjusted R-Square of the fit is shown in Table 4.

The value of parameter *b* was replaced in Eqs. (3) and (4) and constant rates of respiration were obtained for each treatment as shown in Table 2.

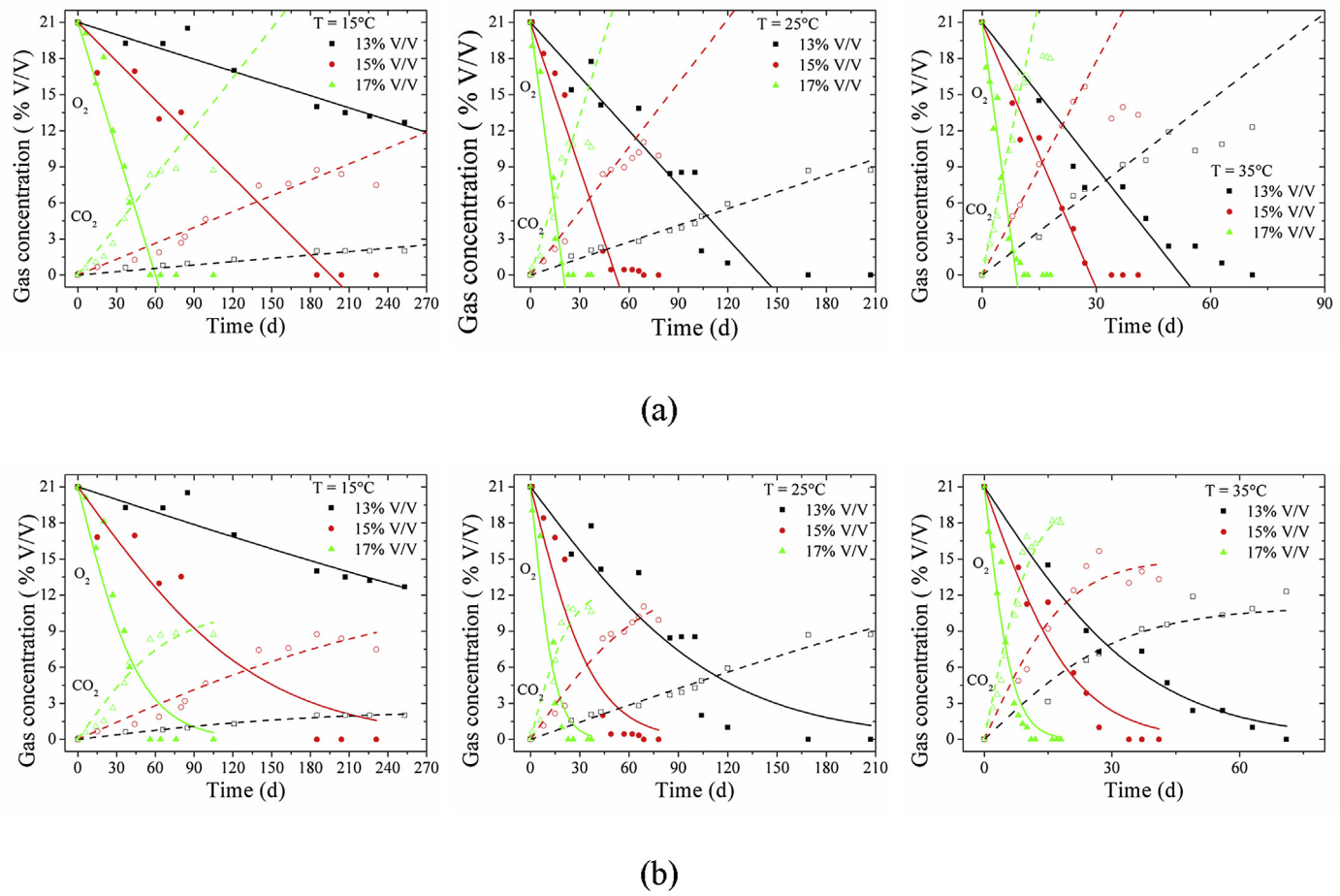

Fig. 1. Observed (points) and fitted CO_2 (dash line) and O_2 (solid line) concentrations of hermetically stored soybean at 15 °C (left), 25 °C (center), 35 °C (right) and 13% (black), 15% (red) and 17% (green) m.c., with the linear correlation (a) and exponential correlation (b). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Parameters values for the linear correlation. (valid for O_2 concentrations above 2% V/V).

m.c. (% w.b) and temperature (°C)	CO_2[a]	O_2[a]
	b	b
13%-15 °C	0.0093	−0.0338
13%-25 °C	0.0460	−0.1500
13%-35 °C	0.2419	−0.4020
15%-15 °C	0.0440	−0.1073
15%-25 °C	0.1773	−0.4052
15%-35 °C	0.5920	−0.7360
17%-15 °C	0.1367	−0.3496
17%-25 °C	0.4391	−1.0472
17%-35 °C	1.5269	−2.3494

[a] Intercept was fixed to 0 for CO_2 and 21 for O_2.

Table 2
CO_2 and O_2 respiration rates (mg/(kgDM d)) computed with the lineal correlation. (valid for O_2 concentrations above 2% V/V).

m.c. (% w.b) and temperature (°C)	R_{CO_2}	R_{O_2}
13%-15 °C	0.130	−0.341
13%-25 °C	0.617	−1.465
13%-35 °C	3.143	−3.799
15%-15 °C	0.614	−1.088
15%-25 °C	2.390	−3.973
15%-35 °C	7.722	−6.982
17%-15 °C	1.941	−3.609
17%-25 °C	6.025	−10.450
17%-35 °C	20.272	−22.684

3.3. Modelling O_2 and CO_2 evolution with an exponential function of time

Fig. 1(b) shows the fitted exponential correlations (Eq. (2a) and (2b)) to the experimental data and estimated parameter (a, k) are shown in Table 3. The adjusted R-Square values of the fit are included in Table 4.

$\frac{d[CO_2]}{dt}$ and $\frac{d[O_2]}{dt}$ were calculated according to the exponential correlations (2a and 2b) and the respiration rates were calculated with Eqs. (3) and (4). Fig. 2 plots the respiration rates as function of time for the different temperature and m.c. combinations for the exponential fitting function.

3.4. Respiration rates models as function of temperature, m.c. and O_2 concentrations

3.4.1. Model I: Oxygen concentration independent

Respiration rates shown in Table 2 were obtained with the linear correlations and are independent of O_2 concentration. Therefore, Model I (Eq. (5)) was fitted to these values obtaining the parameters shown in Table 5.

Table 3
Parameters values for the exponential correlation.

m.c. (% w.b) and temperature (°C)	CO_2		O_2
	a	k	k
13%-15 °C	4.77799	0.01091	0.00334
13%-25 °C	42.10279	0.00454	0.01718
13%-35 °C	26.064	0.04489	0.05098
15%-15 °C	25.42369	0.00749	0.01403
15%-25 °C	27.32983	0.0284	0.05082
15%-35 °C	29.9569	0.10344	0.09362
17%-15 °C	21.57568	0.02819	0.04077
17%-25 °C	25.48771	0.08168	0.13347
17%-35 °C	42.27352	0.164	0.29896

Table 4
Adjusted R-Square values for the linear and exponential correlations for CO_2 and O_2 concentrations at different temperatures and moisture contents.

Temperature (°C)	Moisture content (% w.b)					
	13		15		17	
	CO_2	O_2	CO_2	O_2	CO_2	O_2
Linear correlation (eq. (1))						
15	0.97835	0.99744	0.95616	0.99338	0.96248	0.98835
25	0.99235	0.97839	0.98872	0.99189	0.96475	0.9857
35	0.99463	0.97636	0.99956	0.98927	0.9896	0.98367
Exponential correlation (eq. (2))						
15	0.98282	0.9311	0.92966	0.94383	0.93185	0.92794
25	0.97467	0.90466	0.97569	0.94593	0.931	0.94899
35	0.96372	0.97746	0.92911	0.95732	0.9621	0.95325

3.4.2. Model II: Oxygen concentration dependent

The respiration rates obtained with the exponential correlation shown in Fig. 2 were fitted to Model IIA (Eq. (6)) and IIB (Eq. (7)), and the estimated parameters and accuracy of the fit are listed in Tables 6 and 7, respectively.

3.5. Validation of the proposed correlations

An independent set of experimental data from Arias Barreto (2016) was used to validate Model I, IIA and IIB. Fig. 3 shows the comparison between the predicted O_2 and CO_2 concentrations of the three models and the experimental data for hermetic storage of soybean at 17% m.c. and 35 °C, and Table 8 shows the statistics of the comparison. The interpretation of the three statistics (MRD, MAD and SE) is that the lower their value, the better the prediction of the model.

Across all models, the prediction for CO_2 was slightly better than the prediction for O_2 for the three statistics considered. Model I showed a good performance when data points above 2% O_2 were considered in its evaluations. However, when all data points were considered, the prediction of Model I worsened significantly, showing the difficulty of using constant respiration rates for predicting gasses concentrations in hermetic storage when O_2 concentration is near to be depleted. Model IIA had a better prediction for CO_2 (2 out of 3 statistics), while Model IIB had a better prediction for O_2, although both models had similar performance. Along the entire storage time, Model I had an average deviation from the observed data of about 6.8% for O_2 and 4.0% for CO_2, Model IIA about 2% for O_2 and 1.7% for CO_2 and Model IIB about 1.7% for O_2 and 2.5% for CO_2.

Table 5
Parameter values for O_2 and CO_2 respiration rate according Model I and their statistics (R, R^2, adjusted R^2) and significance (P value).

Gas	$\log\lvert R\rvert = a_1 + a_2T + a_3M$			R	R^2	Adj.R^2	P value
	a_1	a_2	a_3				
O_2	−3.901	0.0442	0.221	0.989	0.979	0.972	<0.001
CO_2	−4.862	0.0584	0.248	0.994	0.988	0.983	<0.001

4. Discussion

4.1. Dynamics of respiration

As many biological processes, the respiration evolution in time is likely to follow a sigmoid, with an initial lag phase, an exponential phase and, finally, a plateau as some resource is depleted (as hermetic storage evolves, O_2 becomes the limiting factor). However, these three phases were not clearly identified (Fig. 1). The lag phase was absent in all treatments. It was observed that respiration started and progressed with a linear or exponential phase. In some experiments the linear or exponential phase continued until O_2 was

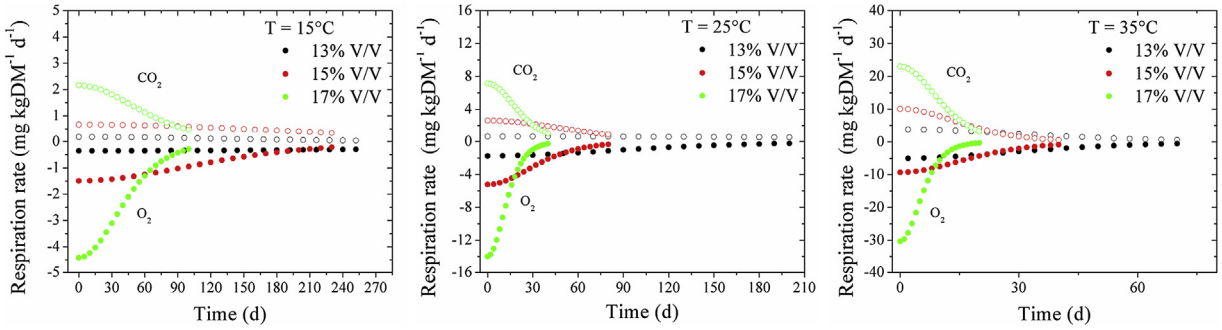

Fig. 2. CO_2 and O_2 respiration rates computed using exponential fitting function for hermetically stored soybean at 15 °C (left), 25 °C (center), 35 °C (right) and 13% (black), 15% (red) and 17% (green) m.c. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 6
Parameter values for O_2 and CO_2 respiration rate according to Models IIA for the three different moisture contents and their statistics (R, R^2, adjusted R^2) and significance (P value).

m.c. (% w.b)	Gas	$R = a_1 + a_2T + a_3O_2 + a_4TO_2$				R	R^2	Adj.R^2	P value
		a_1	a_2	a_3	a_4				
13	O_2	0.972	−0.0437	0.124	−0.010	0.962	0.926	0.924	<0.001
	CO_2	−1.020	0.051	−0.088	0.007	0.914	0.835	0.831	<0.001
15	O_2	0.468	−0.045	0.229	−0.020	0.984	0.968	0.967	<0.001
	CO_2	0.595	0.009	−0.429	0.025	0.959	0.919	0.917	<0.001
17	O_2	0.617	−0.0687	0.888	−0.0712	0.976	0.952	0.951	<0.001
	CO_2	−5.813	0.379	−0.577	0.0420	0.918	0.843	0.839	<0.001

Table 7
Parameter values for O_2 and CO_2 respiration rate according to Model IIB and their statistics (R, R^2, adjusted R^2) and significance (P value).

Gas	$log\|R\| = a_1 + a_2T + a_3O_2 + a_4M$				R	R^2	Adj.R^2	P value
	a_1	a_2	a_3	a_4				
O_2	−4.271	0.0417	0.0502	0.200	0.863	0.745	0.743	<0.001
CO_2	−5.237	0.0537	0.0244	0.255	0.934	0.872	0.871	<0.001

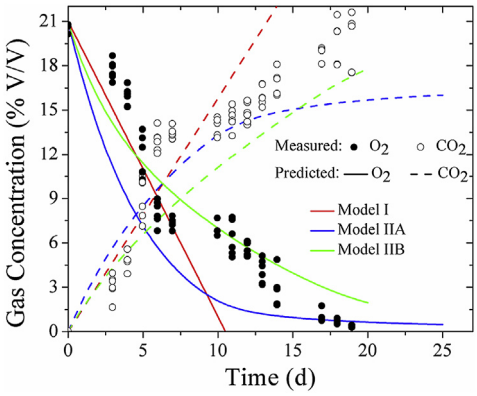

Fig. 3. Comparison between the predicted O_2 (solid line) and CO_2 (dashed line) concentrations of Model I (red), Model IIA (blue) and Model IIB (green) and an independent experimental data (points) for hermetic storage conditions of 17% m.c. and 35 °C. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

depleted, and in others the plateau phase started below 2–3% O_2. Similar behavior was reported by Lacey et al. (1994) in wheat experiments.

The different shapes of the respiration curves at the different temperature and m.c. could be explained because what is called "respiration" in this work is, in fact, a series of metabolic and non-metabolic processes governed, mainly, by the seed hydration level.

In most grain postharvest engineering works, respiration is used as a synonym of O_2 uptake and CO_2 release. However, this is not an accurate terminology from physiological point of view, since at low m.c. the metabolisms of the seeds and associated microorganisms are inactive. Vertucci (1989) described three phases in term of hydration level of the seed and O_2 uptake processes: non-metabolic, enzymatic, and mitochondrial electronic transport. At low m.c. (water activity (a_w) < 0.5), O_2 uptake and CO_2 release are very low and mostly due to oxidative reaction (likely peroxidation). This could be considered as a sort of non-metabolic basal O_2 uptake, not truly respiration. As m.c. increases some enzymatic reactions that consume O_2 become active in the cells, and above the limit of microbiological activity (a_w > 0.7), fungi first, and later yeast and bacteria become active too (Bern et al., 2002; Hamer et al., 1991),

Table 8
Mean relative deviation (MRD), mean absolute deviation (MAD) and standard error (SE) of the goodness of fit for O_2 and CO_2 values for the three evaluated models (n is number of compared data points).

Statistic	Model (n)	O_2	CO_2
MRD	M I (14)	6.5805	0.3447
	M I (7)*	0.2109 [a]	0.3293
	M IIA (14)	0.4616	0.2802
	M IIB (14)	0.8964	0.2565 [a]
MAD	M I (14)	6.8028	4.0218
	M I (7)*	2.0528	1.7495 [a]
	M IIA (14)	3.0197	2.0661
	M IIB (14)	1.6688 [a]	2.5137
SE	M I (14)	8.7979	5.0805
	M I (7)*	2.7624	2.1073 [a]
	M IIA (14)	3.8096	2.5222
	M IIB (14)	1.9839 [a]	2.9838

*Considering only data points with O_2 concentration above 2%.
[a] Best model for the considered statistic.

contributing greatly to the overall respiration process. If seed further increases in m.c. (a_w > 0.95) the physiological mechanism of germination is activated, mitochondrial respiration starts, and the contribution of the seed becomes predominant in the overall O_2 consumption and CO_2 release processes (Vertucci, 1989). Thus, according to the m.c. of the seed, the shape of the respiration curve during hermetic storage could be different since different O_2 up taking processes could be active, and also the O_2 restrictive condition could affect differentially the three processes described above. In the present study, the range of m.c. selected (a_w < 0.87) would involve the combination of processes 1 and 2 described above, out of the range of seed mitochondrial respiration.

4.2. Respiration rates

4.2.1. Comparison of soybean respiration rates with other products

The uptake of O_2 and release of CO_2 reported in this study (Table 2) was from 0.341 to 22.684 mg O_2/(kgDM d) and from 0.130 to 20.272 mg CO_2/(kgDM d) (for storage conditions of 13–17% m.c. and 15–35 °C). Sood (2015) reported a soybean CO_2 production rate of 15.7 mg CO_2/(kgDM d) for storage conditions of 14% m.c. and 35 °C, in the range of what is reported in the present study. These respiration rates of soybeans were lower than those reported in other studies for other grains but under similar temperature and a_w conditions. Karunakaran et al. (2001) reported rates between 23 and 463 mg CO_2/(kgDM d) for stored wheat in the range of 12.7–19% m.c. and 25 °C (a_w of 0.58 and 0.87, without O_2 restrictive conditions). Pronyk et al. (2004) reported CO_2 production rates of canola (in nonrestrictive O_2 condition) of 172 and 290 mg CO_2/(kgDM d) for 12% (a_w 0.80) and 14% m.c. (a_w 0.85), respectively, and a temperature range between 25 and 30 °C, and of 185, 192 and 500

mg CO_2/(kgDM d) for 10% (a_w 0.75), 12% (a_w 0.80) and 14% m.c. (a_w 0.85), respectively, for a higher temperature range (30 and 35 °C). Lacey et al. (1994) reported respiration rates of wheat from 53 to 474 mg CO_2/(kgDM d) for a_w of 0.85 and temperature of 20 °C. On the contrary, Jian et al. (2014) reported higher respiration rates of soybean (from 116.7 to 126.7 mg CO_2/(kgDM d)) than wheat (from 66.0 to 134.3 mg CO_2/(kgDM d)) and canola (from 22.4 to 118.7 mg CO_2/(kgDM d)), but the m.c. of the soybean seed was 23%, equivalent to 0.90 a_w. The m.c. evaluated by Jian et al. (2014) is substantially above the range of safe storage condition (13.0–13.5%), and even above the recommended harvest m.c. (5–16%, Bragachini et al., 2012). Such high m.c. would involve intensive microbial respiration (Bern et al., 2002; Hamer et al., 1991) and physiological processes (Vertucci, 1989) that did not occur in the present study.

The lower respiration rate of soybeans in comparison with other products (at the same a_w), such as cereal grains, could be due to some physiological or compositional condition of the oilseeds that affects the different O_2 uptake processes described in the previous section, or because of some particular condition in the seed coat that disfavor microbiological activity and, hence, overall respiration rate. Supporting these speculations, Lacey et al. (1994) also found a lower respiration in oilseeds (rapeseed and linseed) than in wheat. Fonseca et al. (2002), stated that the ratio CO_2 produced to O_2 consumed, known as the respiratory quotient (RQ), changes according to the main substrate used in the respiration process (from <1 for lipids to 1 for carbohydrates and >1 for acids). Thus, if according to the composition of the seed different metabolic pathways are predominant resulting in different QRs, respiration rates could also be different. Evidences at large scale of hermetic storage also indicate lower respiration activity of soybean in comparison with cereal grains. Cardoso et al. (2008) measured CO_2 concentration lower than 1.5% in several soybean silo bags (180–200 t) during 4–9 months of storage with m.c. between 10 and 15%. In a similar study, Rodríguez et al. (2008) measured CO_2 concentrations substantially higher (up to 20%) in wheat silo bags with m.c. from 13 to 14%. The higher CO_2 concentration of wheat silo bags in comparison with soybean silo bags could be partially attributed to the higher temperature during the storage season of the first ones (wheat is harvested during the early summer while soybean during the autumn). However, soybean silo bags in general always reach lower CO_2 concentration than cereal grain when stored at the same a_w condition, regardless the season.

Other source of variation in respiration rate is the initial level of physical damage of the seed. Physically damaged seeds promote higher respiration rates, possible by increased microbiological activity. Bern et al. (2002) proposed a correlation to predict CO_2 evolution and dry matter for shelled corn, including a multiplier to account for the effect of visible mechanical damage. The higher the level of damage the higher the respiration rate. Navarro et al. (2012) correlated the respiration rate of peanut seeds with the physical damage level of the seeds, and observed that, unless broken or damaged peanut seeds were present, the respiration rate of peanut was low. In the present study the physical damage level of soybean seeds was very low (germination test was >95% for all the samples), which would also explain the low level of respiration rate. However, this level of damaged seeds is representative of commercial soybean samples in Argentina, where modern combines harvest the crop with minimum physical damage of the seeds, usually around 3% (Kowalczuk, 1999; Méndez and Roskopf, 2006).

The respiration rate of the product during hermetic storage determines the time it takes to accomplish anaerobic conditions. Comparing this study results with literature data, the time required for O_2 depleting in hermetic storage was longer for soybean than for other grains (Table 9). For storage conditions of soybean of 25 °C and 0.80 a_w it took 50 days for depleting, while for paddy rice at 24 °C and 0.76 a_w it took 35 days. Similarly, for storage conditions of soybean of 35 °C and 0.86 a_w it took 11 days, while for corn at 30 °C and 0.84 a_w it took 5 days and for wheat at variable temperature and 0.86 a_w it took only 4 days. The maximum CO_2 concentration observed also resulted substantially lower than that reported for cereal grains. For instance, soybean stored with a_w between 0.84 and 0.87 resulted with a maximum CO_2 concentration from 8 to 18%, while in corn and wheat the maximum concentration exceeded 40%.

4.2.2. Effect of temperature, moisture content and oxygen

Respiration rate increased with temperature and m.c. (Fig. 1). The same effect of temperature and m.c. on respiration was reported by several authors for different agricultural commodities. Hamer et al. (1991) observed that respiration of wheat increased with temperature and m.c., markedly above an $a_w > 0.85$. Diawara et al. (1986) reported a similar effect of m.c. on respiration of paddy rice, and Weinberg et al. (2008) on corn. Coincidently, Lacey et al. (1994) concluded that respiration increased linearly with temperature up to 35 °C and that also increased with time and m.c. in barley, wheat, rapeseed and linseed.

The m.c. range explored in the present study (13–17%) represents the typical condition at which most of the commercial soybean would be stored. The commercial standards set a maximum m.c. between 13.0 and 13.5%, thus, harvesting soybean below 13% would imply a substantial economic loss for farmers. On the other hand, harvesting soybean above 17% m.c. would imply higher risk of seed damage by the combine (Bragachini et al., 2012), higher storage risk until seed can be dried to safe storage condition (13–13.5%), and extra drying costs. Respiration rates of soybeans out of the range explored in this study could be substantially different, especially due to the effect of a_w on microbiological activity. At lower m.c. than 13% ($a_w < 0.7$) microbial activity would be further diminished and, hence, lower respiration rates should be observed. On the contrary, respiration rates of soybeans with m.c. higher than 17% ($a_w > 0.85$) would result in higher microbial activity (even anaerobic) and, hence, higher rates.

In this study, respiration rate was more affected by m.c. than by temperature. Across all treatments, CO_2 release and O_2 uptake increased 111% and 91%, respectively, for each 1% point of increase in m.c. On the other hand, for each 1 °C of increase in temperature, respiration rate was modified in 58% for CO_2 and 38% for O_2 (Table 2).

Respiration rate was not affected by O_2 until certain critical concentration limit was reached, and that limit seems to be around 2%. This could be graphically observed in Fig. 1, and also in Table 4, which shows that had a very high Adjusted R-Square for nonrestrictive O_2 conditions (above 2%). The fact that respiration rates at different temperatures and m.c. only started to decrease when O_2 concentration dropped below 2%, would imply that hermetic storage must have a high level of airtightness to result in benefits for the conservation of the soybean seed. In Argentina, about 35% of the soybean is stored every year in silo bags (about 20 million tonnes). Silo bags are considered a very successful storage system for this product, since no significant quality losses are reported when the soybean is stored a 13.5% m.c. and the silo bag maintains a minimum airtightness to prevent the entrance of rainwater (Taher et al., 2014a, 2014b).

In general it can be stated that the silo bag should have a high airtightness level to allow reaching low enough O_2 concentration to obtain a benefit of the O_2 depleted environment, implying that a good sealing of the end of the bag should be achieved, and any perforation in the plastic cover should be patched immediately. Additionally, the incorporation of O_2 barrier in the silo bag liner would help to obtain full benefits in the conservation of the stored

Table 9
Time required for oxygen depleting during hermetic storage at different temperature and a_w conditions.

Grain	Temperature (°C)	Moisture content (% w.b)	a_w	Hours (days)	CO_2 max. (%)	Reference
Soybean	15	13	0.70[a]	(270)[b]	3[d]	This study
	25	13	0.72[a]	3900 (165)[c]	9	
	35	13	0.74[a]	1600 (70)[c]	12	
	15	15	0.78[a]	4400 (183)[c]	8	
	25	15	0.80[a]	1600 (70)[c]	11	
	35	15	0.81[a]	480 (20)[c]	15	
	15	17	0.84[a]	1200 (53)[c]	8	
	25	17	0.86[a]	430 (18)[c]	11	
	35	17	0.87[a]	260 (11)[c]	18	
Corn	30	14	0.74[a]	800 (33)	18	Weinberg et al. (2008)
	30	16	0.84[a]	120 (5)	42	
	30	18	0.90[a]	<50 (2)	75	
	30	20	0.94[a]	<20 (<1)	82	
	30	22	0.97[a]	<10 (<1)	90	
Paddy rice	24	15[a]	0.76	840 (35)	10[e]	Diawara et al. (1986)
	24	17.5[a]	0.86	230 (<10)	17[e]	
Wheat	Variable	17.9	0.83[a]	1600 (70)	40	Hyde and Oxley (1960)
	Variable	18.7	0.86[a]	900 (38)	53	
	Variable	19.5	0.88[a]	600 (25)	65	
	Variable	20.3	0.90[a]	240 (10)	77	
	Variable	21.8	0.93[a]	95 (4)	85	
	Variable	24.4	0.96[a]	70 (3)	95	

[a] Estimated based on Modified Chung-Pfost equilibrium moisture content model and ASABE (2001) parameter values.
[b] O_2 concentration only reached 12% after 270 days.
[c] Storage time corresponding to the first measuring with depleted O_2.
[d] O_2 was reduced only to 12%.
[e] Concentrations did not reach the maximum.

product.

However, due to that most of the soybean is harvested at a m.c. of 13.5% or lower during the fall, and that the temperature of the seeds during fall and winter in the silo bags is below 15 °C (Cardoso et al., 2008), the respiration rate is so low (about 0.341 mg O_2/(kgDM d) – Table 2) that O_2 will not be depleted during the expected storage time of 6 months. The experimental results shown in Table 9 indicates that soybean hermetically stored at 13% m.c. and at 15 °C only reached 12% O_2 after 9 months of storage. This is in agreement with the field data reported by Cardoso et al. (2008), which showed that the CO_2 concentration in soybean silo bags is usually below 4%, indicating low respiration, while in wheat silo bags much higher concentrations were reported, exceeding 20% (Rodríguez et al., 2008). This would indicate that, in the case of soybean seeds, the success of silo bag storage is more related to the combination of the low temperature of the seed during the fall, winter and early spring and to the lower respiration rate of the soybean seeds in comparison with other products.

4.3. Respiration models

Model I is the simplest model since it only takes into account temperature and m.c. When this model was fitted for the entire set of data it was observed that the predicted values below 2% O_2 were off the observed values, thus the model was only fitted for hermetic storage condition with O_2 concentration above 2%. Under these conditions, the model was able to predict O_2 and CO_2 concentrations with high accuracy (adjusted R-Square of 0.972 and 0.983, respectively). However, the main limitation on this respiration correlation for modelling hermetic storage is that an empirical attenuation of the respiration has to be implemented when O_2 concentration drops below 2%.

Model II takes into account temperature and O_2 concentration and it was able to predict, with high accuracy, the respiration of soybean seeds at the three m.c. experimented for the full range of O_2 concentration. The range of adjusted R-Square was 0.924–0.967 and 0.831–0.917 for O_2 and CO_2, respectively. However, the main limitation of this respiration correlation for modelling hermetic storage is that Model IIA was fitted for three specific m.c. (13, 15 and 17%), thus interpolation must be implemented if it want to be used for other intermediate m.c. values.

Model IIB takes into account temperature, m.c. and O_2 concentration and it was able to predict, with lower accuracy than Model I and Model IIA, the respiration of soybean seeds for the full range of O_2 concentration. The adjusted R-Square was 0.743 and 0.871 for O_2 and CO_2, respectively. Since no evidence in the literature was found of a respiration correlation for grains and oilseeds dependent on temperature, m.c. and O_2 concentration, there is no referential value for the goodness of fit to compare with Model IIB. This model is particularly useful and easy to implement in simulation models of hermetically stored grain as m.c., temperature, CO_2 and O_2 concentrations vary during storage.

The validation of the three models with an independent set of experimental data showed that the assumption of Model I (respiration rate does not depends on O_2 concentration) provided good results only when O_2 concentration was above 2%. This would imply that respiration rate is not mainly affected by O_2 until certain critical concentration limit is reached, and that limit seems to be around 2%. Models IIA and IIB (O_2 concentration dependent) had a similar behavior and were able to predict O_2 and CO_2 evolution with high accuracy during the entire range of O_2 concentration.

These models are based on experimental data with three levels of temperature and seed m.c., thus it is advised to use the model between the range of the experimental conditions (13–17% m.c. and 15–35 °C). Additionally, even though these two variables seem to be the most influential there are other factors that could affect respiration rate, such as physical damage of the seed and initial microbial concentration among others, and this also should be considered for the use of the model.

5. Conclusions

The respiration rate of soybean seeds obtained in this study resulted significantly lower than the rates reported in the literature for other grains at similar temperature and a_w storage condition. This could be due to some physiological or compositional condition of the oilseeds that affects the different O_2 uptake processes, or because of some particular condition in the seed coat that disfavor microbiological activity.

Soybean respiration rate was dependent on temperature, moisture content and O_2 concentration of the hermetic interstitial air. However, respiration rate was not mainly affected by O_2 until a critical concentration limit of about 2% was reached. Respiration rate increased with storage m.c. and temperature. Values of respiration rates between 13 and 17% m.c. and 15–35 °C were from 0.341 to 22.684 mg O_2/(kgDM d) and from 0.130 to 20.272 mg CO_2/(kgDM d), respectively.

The fact that respiration rates at different temperatures and m.c. resulted constant until O_2 dropped below 2% would imply that hermetic storage must have a high level of airtightness to have benefits in the conservation of the soybean seed. The silo bag system used in Argentina for successful storage of soybean usually do not reach O_2 concentrations below 2%, implying that the benefit would be more related to the low respiration rate of the oilseed and the low temperature during storage than to the hermetic storage itself.

For hermetic storage simulations in which O_2 concentration is not expected to drop below 2%, the simplest model (Model I) could be used, which only depends on temperature and seed m.c. If the O_2 concentration of the hermetic system is expected to be depleted, Model I would under estimate the time at which O_2 is consumed, and thus Model IIA or IIB are recommended instead.

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