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Changes in soybean quality under controlled environmental conditions using static grain respiration measurement system

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ABSTRACT. *Grain quality evaluation during storage has become an important field of study to control postharvest losses. Lipid oxidation (LO) and dry matter loss (DML) are considered the main causes of deterioration in quality due to its impact on chemical, sensorial and nutritional properties of soybeans. Prolonged storage time during unfavorable conditions, such as high temperature and moisture content, is responsible for accelerating LO and DML rates* (v_{DML}). *To understand changes in soybean quality, storage tests were performed by placing 22% moisture content soybeans in two different sealed chambers at 30°C for 40 days. DML was quantified by measuring respired carbon dioxide (CO_2, ppm). This measurement was used to calculate mass of respired CO2 (g CO_2/kg beans d.b.) and correlated with DML. To obtain information about the oxidative process, samples were collected every 5 days for further chemical analyses. The objective was to quantify changes in quality of soybeans under adverse storage conditions. In addition, an alternative approach to measure DML using a static grain measurement system (S-GRMS) was evaluated. Data and samples collected, as well as the improved S-GRMS, will be important to design further experiments to estimate quantitative and qualitative grain losses during storage. Results of future experiments will be essential for understanding deterioration processes in soybean and building guidelines to prevent postharvest losses.*

Keywords. *Dry matter loss, grain quality, lipid oxidation, postharvest losses, safe storage, soybean.*

Introduction

Postharvest loss is a global concern due to population growth and its food demand. Global soybean production in 2020/21 is forecast at 362.8 million tons (USADA, 2020) and a significant quantitative and qualitative amount of this production is lost every year. Moreover, in recent times, the traceability of products and activities in the supply chain have become new factors in food and agribusiness. Increasingly, consumers and industry are searching for evidence of quality and safety through the entire food chain (Narayan et al., 1988; Opara, 2003). For this reason, postharvest losses and grain quality evaluation from harvest through storage have become important fields of study.

During storage, grains are susceptible to physical, chemical and biological changes depending on storage conditions and time, and characteristics of the stored product (Narayan et al., 1988). High levels of temperature and moisture content, for instance, are responsible for accelerating the respiratory metabolism and oxidation process (Yang et al., 2014). If these changes exceed a certain limit, the beans are considered deteriorated. Dry matter loss (DML) and lipid oxidation (LO) are considered the main causes of these physical-chemical changes, resulting in weight and value loss. Therefore, acceptability and suitability of soybeans are related directly to overall quality during storage (Narayan et al., 1988). Also, substantial quality loss after harvest may occur between field harvest and initial drying at grain terminals, especially when harvest occurs during hot and wet periods of time (Danao et al., 2015).

Because of its intrinsic characteristics and composition, soybeans are among the most valuable crops in the world, being used to feed billions of livestock (Nwokolo, 1996) as well as substantial global vegetable oil supplies (Alencar et al., 2010). Given its importance, several studies have been conducted to understand and improve soybean storage conditions to ensure its quality and reduce losses. It is important to estimate a safe storage time for grains by considering environmental conditions. One way to perform this estimation is using a static grain respiration measurement system (S-GRMS). This system consists of grain placed in a hermetically sealed chamber, with controlled environment conditions to measure respired CO_2 and evaluate DML during storage.

One way to quantify DML is by correlating respired carbon dioxide (CO_2) accumulated in a sealed chamber with substrate loss. This correlation is estimated based on the respiration process, where for each mole of glucose (180 g mol^{-1}) consumed, 6 moles of CO_2 (6 x 44 g mol^{-1}) are produced. Several studies have attempted to estimate time to reach 0.5% DML, which is considered the threshold used for maximum allowable storage time guidelines for shelled corn by ASABE Standard D535 (R2019) . These guidelines have been also used by many authors as an estimation for safe storage time of soybeans (Rukunudin et al., 2004; Steele, 1967).

To develop a set of safe storage times for soybeans, various researchers reported the effects of different storage conditions on DML rates (v_{DML}). For instance, Jien et al. (2014) found v_{DML} as 0.003% to 0.008% day^{-1} for 23% moisture content soybeans storage at 15°C to 35°C in a S-GRMS for 30 days. Similarly, Pereira da Silva (2018), who also used a static system to estimate DML, reported 0.0157% day^{-1} v_{DML} for soybeans storage at 30°C with 18% moisture content. However, there is still a lack of data of soybeans storage at warmer conditions. Therefore, more studies should be conducted to understand deterioration processes in oilseeds and building guidelines to prevent postharvest losses in tropical climates.

Soybeans are composed of 20% oil (d.b.), which contains a significant level of unsaturated fatty acids (approximately 85% of total oil), including palmitic acid (7% - 14%), oleic acid (19% - 30%), linoleic acid (44% - 62%) and linolenic acid (4% - 11%) (Carrera et al., 2011). Because of this profile, soybean oil is highly susceptible to oxidation, resulting in physical and biochemical changes in its properties, which reduces the quality of extracted crude oil and finished refined, bleached and deodorized oil (Frankel et al., 1987).

The overall mechanism of lipid oxidation consists of three phases. The first phase is initiation where free radicals (R•) are formed from an unsaturated lipid molecule (RH). In the propagation phase, free radical chains react with oxygen to generate peroxy radicals (ROO•), which can further react with unattached unsaturated fatty acids to form hydroperoxide (ROOH) (Frankel, 1984). In the termination phase, two radicals react resulting in products (R-R and ROOR) that do not sustain the propagation phase (Ahmed et al., 2016).

Initiation:	$RH \rightarrow R\bullet + H\bullet$
Propagation:	$R\bullet + O_2 \rightarrow ROO\bullet$ $ROO\bullet + RH \rightarrow ROOH + R\bullet$
Termination:	$R\bullet + R\bullet \rightarrow R\text{-}R$ $ROO\bullet + R\bullet \rightarrow ROOR$

During the propagation phase, secondary products are formed from the decomposition of lipid hydroperoxides. Hydroperoxide is a primary oxidation product, which is tasteless and odorless, and therefore, has no significant impact on sensorial quality. However, hydroperoxides are generally unstable and can lead to the formation of secondary oxidation products, that are volatiles such as aldehydes (hexanal and propanal), ketones, alcohols, hydrocarbons, and organic acids, which adversely affect lipid quality (Ahmed et al., 2016).

Given the complexity and dynamism of the LO reaction, it is important to evaluate primary and secondary products several times during storage. One way to measure hydroperoxides is through peroxide value (PV). However, PV is limited to the initial stages of oxidation and a different methodology needs to be used to measure secondary products (Van der Merwe, 2003). This measurement can be done efficiently using headspace gas chromatography, which is considered the most sensitive methodology for estimsting oxidative deterioration (Frankel et al., 1987).

Considering DML and LO as the main concerns for quality and safety during grain storage, determining those changes can help to create guidelines for handling and storing grains under different conditions. Therefore, it is essential to identify an efficient way to evaluate the changes in lipid profiles and losses in dry matter during storage time. For this purpose, this study approached two main objectives that aimed to provide important information and data for future tests:

1. To improve efficiency of S-GRMS research by comparing a conventional respiration chamber to an alternative one.
2. To understand changes in soybean quality by collecting data for estimations of v_{DML} and for further chemical analysis of LO.

Material and Methods

Soybeans and sample preparation

Soybeans were obtained from the Crop Sciences Research and Education Farm at the University of Illinois at Urbana-Champaign in 2019. Beans were harvested with moisture content of 12.96% (w.b.) and stored at 4°C until testing. Soybeans (4.8 kg) were cleaned to remove split or damaged beans and impurities using a sieve (Grainman 10/64" x 3/4", Miami, FL, USA). After cleaning, the sample was acclimated in an incubator at 30°C ± 2°C for 3 days.

To ensure the desired moisture content (22% w.b.) during storage tests, initial moisture was estimated using a portable moisture meter (Model No. SW16060, John Deere, Moline, IL, USA) and moisture content was adjusted by adding a calculated amount of deionized water needed to rewet the beans. Around 580 g water was added gradually to the 4.8 kg sample. To provide complete and uniform water absorption, soybeans were placed in 2 L bottles, which were placed on roller mixers (Model No. MX-T6-S, Scilogex, Rocky Hill, CT, USA) set to 60 rpm for 60 min. Final moisture was checked via moisture meter and determined gravimetrically (ASABE Standard S352.2, R2017) (fig. 1).

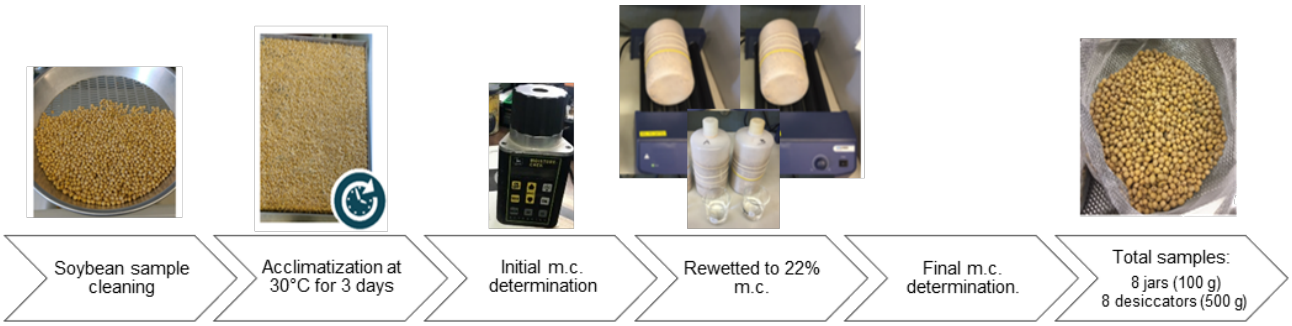

Figure 1. Soybean sample preparation

Static grain respiration measurement system

The static grain respiration measurement system (S-GRMS) was composed of a hermetically sealed chamber, where the soybean sample was placed with a sensor package (Catalog No. K33-BLG, CO2Meter, Inc., Ormond Beach, FL, USA) to monitor CO_2 concentration, temperature and relative humidity (fig. 2a). The chamber was placed inside two temperature-controlled incubators (Model No. 3033, Steri-Culti 200, Forma Scientific, Inc., Marjetta, OH, USA), set to 30°C ± 2°C (fig. 2b). Due to limited space inside the incubators and number of samples required for LO analyses, two types of chambers were tested and compared: a hermetic 10 L glass desiccator with 500 g soybeans, as used by Pereira da Silva (2018), and a 2 L glass jar with 100 g sample (fig.2b). This amount of sample was calculated to ensure proportional soybean mass in both chambers and therefore similar conditions between them, with same volume of air available for respiration. If there was no significant difference between jars and desiccators, jars will be used in future tests due to lower cost and reduced size

compared to the conventional chamber. In this way, it would be possible to perform more replications and run several experiments simultaneously within the limited space of the incubator.

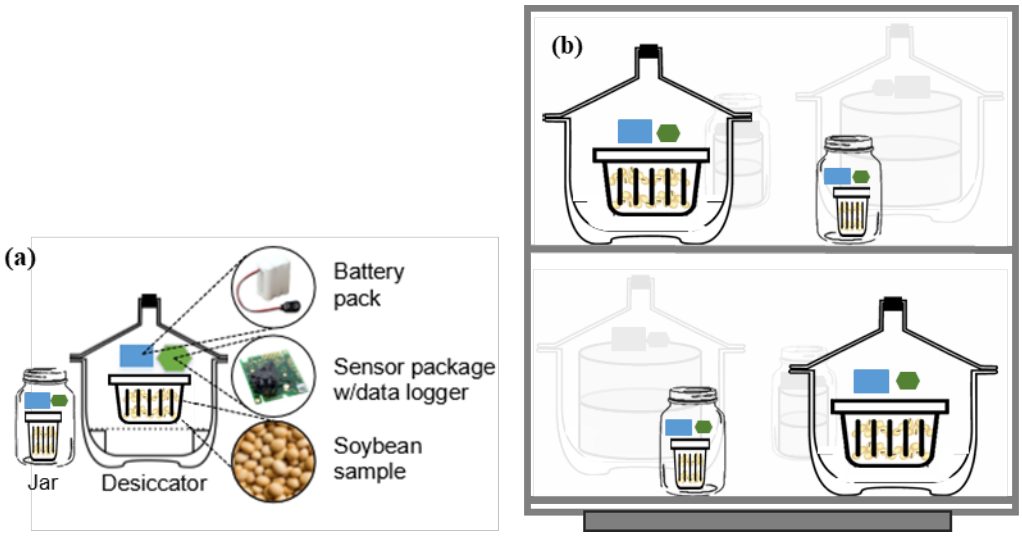

Figure 2. Representation of two S-GRMS (a) with jar and desiccator as storage chambers holding 100 and 500 g sample, respectively, a sensor package and a battery pack. Dessicators and jars were placed inside the incubator (b) for temperature control.

Respiration experiment

Each respiration experiment consisted of eight jars and eight desiccators to provide samples for LO analyses every 5 days over a maximum storage period of 40 days. Thus, a sample from each jar and desiccator was taken at 5 day increments, consecutively, until the eighth jar and desiccator reached 40 days of storage. Samples from each chamber were vacuum sealed and refrigerated at -18°C for future chemical analyses.

To estimate DML, accumulated CO_2 was measured by ten calibrated sensors placed inside jars and desiccators 3, 5, 6, 7 and 8 to obtain data for days 15, 25, 30, 35 and 40, respectively. These data allowed comparison of v_{DML} between jars and desiccators to determine if desiccators can be replaced by jars. Moreover, the information from the sensors eventually will be used to establish a relationship between v_{DML} and oxidation rates, once LO analyses are performed.

Dry matter loss

To observe CO_2 concentrations (C_{CO_2}) inside the chambers, sensors recorded data every 10 min. The amount of accumulated gas was converted to mass basis, using the ideal gas law (equation 1), and DML (%) was calculated according to the relationship between moles of glucose consumed and moles of CO_2 respired during the storage process (equation 2).

$$\sum m_{CO_2} = (C_{CO_2}) \left(\frac{P\ V\ M_{CO_2}}{R\ T} \right) \quad (1)$$

Where $\sum m_{CO_2}$ is the accumulated mass of respired CO_2 detected by the sensor, P is the pressure (1 atm), V the volume (10 L for desiccators and 2 L for jars), R the ideal gas constant (0.08205 L atm K^{-1} mol^{-1}), T the temperature (K), and M_{CO_2} the molecular weight of CO_2 (44 g mol^{-1}).

$$DML = \left(\frac{\sum m_{CO_2}}{m} \right) \left(\frac{1 \text{ mol } C_6H_{12}O_6}{6 \text{ mol } CO_2} \right) \left(\frac{M_{C_6H_{12}O_6}}{M_{CO_2}} \right) 100\% \quad (2)$$

Where $\sum m_{CO_2}$ is accumulated mass of respired CO_2 obtained from equation 1, m is the total sample mass inside the chamber (d.b.) and M represents the molecular weight of $C_6H_{12}O_6$ (180 g mol^{-1}) and CO_2 (44 g mol^{-1}), respectively. The DML of soybeans from each jar and desiccator was determined at the sampling day (15, 25, 30, 35 and 40 days).

Dry matter loss rate and safe storage time

To estimate v_{DML}, DML was plotted and regressed with time for each jar or desiccator using Data Analysis ToolPak in MS Excel (Version 2016, Microsoft Corporation, Redmond, WA, USA). The slope of the line was the estimated value of

v_{DML}, expressed as DML per unit time (% d^{-1}). Larger values of v_{DML} indicate soybeans were losing dry matter at higher rates, which usually characterizes a secondary stage (Phase II) of the DML curve (fig. 3). However, as reported by Ochandio et al. (2012) and Rukunudin et al. (2004), Phase II starts after a lag period (Phase I), characterized by a low v_{DML}. Over time, high rates of Phase II tends to decrease and become close to zero in response to the accumulated CO_2 and limited O_2 inside the static respiration system (Saltveit, 2019). This shows there was a stabilization in DML, and indicated a final stage (Phase III) of aerobic respiration was reached (fig. 3).

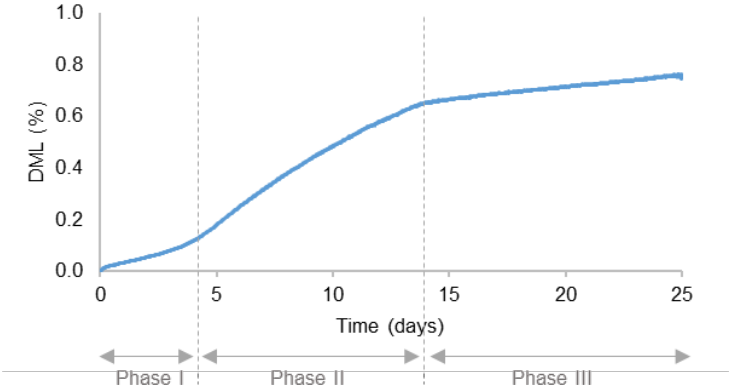

Figure 3. Representation of a pattern behavior of DML curve classified in Phase I, Phase II and Phase III.

Safe storage time ($t_{0.5\%DML}$) was determined as the elapsed time for the soybean samples to reach 0.5% DML, which is considered the threshold for maximum allowable storage time.

Statistical analyses

Paired *t*-tests were conducted to detect differences between respiration chambers for response variables of safe storage time ($t_{0.5\%DML}$) and v_{DML}. The results were tabulated as mean values of dry matter loss ($\overline{\text{DML}}$), dry matter loss rates ($\overline{v_{DML}}$) and time to reach 0.5% DML ($\overline{t_{0.5\%DML}}$), and standard deviations (SD). The *t*-tests were conducted using Data Analysis ToolPak in MS Excel (Version 2016, Microsoft Corporation, Redmond, WA, USA), based on two populations (desiccator, jar), α = 0.05, *t*-table (t_t) and *t*-statistic (t_{stat}). The null hypothesis (H_0) was rejected when $t_{stat} > t_t$, considering:

$$H_0: \mu_{dessicator} = \mu_{jar} \quad (2)$$

$$\text{Alternative hypothesis } (H_a): \mu_{dessicator} \neq \mu_{jar} \quad (3)$$

$$t_t = (\alpha, (n-1)) \quad (4)$$

$$t_{stat} = \frac{\mu_{desiccator,jar}}{SE_{desiccator,jar}} \quad (5)$$

where,

$$\mu_{desiccator,jar} = \frac{\sum_{i=1}^{k}\left(v_{\text{DML},desiccator} - v_{DMLjar}\right)_i}{\sum_{i=1}^{k} n_i}$$

and,

$$SE_{desiccator,jar} = \frac{SD_{desiccator,jar}}{\sqrt{n}}.$$

Results and Discussion

During the experiment, an air leak was detected on the desiccators of 15 and 30 days of storage time. Soybean sample from those desiccators did not have an expected increase in DML over time, achieving a maximum of 0.23% and 0.01%, respectively (table 1). Therefore, the statistical analysis was performed using paired data for 25, 35 and 40 days of storage. This resulted in only 2 degrees of freedom for paired *t*-tests.

DML over storage time curves for both chambers are represented in figure 4. Curves of S-GRMS using desiccators (fig. 4a) were very consistent over time, but for jars (fig. 4b) there was a higher variability. Desiccators that experienced air leakage (days 15 and 30) can be easily identified in figure 4a, due to its significant reduction in accumulated CO_2 concentration, close to zero for 30 days and a lower increase for 15 days. The variability from the data using jars as a chamber may be caused by undetected air leaks (e.g. for day 30 and day 40, compared with day 35), as their accumulated CO_2 concentration was subdued.

The results of paired *t*-tests comparing jars and desiccators resulted in no differences in DML (p = 0.26). However, it is evident in table 1 that overall $\overline{\text{DML}}$ of samples in jars (0.75%) was higher than the values observed for desiccators (0.64%). Therefore, with two degrees the results of statistical tests were not conclusive.

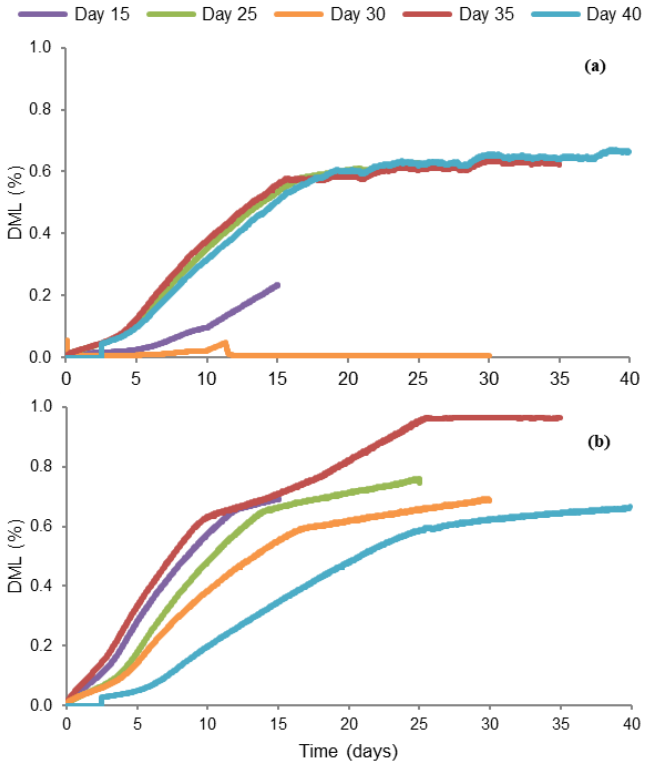

Figure 4. DML over storage time for soybeans stored at 30°C and 22% moisture content in S-GRMS using two chambers: (a) desiccators and (b) jars.

Table 1. Achieved dry matter losses of 22% moisture soybeans stored at 30°C in S-GRMS using desiccators and jars as chambers.

Storage time (day)	Dry matter loss (%)	
	Desiccator	Jar
15*	0.23	0.69
25	0.63	0.74
30*	0.01	0.69
35	0.62	0.97
40	0.66	0.67
Mean ± Standard Deviation* $\overline{v_{DML}}$ ± SD	0.64 ± 0.02**a**	0.75 ± 0.11**a**

*Data for samples at day 15 and day 30 excluded for desiccator mean

Means in a row followed by the same lowercase letter were not different from each other (p > 0.05).

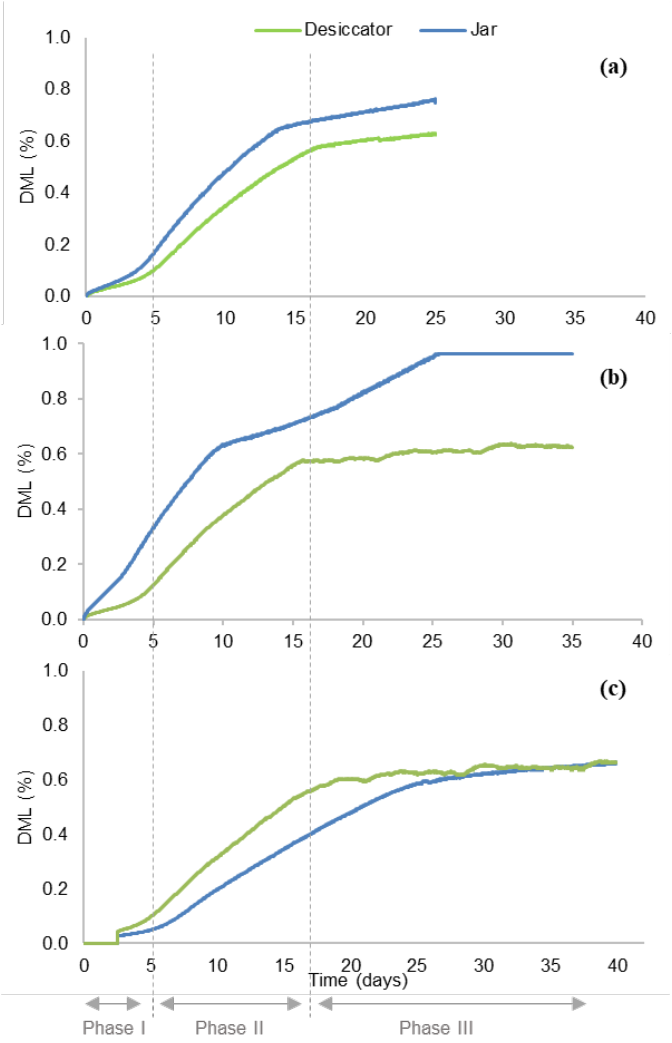

Figure 5. DML over time for soybeans stored in S-GRMS using jars and desiccators as respiration chambers with (a) 25, (b) 35 and (c) 40 days of storage time at 30°C and 22% moisture content.

Overall, DML curves (fig. 5) showed three distinct phases of respiration. At the start of each test, v_{DML} was relatively low for about 3-5 days (Phase I) and increased rapidly following that period (Phase II). After 15-18 days of storage, there was a stabilization in deterioration rates (Phase III).

Except for the jar at 35 days of storage, all chambers presented relatively lower values of v_{DML} in Phase I compared to Phase II (table 2). This suggests that Phase I is a lag phase, which might be a result of the time required for the respiration measurement system to equilibrate to constant temperature and relative humidity in addition to the rewetted beans acclimating to the new environment conditions (Trevisan, 2017). The Phases I and II behavior in which there is an initial low v_{DML} followed by an increasing phase was noted also by Ochandio et al. (2012) and Rukunudin et al. (2004). Pereira Da Silva (2018) reported a rapid increase in respiration rates only after 120-150 h (5-6.25 days) for beans storage at 30°C with 18% moisture content in S-GRMS.

As Phase II attenuated, the third phase started. In the static system, CO_2 accumulated until it reached a concentration that affected the aerobic respiration rate. At this point, O_2 became limiting over time and CO_2 concentration inside the chamber stabilized at a high level, which restrained further respiration (Saltveit, 2019) and thus DML. Therefore, the rate of DML accumulation declined and became closer to zero.

The observed behavior in v_{DML} was organized by Overall and Phase I and Phase II (table 2). Although Phase III started on approximately the same day for all tests, its length depended on total storage time. For example, Phase III was much longer for the 40 day samples than for 25 or 35 days of storage. Therefore, it was not possible to compare Phase III means.

Table 2. Dry matter loss rates (slope ± standard error) and overall mean ± standard deviation of 22% moisture soybeans stored at 30°C in desiccators and jars.

Storage time	Dry matter loss rates ± Standard Error					
(day)	v_{DML} ± Sv_{DML} (10^{-3} % day^{-1})					
	Overall		Phase I (0 to 5 days)		Phase II (5 to 15 days)	
	Desiccator	Jar	Desiccator	Jar	Desiccator	Jar
25	30.45 ± 0.065	37.47 ± 0.098	19.08 ± 0.064	29.74 ± 0.119	34.84 ± 0.063	46.5 ± 0.053
35	24.19 ± 0.081	36.38 ± 0.112	20.79 ± 0.086	62.79 ± 0.126	36.99 ± 0.049	55.68 ± 0.186
40	20.15 ± 0.067	20.14 ± 0.035	17.73 ± 0.085	9.77 ± 0.016	31.99 ± 0.067	20.65 ± 0.080
Mean ± Standard Deviation, $\overline{v_{DML}}$ ± SD	24.93 ± 0.005**a**	31.34 ± 0.010**a**	19.20 ± 0.002**b**	34.10 ± 0.027**b**	34.61 ± 0.003**c**	40.95 ± 0.018**c**

Means of desiccators and jars within Overall, Phase I and Phase II columns followed by the same lowercase letter were not different from each other ($p > 0.05$).

Estimated average safe storage time for soybeans at 22% moisture content and 30°C stored in desiccators was 14.03 ± 0.81 days and 12.93 ± 6.98 days for jars, with the much larger SD caused by the jar sample taken at 40 days (table 3). Values for $t_{0.5\%DML}$ were reported by Pereira da Silva (2018), who estimated approximately 22 days for $t_{0.5\%DML}$ of soybeans at 30°C and 18% moisture content stored in desiccators. Higher moisture content accelerates DML, therefore, it was expected the estimated safe storage time to be lower with 22% moisture content.

Table 3. Safe storage time and overall mean ± standard deviation of 22% moisture soybeans stored at 30°C in desiccators and jars.

Storage time	Safe storage time	
(day)	$t_{0.5\%DML}$ (day)	
	Desiccator	Jar
25	13.95	10.47
35	13.26	7.52
40	14.88	20.81
Mean ± Standard Deviation, $t_{0.5\%DML}$ ± SD	14.03 ± 0.81**a**	12.93 ± 6.98**a**

Means in a row followed by the same lowercase letter were not different from each other ($p > 0.05$).

Because of high variability among jars, and the small number of replicate pairs, there were no significant differences detected when comparing v_{DML} and $t_{0.5\%DML}$ from the two respiration chambers. However, given the relative differences seen in figure 3, it is not advisable to replace desiccators with jars in future static respiration tests until further testing is conducted.

Conclusion

This study provided information regarding v_{DML}, safe storage time, as well as dry matter loss (DML) profiles when soybeans at 22% moisture content were stored at 30°C for 40 days of in a static grain respiration measurement system (S-GRMS). There were three distinct phases observed in the DML curves observed during static respiration tests. Phase I was a lag period, with low DML rates (v_{DML}), followed by an exponential increase in v_{DML} (Phase II) which tended to decline after a period of time in response to oxygen depletion, and subsequently a stable third phase (Phase III) where aerobic respiration was limited. Also, samples collected during this experiment will be evaluated for estimation of LO. Results from LO will be compared with DML to estimate quantitative and qualitative losses in stored soybean.

The information obtained from this study can be used as a guide for future experimental design of S-GRMS to increase understanding of effects of storage conditions on grain deterioration. In the future, more replications should be performed to evaluate the substitution of a desiccator as a respiration chamber with jars for respiration tests of grains at different storage conditions.

Acknowledgments

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