DRY MATTER LOSS RATES OF SOYBEANS:
EFFECTS OF RESPIRATION MEASUREMENT SYSTEM, DAMAGE BY SPLITS,
AND MOISTURE CONTENT AT ELEVATED TEMPERATURES

BY

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THESIS

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ABSTRACT

Soybean quality is affected by temperature \((T)\), moisture content \((w)\) and split beans content \((x_s)\), elevated levels of which can decrease safe storage time \((t_s)\) because of accelerated grain deterioration. Depending on the final use of soybean, \(t_s\) can be based on dry matter loss \((DML)\), germination, or mold growth. \(DML\) can be estimated by measuring the amount of respired carbon dioxide \((CO_2)\) during grain storage, and so has become the basis of previous recommendations for \(t_s\) of grains, oilseeds, and feedstocks.

The main objective of this thesis research was to measure dry matter loss rates \((v_{DML})\) of soybeans in a series of respiration tests using two different grain respiration measurement systems (GRMS) and to understand the effects of GRMS itself, \(x_s\), and \(w\) at elevated \(T\). Several researchers have attempted to determine \(v_{DML}\) or time to reach a particular \(DML\) threshold \((t_{DML})\) in grain respiration studies. Two measurement approaches have been used to measure respired \(CO_2\): static and dynamic systems. In a static grain respiration measurement system (S-GRMS), grain is placed in a sealed chamber wherein a limited amount of oxygen gas is available for respiration. The respired \(CO_2\) accumulates in the sealed chamber and is measured over time. In a dynamic grain respiration measurement system (D-GRMS), air passes continuously through a bed of grain so the oxygen supply for respiration is maintained. The constant airflow carries the respired \(CO_2\) into a measurement system. The availability of oxygen for respiration or GRMS used in the study is expected to affect \(v_{DML}\) and \(t_{DML}\) estimates, but the effect by GRMS used has not been quantified before.

The specific objectives of the research were to: (1) determine the effect of GRMS on \(v_{DML}\) estimates for 18% moisture content (m.c.) soybeans stored at 30°C; (2) develop a damage multiplier \((M_D)\) for soybeans with an \(x_s\) range of 4 to 16% (w/w) from a baseline of 0% (w/w) stored with 18% m.c. and 35°C using S-GRMS; and (3) estimate \(v_{DML}\) of 14, 18, and 22% m.c. soybeans stored at 30°C using D-GRMS. The \(w\) and \(T\) parameters in these tests were chosen based on typical soybean harvest and initial storage conditions in Mato Grosso, Brazil, which has produced 32 million tons of soybeans per year, in recent years. Since soybeans are an important source of plant-based proteins and oils, a preliminary test to correlate \(DML\) to changes in chemical composition and lipid oxidation byproducts was also conducted. Secondary byproducts of lipid oxidation were measured via change in peroxide value \((\Delta PV)\) and 2-Thiobarbituric Acid...
(ΔTBA) value of soybean samples from before and after respiration tests. $v_{DML}$ values from the third objective were also used to estimate time to reach 0.5% DML ($t_{0.5}$), the threshold used for maximum allowable storage time (MAST) guidelines for shelled corn by the ASABE Standard D535 and “approximate” MAST guidelines for soybeans in many university extension publications.

Results showed that $v_{DML}$ estimates for 18% m.c. soybeans at 30°C when measured using D-GRMS were 1.20 times higher than those from S-GRMS. While the difference between GRMS at this single set of storage conditions was found to be non-significant ($p = 0.09$), respiration and $v_{DML}$ reported in the literature for grains stored in S- and D-GRMS vary greatly. Estimates with S-GRMS system tend to be lower than for D-GRMS. Thus, care should be taken when using $v_{DML}$ rates from literature to estimate $t_s$.

Damage by splits was expected to have a greater effect on $v_{DML}$. Soybeans are inherently prone to cracking, splitting, lipid oxidation, and protein degradation – all of which lead to accelerated dry matter and quality losses. Results showed that $v_{DML}$ increased with increasing $x_s$ and that average $v_{DML}$ increased by 1.10 to 1.70 times greater than the base case (0% splits) when $x_s$ increased from 4 to 16%. $M_D$ was defined as the $v_{DML}$ of 0% splits soybeans relative to $v_{DML}$ with $x_s$ % splits, and it decreased from 1.0 to 0.60 as $x_s$ increased from 0 to 16% splits. $M_D$ for soybean was found to be 1.25 times as sensitive to $x_s$ when compared to the $M_D$ for corn, which decreases from 2.08 to 1.42 as damaged kernels content increased from 0 to 16% (w/w). These results and the procedure developed for quantifying a soybean $M_D$ are useful in the future as $v_{DML}$ data for a wider range of $w$ and storage conditions become available.

$v_{DML}$ was found to increase significantly with $w$ at 30°C – approximately by a factor of four as $w$ increased from 14 to 18% m.c. and by a factor of 14 when $w$ increased from 14 to 22% m.c.. Using $v_{DML}$ of 14 to 22% m.c. clean soybeans at 30°C, estimates of $t_{0.5}$ were calculated and found to be four to five times longer than recommended MAST for corn and soybeans at the same $T$, $w$, and water activity ($a_w$). The discrepancy between estimated $t_{0.5}$ and recommended MAST values can be attributed to the fact that the soybeans used in this study were clean, intact soybeans, while the MAST values were for corn with a typical damage content of 30%, and for soybeans at the same $a_w$ of corn at this damage level.

Results from proximate analyses showed that $DML$ had a -0.40 correlation coefficient with a decrease in carbohydrates change content ($\Delta C$, $p = 0.18$). Results from testing for lipid
oxidation byproducts suggested that the first and second stages of oxidation occurred during the respiration tests, but a correlation coefficient of 0.35 was found between $DML$ and $\Delta PV$ ($p = 0.65$) and of -0.38 between $DML$ and $\Delta TBA$ ($p = 0.62$). However, to gain a better understanding of lipid oxidation byproducts and their rate of increase with $v_{DML}$, samples must be tested throughout a respiration test – as was done with $DML$ measurements – for which the current D-GRMS was not designed. Nevertheless, these preliminary results support the idea that $DML$ measurements may provide an indirect measure of lipid quality of soybeans during storage.

The research reported in this thesis provide detailed development and testing of robust GRMS, test protocols, and data analyses for the development of MAST guidelines, which are sorely needed to mitigate postharvest losses of soybeans during storage, especially in expanding soybean production in sub-tropical regions of the world. The methodologies and analyses presented here are directly applicable to other crop systems, such as wheat, rice, and pulses – all of which are important sources of protein and nourishment to an ever-growing global population.
ACKNOWLEDGMENTS

First, I would like to thank God Almighty for giving me the knowledge, resilience, strength, and ability to undertake this research and to persevere through the trials in order to complete it satisfactorily. Without his blessings, this achievement would not have been possible.

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I also would like to thank all of the staff of the Department of Agricultural and Biological Engineering, especially Mrs. Tracy Anne Wikoff, Mrs. Jamie H. Price, Mr. Tim Lecher, and Mr. Steve E. Ford, who were always ready to help me. Mr. Tim Lecher has always done an excellent job at addressing every technical request I had.

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CHAPTER 1. INTRODUCTION

Soybean (*Glycine max* (L.) Merr.) belongs to the genus *Glycine* in the family Leguminosae, and subgenus *Soja* (Nwokolo, 1996). Soybean is one of the most valuable crops in the world due to its high protein and oil contents, about 40 and 20% (dry basis, d.b.), respectively (Asbridge, 1995). It is used as protein source feed for livestock and poultry, as a protein and oil dietary source for people, and in several processes and products for industrial manufacturing. However, a significant amount of the world soybean production is lost following harvest through distribution and consumption (Aulakh and Regmi, 2013). Those losses of grain have been the reason why many researchers are trying to find different ways to minimize them. One way to minimize postharvest losses is to provide an estimate of the maximum allowable storage time (MAST) guidelines for every cereal grain, oilseed, and pulse at different representative combinations of storage temperature (*T*) and moisture content (*w*). MAST is cumulative, and personnel inside the supply chain do not always understand this. For example, corn is harvested at 20% moisture content (m.c.) at 24°C and is stored temporarily in a truck for 48 h prior to unloading at a storage facility such as in some developing countries where goods are transported via trucks on poor road conditions. According to the ASABE Standard D535 (R2014), this corn has a MAST of 12 d, after which 0.5% dry matter loss (*DML*) and a decrease by U.S. Corn Grade (i.e., from Grade No. 1 to Grade No. 2) likely has been reached. Keeping the corn in the truck under these storage conditions depletes its “shelf-life” by two-twelfths, or 16.7%, even after drying down for long-term storage. If the corn were then dried to 16% m.c. and cooled to 10°C, the cumulative nature of MAST means it would be reduced from 339 d to 282 d.

However, in order to estimate MAST for a specific grain, it is important to understand what the factors that affect deterioration are, how to measure deterioration at different levels of those factors, and how to correlate the measurements to calculate safe storage time (*t_s*). This thesis is an important step towards the development of MAST estimation for soybeans, where deterioration was measured based on dry matter loss rates (*v_{DML}*). During storage, grain losses may occur due to the presence of spoilage organisms, such as insects, mites, rodents, and molds, or to unfavorable storage conditions, such as high *w* and *T* (Coker, 1994). However, even under favorable conditions, grains will lose dry matter by respiration. Respiration is a catabolic oxidative reaction of complex substrate molecules, such as
glucose (C\(_6\)H\(_{12}\)O\(_6\)), to simpler ones, such as carbon dioxide (CO\(_2\)) and water (H\(_2\)O). In addition to this reaction, energy and intermediate molecules are also produced. In anaerobic respiration process, for every mole of C\(_6\)H\(_{12}\)O\(_6\) reacting with six moles of oxygen (O\(_2\)), six moles each of CO\(_2\) and H\(_2\)O are produced (Kader & Saltveit, 2002a). Therefore, the amount of carbohydrates lost and, by extension, DML, can be estimated by the consumption of O\(_2\) or the production of CO\(_2\).

Relatively few studies of soybean respiration rates based on monitoring CO\(_2\) production (\(v_{CO_2}\)) have been reported over the past seventy years (Ramstad & Geddes, 1942; Rukunudin et al., 2004; Sorour & Uchino, 2004; Mendes et al., 2009; Jian et al., 2014; Hartmann Filho et al., 2016; Trevisan, 2017). To measure \(v_{CO_2}\), two typical grain respiration measurement systems (GRMS) are used, which can be described as being static or dynamic. In a static system (S-GRMS), the soybeans are placed in a hermetically sealed vessel, in which the limited concentration of O\(_2\) is consumed and CO\(_2\) is produced and accumulated. This system can simulate typical storage conditions in silo bags. Alternatively, a dynamic system (D-GRMS) simulates aerated bulk storage, wherein air passes through the soybeans carrying respiration products (CO\(_2\) and H\(_2\)O) and maintaining O\(_2\) concentration (Saltveit, 2016). Each measurement system is useful for estimating DML in storage: static for silo bags and dynamic for aerated bins.

An increase in \(w\), \(T\) and mechanical damage (\(D\)) for stored grains increases \(v_{CO_2}\) and consequently, \(v_{DML}\). A number of studies have related these factors to decreased quality of stored soybeans and corn in both static and dynamic systems (Johnson et al., 1963; Steele, 1967; Fernandez et al., 1985; Al-Yahya, 1991; Bern et al., 1999; Gupta et al., 1999; Rukunudin et al., 2004; Weinberg et al., 2008; Jian et al., 2014). Yet, the effect of \(D\) is expected to be significantly higher in soybeans than corn, because a soybean seed can split more easily and expose its two cotyledons leading to an increase in \(v_{DML}\). Rukunudin (1997) used 48 wk-old stored soybeans, regressed total damaged seeds with DML, and determined values of DML for specific U.S. Soybean Grades (1, 2, and 3) corresponding to 2, 3 and 5% total damaged seeds to be 0.55, 0.82, and 1.4% DML, respectively.

Based on \(v_{DML}\), MAST of corn and soybean can be estimated as the elapsed time to reach 0.5% DML (\(t_{0.5}\)). This threshold was first proposed by Steele (1967) when he observed that one U.S. Corn Grade loss coincided with 0.5% DML. Several researchers had observed different correlations between grade loss and DML for corn and wheat depending on mold presence.
(White et al., 1982a; Friday et al., 1989; Wilcke et al., 1993), aflatoxin contamination (Marin et al., 1999), and other quality metrics (Hall and Dean, 1978). Nevertheless, several researchers have adopted a 0.5% DML threshold despite these concerns. The only widely accepted and used MAST reference in the grain industry is ASABE Standard D535 (R2014) for shelled corn based on $v_{CO_2}$ data collected at $T$ ranging from 15 to 26°C and $w$ of 18.8 to 28%.

In terms of quality, deterioration of soybeans stored under different conditions has been reported as changes in $v_{DML}$ or in physical, mechanical and chemical properties. These properties are further elaborated in the next chapter; however, deterioration is aggravated with increased $w$, $T$, $D$ and storage time ($t$) (Ramstad & Geddes, 1942; Parrish & Leopold, 1978; Narayan et al., 1988; Cárabaz-Trejo et al., 1989; Paredes-López et al., 1991; Bern et al., 1999; Braccini et al., 1999; Kong et al., 2008; Mendes et al., 2009; Kamizake et al., 2014). Due to its high oil and protein content changes in free fatty acid ($FFA$), peroxide value ($PV$), and amino acid profiles are the main chemical analyses used to characterize stored soybeans lipid and protein degradation (White et al., 1976; Alencar et al., 2010; Kong & Chang, 2013; Yang et al., 2014; Hartmann Filho et al., 2016). From these several studies, none have correlated the different storage conditions of soybeans with deterioration based directly on $DML$ and chemical changes.

Therefore, the main objective of this thesis was to better understand the effects of GRMS, damage by splits content ($x_s$), and $w$ on $v_{DML}$ of soybeans at elevated temperatures. The storage conditions chosen in this thesis cover typical soybean harvest and initial storage conditions in Mato Grosso Brazil, which has produced 32 million tons of soybeans per year, in recent years. Soybean harvest $w$ in this region can range from 14 to 22% m.c. (wet basis, w.b.) and average ambient $T$ of 30 to 35°C (Danao et al., 2015).

The specific objectives and organization of this thesis were:

1. determine the effect of GRMS on $v_{DML}$ estimates for 18% m.c. soybeans stored at 30°C (Chapter 3);
2. develop a damage multiplier ($M_D$) for soybeans with $x_s$ ranging from 4 to 16% (w/w) from a baseline of 0% (w/w) stored with 18% m.c. and 35°C using S-GRMS (Chapter 4); and
3. estimate $v_{DML}$ of 14, 18, and 22% m.c. soybeans stored at 30°C using D-GRMS (Chapter 5).
In addition to these objectives, since soybeans are an important source of plant-based proteins and oils, a preliminary test to correlate $DML$ to changes in chemical composition and lipid oxidation byproducts was also conducted. Byproducts of lipid oxidation byproducts were measured via a change in peroxide value ($\Delta PV$) and 2-Thiobarbituric Acid ($\Delta TBA$) value of soybean samples from before and after respiration tests. $v_{DML}$ values from the third objective were also used to estimate time to reach 0.5% $DML$ ($t_{0.5}$), the threshold used for MAST guidelines in ASABE Standard D535 (R2014) and “approximate” MAST guidelines for soybeans in university extension publications (Hellevang, 2014; Sadaka, 2014; Stahl, 2014).

Chapters 3, 4, and 5 of this thesis were previously presented at the ASABE Annual International Meetings in 2017 and 2018 and can be found in ASABE’s Technical Library as Paper Nos. 170075, 1801406, and 1801413, respectively.
CHAPTER 2. LITERATURE REVIEW

2.1. Soybean

Soybean (Glycine max (L.) Merr.) is part of the subgenus Soja, a member of the genus Glycine and family Leguminosae (Figure 2.1). It is an erect, small, hirsute plant, with trifoliate and alternate arrangement of leaves and ovate to lanceolate leaflets. Its mature flower can be purple or white, and the pods are oblong, slightly elongated, hairy, and when they are immature their color is green turning to yellowish-brown when mature. Per pod, there are two to three ovoid to subspherical seeds, where the cotyledons colors are usually yellow when mature and green immature (Nwokolo, 1996).

![Figure 2.1. Soybean plant (Illustration by Nestly Hoeg, purchased on September 11, 2018).](image)

The 2016/17 soybeans world’s production was 350.76 million metric tons with the United States, Brazil, Argentina and China as major producers (USDA, 2018). Soybean is one of the most valuable crops in the world, used as a protein source feed for billions of livestock (Nwokolo, 1996). The value of this crop is due to its high content in protein and oil, about 40% and 20%, respectively. Nutrient values of mature raw soybeans show high composition on protein (36.49 g per 100 g) and fat (19.94 g per 100 g) (Table 2.1). The main products from this oilseed are a high-quality protein meal and edible oil products (Asbridge, 1995). The global
oilseed meal and vegetable oil production and consumption are expected to grow in the next years, which is relevant to increase production and to decrease losses (USDA, 2018).

### Table 2.1. Nutrient values and weights for edible portion of mature raw soybeans.

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Unit</th>
<th>Value per 100 g</th>
<th>Nutrient</th>
<th>Unit</th>
<th>Value per 100 g</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Proximate</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water</td>
<td>g</td>
<td>8.54</td>
<td>Carbohydrate, by difference</td>
<td>g</td>
<td>30.16</td>
</tr>
<tr>
<td>Energy</td>
<td>kcal</td>
<td>446</td>
<td>Fiber, total dietary</td>
<td>g</td>
<td>9.3</td>
</tr>
<tr>
<td>Protein</td>
<td>g</td>
<td>36.49</td>
<td>Sugars, total</td>
<td>g</td>
<td>7.33</td>
</tr>
<tr>
<td>Total lipid (fat)</td>
<td>g</td>
<td>19.94</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Minerals</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcium, Ca</td>
<td>mg</td>
<td>277</td>
<td>Potassium, K</td>
<td>mg</td>
<td>1797</td>
</tr>
<tr>
<td>Iron, Fe</td>
<td>mg</td>
<td>15.70</td>
<td>Sodium, Na</td>
<td>mg</td>
<td>2</td>
</tr>
<tr>
<td>Magnesium, Mg</td>
<td>mg</td>
<td>280</td>
<td>Zinc, Zn</td>
<td>mg</td>
<td>4.89</td>
</tr>
<tr>
<td>Phosphorus, P</td>
<td>mg</td>
<td>704</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Vitamins</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin C, total ascorbic acid</td>
<td>mg</td>
<td>6.0</td>
<td>Folate, DFE</td>
<td>μg</td>
<td>375</td>
</tr>
<tr>
<td>Thiamin</td>
<td>mg</td>
<td>0.874</td>
<td>Vitamin A, RAE</td>
<td>μg</td>
<td>1</td>
</tr>
<tr>
<td>Riboflavin</td>
<td>mg</td>
<td>0.870</td>
<td>Vitamin A, IU</td>
<td>IU</td>
<td>22</td>
</tr>
<tr>
<td>Niacin</td>
<td>mg</td>
<td>1.623</td>
<td>Vitamin E (α-tocopherol)</td>
<td>mg</td>
<td>0.85</td>
</tr>
<tr>
<td>Vitamin B-6</td>
<td>mg</td>
<td>0.377</td>
<td>Vitamin K (phytolquinone)</td>
<td>μg</td>
<td>47</td>
</tr>
<tr>
<td><strong>Lipids</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fatty acids, total saturated</td>
<td>g</td>
<td>2.884</td>
<td>Fatty acids, total trans</td>
<td>g</td>
<td>0.000</td>
</tr>
<tr>
<td>Fatty acids, total monounsaturated</td>
<td>g</td>
<td>4.404</td>
<td>Fatty acids, total polyunsaturated</td>
<td>g</td>
<td>11.255</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>mg</td>
<td>0</td>
<td></td>
<td></td>
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</tbody>
</table>


Raw soybean contains antinutritional factors that affect the digestion of its protein in the human intestinal tract. Some proteins have their full nutritional potential achieved only after heat has been applied due to the presence of the antinutritional factors. In soybeans, factors that are inactivated by moist heat are protease inhibitors, lectins, goitrogens, and antivitamins. However, other antinutritional factors are heat-stable and can decrease the protein quality of soybeans; these include saponins, tannins, estrogens, and flatulence factors, lysinoalanine, allergens, and phytate. Protease inhibitors are the main antinutritional factor in soybean and they are responsible for inhibiting a range of proteases, including trypsin and chymotrypsin. According to Liener et al. (1988), trypsin inhibitors present in raw soybean can perturb the normal human
pancreatic function, low levels of which have little effect in humans. Therefore, for safe consumption of soybean, moist heat treatment needs to be applied during processing (Liener, 1994; Nwokolo, 1996).

The USDA provides quality grading standards for each grain product. For example, corn grading is based on three classes of corn, yellow, white and mixed, where the grading evaluation depends on total damaged kernels, heat-damaged kernels (discolored and damaged by heat), broken corn and foreign material (USDA, 1996). However, soybean grading is established only on two classes, yellow and mixed, and in addition to damaged kernels and foreign material, it is also evaluated in terms of splits and beans with other colors (Table 2.2).

Table 2.2. U.S. Standard Grade and requirements for soybeans (USDA, 2007).

<table>
<thead>
<tr>
<th>Grading factors</th>
<th>U.S. Grade No.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Maximum percent limits of:</td>
<td></td>
</tr>
<tr>
<td>Damaged kernels:</td>
<td></td>
</tr>
<tr>
<td>Heat (part of total)</td>
<td>0.2</td>
</tr>
<tr>
<td>Total</td>
<td>2.0</td>
</tr>
<tr>
<td>Foreign material</td>
<td>1.0</td>
</tr>
<tr>
<td>Splits</td>
<td>10.0</td>
</tr>
<tr>
<td>Soybeans of other colors[a]</td>
<td>1.0</td>
</tr>
<tr>
<td>Maximum count limits of:</td>
<td></td>
</tr>
<tr>
<td>Other materials:</td>
<td></td>
</tr>
<tr>
<td>Animal filth</td>
<td>9</td>
</tr>
<tr>
<td>Castor beans</td>
<td>1</td>
</tr>
<tr>
<td>Crotalaria seeds</td>
<td>2</td>
</tr>
<tr>
<td>Glass</td>
<td>0</td>
</tr>
<tr>
<td>Stones[b]</td>
<td>3</td>
</tr>
<tr>
<td>Unknown foreign substance</td>
<td>3</td>
</tr>
<tr>
<td>Total[c]</td>
<td>10</td>
</tr>
</tbody>
</table>

U.S. Sample grade are soybeans that:
(a) Do not meet the requirements for U.S. Nos. 1, 2, 3, or 4; or
(b) Have a musty, sour, or commercially objectionable foreign odor (except garlic odor); or
(c) Are heating or otherwise of distinctly low quality.

[a] Disregard for mixed soybeans.
[b] In addition to the maximum count limit, stones must exceed 0.1 percent of the sample weight.
[c] Includes any combination of animal filth, castor beans, crotalaria seeds, glass, stones, and unknown foreign substances. The weight of stones is not applicable for total other material.
2.2. Respiration and dry matter loss of grains

Grain continues to perform their metabolic functions even after harvest, such as the respiration. Respiration is an oxidative reaction of a complex organic compound, such as C₆H₁₂O₆, to simple compounds, such as CO₂ and water H₂O (Equation 2.1). This reaction also produces energy in form of adenosine triphosphate (ATP) and kilocalories (kcal) and requires intermediate molecules to occur, which are adenosine diphosphate (ADP) and inorganic phosphate (Pi) (Kader & Saltveit, 2002a).

\[
C_6H_{12}O_6 + 6O_2 + 38 ADP + 38Pi \rightarrow 6CO_2 + 6H_2O + 38 ATP + 686 kcal
\]  
(2.1)

From this equation, it is possible to correlate the respiration process with substrate losses or DML. For every respired mole of C₆H₁₂O₆ (180 g mol⁻¹), six moles of oxygen (6 x 32 g mol⁻¹) is consumed and six moles of CO₂ (6 x 44 g mol⁻¹) is produced. Thus, based on CO₂ production:

\[
DML = \sum m_{CO_2} \left( \frac{1 \text{ mol } C_6H_{12}O_6}{6 \text{ moles } O_2 \text{ or } CO_2} \right) \left( \frac{M_{C_6H_{12}O_6}}{M_{O_2 \text{ or } CO_2}} \right) \%
\]  
(2.2)

where \(\sum m_{CO_2}\) is the accumulated mass of respired CO₂, and \(M\) is the molar mass.

Respiration has been expressed in terms of decreased consumed oxygen (O₂) levels, \(m_{O_2,s}\) [mg O₂ consumed (kg dry matter)⁻¹], or increased respired CO₂ levels, \(m_{CO_2,s}\) [mg CO₂ (kg dry matter)⁻¹], to estimate DML (%). The consumption of O₂ or production of CO₂ will increase or decrease depending on a host of factors. Intrinsic factors include the type and genotype of the grain, its development phase during harvest, chemical composition, \(D\) and \(w\) or water activity \(a_w\). Extrinsic factors are based on environment conditions, such as \(T\), O₂, CO₂, carbon monoxide and ethylene concentrations, hydrocarbons and stress (Kader & Saltveit, 2002a). As with most chemical reactions, respiration increases with increase in \(T\) and \(w\), and high levels of this storage conditions results in a lower postharvest or shelf-life of a commodity (Steele et al., 1969; Kustermann & Scherer, 1982; Sorour & Uchino, 2004; Jian et al., 2014). This increase in DML demonstrates the importance of monitoring respired CO₂ during grain storage to develop safe storage systems to reduce postharvest losses (Huang et al., 2013).

2.3. Grain respiration measurement systems

\(ν_{CO_2}\) are typically measured by monitoring respired CO₂ using either of two types of systems – static or dynamic. The main difference between these systems is the availability of air or, specifically, O₂. In S-GRMS, grain is placed in an airtight chamber in which O₂ is consumed
while products of respiration, CO₂, and H₂O (in the form of vapor) increase over time. The chamber must be hermetically sealed to allow accurate measurement of CO₂ with a gas chromatograph, infrared CO₂ analyzer, gas pressure sensor, or CO₂ absorbent methodologies (Saltveit, 2016). While S-GRMS have the advantage to be easy to set up, they never equilibrate, so the depletion of O₂, the accumulation of respired CO₂, the presence of other gases (e.g., ethylene) and T – all of which influence $v_{CO_2}$ – increase as a result of the exothermic respiration process (Kader & Saltveit, 2002a).

Lacey et al. (1994) stated that increased CO₂ and decreased O₂ concentrations can restrain aerobic respiration and enable other respiration pathways including anaerobic. Kader and Saltveit (2002b) also noted that increased CO₂ and decreased O₂ concentrations enhance physiological disorders and susceptibility to decay. Thus, these S-GRMS typically run for a short period of time and accuracy of $v_{CO_2}$ measurement depends on the quality of the hermetic seal and method of measuring CO₂.

Some researchers tried creative ways to replenish O₂ inside the chamber during respiration tests. White et al. (1982b) opened the grain-filled flask after each gas sampling and flushed the flask with air for 2-5 min. Lacey et al. (1994) used a laboratory electrolytic respirometer developed by Tribe and Maynard (1989) that enabled O₂ to be replenished over time inside a sealed chamber. As O₂ was consumed by the grain, respired CO₂ was absorbed by an alkali solution. The decrease in gas pressure triggered an electrode to come into contact with an anode, and O₂ was generated at the anode as copper was deposited on the cathode.

In D-GRMS, air flows continuously through a bed of grain, delivering a constant supply of O₂ and extracting respiration products from it. Similar to a S-GRMS, respired CO₂ can be measured using a gas chromatograph, infrared CO₂ analyzer, or extracted using a CO₂ absorbent. This system can be run for extended periods and the gas mixture of the air supply is typically conditioned to maintain constant T, relative humidity ($\phi$), and O₂ levels during the respiration test. The system requires constant monitoring of airflow conditions to maintain the environment in equilibrium over a long period of testing (Kader & Saltveit, 2002a). Another way to measure $v_{CO_2}$ in a D-GRMS is to absorb CO₂ in a suitable material and monitor its accumulation over time. This method is a direct gravimetric option that eliminates high-precision air flow rate measurement. It has been documented in Rukunudin (1997), Sood (2015), and Trevisan (2017).
2.4. Effects of moisture content, temperature, and damage on respiration rates from different measurement systems

Since the 1940s, several researchers have reported grain deterioration of stored corn and soybeans based on monitoring respired CO$_2$, where most studies measured $v_{CO_2}$ or $t_{0.5}$ using a dynamic system. (Table 3.1 in Chapter 3 summarizes these studies). The variety of studies conducted using different storage conditions and measurement systems makes it difficult to determine the individual level effect of $T$, $w$, and GRMS on $v_{DML}$.

From the reported $v_{CO_2}$, trends show increases in $w$ and $T$ increase $v_{CO_2}$, and consequently $v_{DML}$, independent of the measurement system used. For example, Steele (1967) conducted dynamic measurements for corn with 18.8 to 28% m.c. at a fix stored temperature of 18°C resulting in $v_{DML}$ of 0.013 to 0.108% d$^{-1}$. This increase in $v_{DML}$ was also observed by Fernandez et al. (1985) and Gupta et al. (1999). In a S-GRMS, this trend holds. Weinberg et al. (2008) measured $v_{DML}$ of 0.009 to 0.033% d$^{-1}$ for corn with 14 to 22% m.c. stored at 30°C using S-GRMS. Al-Yahya (1991) reported increasing $v_{DML}$ for 15 to 25°C corn at 21.6% m.c., from 0.036 to 0.045% d$^{-1}$. The same behavior has been observed in soybeans. For example, 9-21% m.c. soybeans stored at 26°C in a D-GRMS had increased $v_{DML}$ from 0.019 to 0.050% d$^{-1}$ (Rukunudin et al, 2004), while soybeans with 23% m.c. stored in a S-GRMS at 15 to 35°C had $v_{DML}$ of 0.003 to 0.020% d$^{-1}$ (Jian et al., 2014).

It is not possible to extract the effect (if any) by the GRMS used in multiple studies due to the wide variety of grain and storage conditions used. For example, 20 to 21% m.c. shelled corn stored at 25°C reached 0.5% DML in 9.25 to 10.9 d, according to Friday et al. (1989) and Al-Yahya (1991), who used a D-GRMS in their respective studies. However, 21% m.c. corn stored in S-GRMS during 10 d at 23°C evolved 0.00062% DML, not even close to 0.5% DML (Ubhi & Sadaka, 2015). For soybeans, Sorour and Uchino (2004) achieved 0.5% DML in 21.62 d in a dynamic system for 22% m.c. beans at 25°C and Rukunudin et al. (2004) reached the same point in 11.5 d for 21% m.c. beans at 26°C. Yet, like Ubhi and Sadaka (2015), Jian et al. (2014) didn’t achieve $t_{0.5}$; after 30 d the maximum DML was only 0.00064% for 23% m.c. soybeans stored in a S-GRMS at 25°C. Therefore, the trend seems to be that $v_{DML}$ of grains stored in a S-GRMS is lower than in a D-GRMS, but there have been no studies conducted of a side-by-side comparison of the two GRMS before.
Controlling \( w \) of a commodity is the main technique used to reduce \( DML \) (Steele, 1967). To achieve acceptable \( t_s \), grains are harvested at the safest known \( w \) or, if harvested at a higher \( w \), they are often dried to the safe \( w \). However, as \( w \) decreases, whether during harvest or drying, \( D \) tends to increase (Johnson et al., 1963). Steele (1967) estimated the relationship between artificial \( D \) percentages on shelled corn and \( DML \) in a D-GRMS, showing how \( DML \) increases with \( D \). Bern et al. (1999), also using a D-GRMS, showed the same relationship between splits soybeans percentages and \( DML \). Thus, \( D \) is a consequence from harvest to storage, and its level affects directly \( DML \).

The effect of \( D \) in soybeans is expected to be significant. In addition to mechanical damage caused by fissures, cracks or heat damage, soybeans easily split and expose embryo and endosperm aggravating \( DML \). Also, compared to other crops, soybean seed deteriorates faster (Priestley et al., 1983) and is more susceptible to hydrolysis of triglycerides and protein degradation (Bern et al., 1999; Alencar et al., 2010; Kong & Chang, 2013).

2.5. Safe storage time

\( t_s \) for grains has been determined according to a variety of quality thresholds. For example, \( t_s \) models for wheat have been developed based on visible mold growth, grain germination, or respiration and, consequently, \( v_{DML} \) (White et al., 1982a; Hamer et al., 1991; Lacey et al., 1994; Schrot et al., 1998; Karunakaran et al., 2001). Based on measured respired CO\(_2\) and germination, White et al. (1982a) set a limit of 0.04% \( DML \) for stored hard red spring wheat to be used as seed with a correlation of 5 to 10% germination loss. The same research showed that 0.1% \( DML \) was unacceptable for wheat, because an increase in deterioration occurred at slow \( v_{CO_2} \) without visible signs of mold growth. However, they measured respiration using a S-GRMS where air was flushed three times per week during the test. As mentioned before, S-GRMS may have a lower respiration rate than D-GRMS, so this could explain why deterioration happened at slow respiration rates. \( t_s \) for hard red spring wheat was also estimated based on germination loss by Schrot et al. (1998) and Karunakaran et al. (2001). Both studies determined the rate of deterioration based on a drop to 90% germination and Karunakaran et al. (2001) additionally measured \( v_{CO_2} \). Mold growth was visible on stored wheat at 20 to 35°C after 10% drop in germination; however, wheat stored at 10 and 15°C did not have visible mold growth even after the germination had dropped to 70% (Karunakaran et al. 2001). The same
study showed that wheat stored at 25°C and 15, 17, and 19% m.c. had $v_{CO_2}$ and germination equal to 23, 37, and 822 mg (kg dry matter)$^{-1}$ d$^{-1}$ and 95, 91, and 32%, respectively.

Seib et al. (1980) estimated $t_s$ for rice based on $DML$ and USDA market grade level. $DML$ was measured for two varieties of rough rice (paddy), long-grain and medium-grain, at 18 and 22% m.c. and 18 to 35°C. To determine $t_s$, a threshold of 0.5% $DML$ was applied to 18% m.c. rice and was dropped to 0.25% $DML$ for 22% m.c. rice; these values were established based on graded samples that did not lower U.S. Grade Nos. 1 and 2. Results from Seib et al. (1980) of stored rough rice at 18% m.c. associated $DML$ less or equal to 0.25, 0.5, 0.75, 1.0 and 1.5% with U.S. Rice Grade Nos. 1, 2, 3, 4, and sample grades, respectively, while at 22% m.c., 0.25, 0.5 and 0.75% were associated with U.S. Rice Grade Nos. 2, 4 and 5, and sample grades. In addition to sample grading, the research concluded that at lower temperatures, medium-grain rice can be safely stored for a longer period than long-grain rice; however, with an increase in $T$ the variety of grain did not affect $t_s$. These examples show that $t_s$ can vary greatly from one grain to another, depending on their valuation and final end use. Hence, to determine $t_s$ and, eventually MAST guidelines, different approaches in grain quality are needed for a better estimation.

There are no standards to estimate safe storage time for soybeans. The only MAST guideline available that estimates $t_s$ is for shelled corn in ASABE Standard D535 (R2014). This guideline has been developed using the time to reach 0.5% $DML$, which is the equivalent to corn losing one USDA market grade level (Steele, 1967). However, Hellevang (2014) from the North Dakota State University Extension Service, developed an “approximate” allowable storage time guideline for soybeans (Table 2.3). This approximation is based on assumptions that two different types of grains (in this case, corn and soybeans) with the same $a_w$ will behave similarly during storage, i.e., the rates of degradation of their macronutrients (carbohydrates, lipids, and proteins) will be similar (Hellevang, 2018; Kenneth J. Hellevang, NDSU Agricultural and Biosystems Engineering, personal communication, 18 July 2017). At a known $t_s$ for corn from ASABE Standard D535 (R2014), the first step in his approximation was to find $a_w$ using corresponding corn isotherms of equivalent $T$ and equilibrium $w$ ($w_e$) for the chosen $t_s$. Secondly, for the same $T$, the approximate $w_e$ that soybeans may be stored will be the value corresponding to the $a_w$ in the corn isotherm. Then, soybeans at this $w_e$ may be safely stored for the same $t_s$ as the known value for corn at the same $T$. Figure 2.2 depicts the process for
### Table 2.3. Approximate allowable storage time for soybeans (from Hellevang, 2014).

<table>
<thead>
<tr>
<th>Moisture Content ( (w, % \text{ w.b.}) )</th>
<th>Approximate allowable storage time (d)</th>
<th>Temperature ( (T, , ^{\circ}C) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>11 ( [a] ) ( 4.4 ) ( 10.0 ) ( 15.6 ) ( 21.1 ) ( 26.7 )</td>
<td>200 ( [a] )</td>
<td>140</td>
</tr>
<tr>
<td>12 ( [a] ) ( [a] ) ( [a] ) ( [a] )</td>
<td>240 ( [a] )</td>
<td>125 ( [a] )</td>
</tr>
<tr>
<td>13 ( [a] ) ( [a] ) ( [a] )</td>
<td>230 ( [a] )</td>
<td>120 ( [a] )</td>
</tr>
<tr>
<td>14 ( [a] ) ( [a] )</td>
<td>280 ( [a] )</td>
<td>130 ( [a] )</td>
</tr>
<tr>
<td>15 ( [a] ) ( [a] )</td>
<td>200 ( [a] )</td>
<td>90 ( [a] )</td>
</tr>
<tr>
<td>16 ( [a] ) ( [a] )</td>
<td>140 ( [a] )</td>
<td>70 ( [a] )</td>
</tr>
<tr>
<td>17 ( [a] ) ( [a] )</td>
<td>90 ( [a] )</td>
<td>50 ( [a] )</td>
</tr>
<tr>
<td>19 ( 190 ) ( [a] ) ( [a] )</td>
<td>60 ( [a] )</td>
<td>30 ( [a] )</td>
</tr>
<tr>
<td>21 ( 130 ) ( [a] ) ( [a] )</td>
<td>40 ( [a] )</td>
<td>15 ( [a] )</td>
</tr>
<tr>
<td>23 ( 90 ) ( [a] ) ( [a] )</td>
<td>35 ( [a] )</td>
<td>12 ( [a] )</td>
</tr>
<tr>
<td>25 ( 70 ) ( [a] ) ( [a] )</td>
<td>30 ( [a] )</td>
<td>10 ( [a] )</td>
</tr>
<tr>
<td>27 ( 60 ) ( [a] ) ( [a] )</td>
<td>25 ( [a] )</td>
<td>5 ( [a] )</td>
</tr>
</tbody>
</table>

\( \text{[a]} \) Allowable storage time exceeds 300 d.

- Allowable storage time is the storage period before quality loss is expected to affect grain quality.
- Airflow through the grain permits maintaining the grain temperature but does not extend the allowable storage time beyond that listed in the table.
- Allowable storage time is cumulative. If 16% moisture soybeans were stored for 35 days at 50°F, one-half of the storage life has been used. If the soybeans are cooled to 40 degrees, the allowable storage time at 40 degrees is only 70 days.

![Figure 2.2: Example of safe storage time \( t_s \) approximation for soybeans using isotherms at 25°C from ASAE Standard D245.6 (R2012) and \( t_s \) for corn in the ASABE Standard D535 (R2014).](image)

**Figure 2.2.** Example of safe storage time \( t_s \) approximation for soybeans using isotherms at 25°C from ASAE Standard D245.6 (R2012) and \( t_s \) for corn in the ASABE Standard D535 (R2014).
an example of soybeans at 13% m.c. and 25°C which can be stored for 56 d, because corn stored at the same $T$ and 16% m.c. has $t_s$ and $a_w$ equal to 56 d and 0.65, respectively; at this $a_w$, the soybean $w_e$ is 13%. Therefore, Table 2.3 is not based on respired CO$_2$ data, but uses the $t_s$ from shelled corn in the ASABE Standard D535 (R2014). In the absence of data for $v_{CO_2}$ (or equivalent $v_{DML}$), this assumption has become the basis for MAST guidelines for soybeans in other university extension publications (Hellevang, 2014; Sadaka, 2014; Stahl, 2014).

As mentioned before, MAST for corn uses the time to reach 0.5% DML as the upper threshold for $t_s$. By definition, $t_s$ (Equation 2.6) from the ASABE Standard D535 (R2014) corresponds to the mathematical product of a constant and five multiplier factors which influence storage time: temperature ($M_T$), moisture content ($M_w$), damage ($M_D$), hybrid ($M_H$), and fungicide treatment ($M_F$). These multipliers are all equal to unity at 15.6°C and 25% m.c., assuming a 30% total damage kernels content from mechanical harvesting for a generic hybrid kernel without fungicide treatment.

$$t_s = 9.583 M_T M_w M_D M_H M_F \quad (2.6)$$

To estimate these multipliers, several studies measured carbon dioxide production of shelled corn at different conditions considering 0.5% DML as upper threshold (Steele et al., 1969; Thompson, 1972; Friday et al., 1989; Stroshine & Yang, 1990; Al-Yahya et al., 1993; Wickle et al., 1993; Bern et al., 2002). These different studies used some confounded multipliers without data collection at lower $w$ and higher $T$, which can overestimate $t_s$.

One example of confounded estimation is the $M_D$ determined by Steele et al. (1967) for 0.5% DML. To estimate this $M_D$ for different DML percentages, Steele et al. (1967) first estimated storage time ($t_i$, Equation 2.7) in hours for shelled corn based on multiple linear regression functions of several sets of data at different levels of $T$ and $w$, and one level of $D$. Then, $M_T$ and $M_M$ were set as the inverse of their respective functions ($M_T = 1/R_T$; $M_M = 1/R_M$), where $R_T$, $R_M$, and $R_D$ are functions of $T$, $w$, and $D$, respectively. $M_D$ was not developed following the same procedure because at multiple tests only one level of $D$ was considered in one harvest season. Therefore, $M_T$, $M_M$ and a calculated lot multiplier ($M_L$) were used to arbitrarily define $M_D$ (Equation 2.8).
\[ t_i = \frac{t_R}{R_T R_M R_D} \]  \hspace{1cm} (2.7)

\[ M_D = \frac{t_i}{t_R M_T M_M M_L} \]  \hspace{1cm} (2.8)

where \( t_R \) is the time in hours for corn to reach determined level of respired CO\(_2\) at reference conditions. Equation 2.8 is based on the observed time ratios to reach 0.5\% \( DML \) for each test.

Based on the estimated data provided by Equation 2.8, Equation 2.9 was fitted by least squares regression to find the values of the coefficients “A” and “B” for 0.1, 0.5 and 1.0\% \( DML \).

\[ M_D = A e^{B \cdot D} \]  \hspace{1cm} (2.9)

The results based on the time to reach different levels of \( DML \) were the exponential equations:

\[ M_D = 1.82 \cdot e^{-0.0143 \cdot D} \text{ for } 0.1\% \text{ DML} \]  \hspace{1cm} (2.10)

\[ M_D = 2.08 \cdot e^{-0.0239 \cdot D} \text{ for } 0.5\% \text{ DML} \]  \hspace{1cm} (2.11)

\[ M_D = 2.17 \cdot e^{-0.0254 \cdot D} \text{ for } 1.0\% \text{ DML} \]  \hspace{1cm} (2.12)

**2.6. Physical aging and deterioration of soybeans**

Physical aging considers changes in physical and mechanical properties of an amorphous polymer. This term is considered different from aging caused by chemical reactions, degradation or changes in crystallinity (Schmidt & Lammert, 1996). The main evidence of aging of leguminous grains are color change, decrease in density, alterations in the structure of hulls and cotyledons, increase in water sorption through hydration, increase in hardness during cooking process, and loss of vigor (Parrish & Leopold, 1978; Narayan et al., 1988; Cárabez-Trejo et al., 1989; Paredes-López et al., 1991; Braccini et al., 1999; Kong et al., 2008; Mendes et al., 2009; Kamizake et al., 2014).

Accelerated aging of soybeans is the term used when beans are stored under high \( T \) and \( \phi \) (Parrish & Leopold, 1978; Priestley & Leopold, 1979; Braccini et al., 1999; Kamizake et al.,
Kamizake et al. (2014) compared water sorption rates and hardness of accelerated aging soybeans, stored at 30°C and ϕ of 84% RH, and natural aging soybeans, ambient T, and ϕ. Confirming previous research (Paredes-López et al., 1991; Braccini et al. 1999), as time increased, water sorption and hardness of beans increased, and these rates are much higher at high T and ϕ storage conditions.

On the other hand, Priestley and Leopold (1979) evaluated total lipid, extractable phospholipid and unsaturated fatty acids in accelerated aging soybeans seeds stored at 40°C and 100% RH for 5 d. The research resulted in a slight increase in total lipid and decrease in phospholipid and unsaturated fatty acids. These results suggest that soybean aging may not be correlated to lipid oxidation. The same results in lipids and polyunsaturated fatty acids were found for natural aging soybeans seeds stored at 4°C and low ϕ for 44 d (Priestley & Leopold, 1983).

Some authors do not refer to different storage conditions effects as accelerated aging, and instead, they assign degradation of lipids and proteins as deterioration factors affecting grain quality. For example, Alencar et al. (2010) evaluated FFA, PV and photometric color indexes of soybeans stored at different conditions. Soybeans at 11.2, 12.8, and 14.8% m.c. were stored at 20, 30 and 40°C for a period of 180 d. The increase in T, w and t resulted in a significant increase in FFA, PV and photometric color. Even with a significant increase in FFA at 20°C, the soybeans at all w did not overcome the Brazilian quality standard of 2% in FFA content of crude soybean oil (ANVISA, 1999). The same results were found for soybeans with 12.8 % m.c. at 30°C; however, at 40°C, it was not possible to maintain satisfactory quality of the beans at any w tested.

Kong and Chang (2013) studied protein degradation of soybeans at different storage conditions. Soybeans were stored at a wide range of ϕ (60 to 80% RH) at three T (22, 30, and 40°C). They found that an increase in T decreased total protein, β-conglycinin (7S) and glycinin (11S) yields caused by deterioration in protein functionality.

2.7. Respiration rates and dry matter loss correlation with chemical changes

Bern et al. (1999) observed that soybeans stored at 26°C and 22% m.c. had νCO₂ and FFA increased during storage time. After about 22 d of storage, respired CO₂ levels of three soybeans varieties were 8.23, 7.1, and 7.94 g CO₂ (kg of dry matter)⁻¹ and FFA means were 0.38, 0.24, and
0.41%. In 41 days, respired CO₂ increased to 18.72, 15.02 and 18.00 g CO₂ (kg of dry matter)⁻¹ and FFA to 0.68, 0.56, and 1.2%.

DeRocher et al. (2005) estimated DML by calculating the dry matter mass from the w of soybeans, which was being maintained at either 9% or 14% m.c. during storage at 10 and 27°C for 12 mo. The estimated DML was then compared to FFA. Mean DML increased over time with increase in w and T. Yet, average FFA was not different for soybeans stored at 10°C with different w, and increased its value at 27°C with increase in w. Basing DML estimates on dry matter mass yielded negative values from time to time, which showed the soybeans were gaining mass instead of losing dry matter. This method, overall, was not a reliable method of estimating DML.

Yang et al. (2014) evaluated \( v_{\text{CO}_2} \), FFA and protein content of soybeans stored at a large ventilated warehouse at approximately 15°C and at other warehouses whose T were 3 to 6°C higher than 15°C. \( v_{\text{CO}_2} \) were measured using a portable gas analyzer. After 12 mo., \( v_{\text{CO}_2} \) and FFA were higher at the higher T; solubility of protein declined at all T tested but was significantly higher for the ventilated warehouse.
CHAPTER 3. COMPARISON OF DRY MATTER LOSS RATES OF SOYBEANS AT 18% MOISTURE CONTENT AND 30°C DETERMINED USING STATIC AND DYNAMIC GRAIN RESPIRATION MEASUREMENT SYSTEM

3.1. Introduction

According to USDA (2017), world oilseed production in 2016/2017 was 566 million metric tons, of which 348 million metric tons were soybeans. A significant quantitative and qualitative amount of this production is lost. For example, soybean loss in Brazil is estimated at 10.3%, including 2.7% in storage stage (Grolleaud, 2002). During storage, grain may be lost due to the presence of spoilage organisms, such as insects, mites, rodents, and molds or to unfavorable storage conditions, such as high \( w \) and \( T \) (Coker, 1994). Even without spoilage, presence of organisms, and under favorable storage conditions, grains continue to respire and lose dry matter. Therefore, to determine the shelf-life or allowable storage times of a grain, it is important to have a method to measure and model its DML under a range of storage conditions.

Respiration is an oxidative reaction of a complex organic compound, such as \( \text{C}_6\text{H}_{12}\text{O}_6 \), to simple compounds, such as \( \text{CO}_2 \) and \( \text{H}_2\text{O} \), that produces energy in the form of \( \text{ATP} \) and kilocalories (\( \text{kcal} \)). \( \text{ATP} \) is regenerated from \( \text{ADP} \) and \( P_i \) (Kader & Saltveit, 2002a):

\[
\text{C}_6\text{H}_{12}\text{O}_6 + 6\text{O}_2 + 38 \text{ADP} + 38P_i \rightarrow 6\text{CO}_2 + 6\text{H}_2\text{O} + 38 \text{ATP} + 686 \text{kcal}
\] (3.1)

From this equation, it is possible to see the relationship between the respiration process and substrate losses or DML. For every mole of \( \text{C}_6\text{H}_{12}\text{O}_6 \) (180 g mol\(^{-1}\)) respired, six moles of \( \text{CO}_2 \) (6 moles x 44 g mol\(^{-1}\)) are produced. \( v_{\text{CO}_2} \) will increase or decrease depending on a host of factors. Intrinsic factors include the type and genotype, development phase during harvest, chemical composition, and \( w \) or \( a_w \) of the grain. Extrinsic factors are based on environment conditions, such as \( T, \text{O}_2, \text{CO}_2, \) carbon monoxide and ethylene concentrations, hydrocarbons and stress (Kader & Saltveit, 2002a). As with most chemical reactions, \( v_{\text{CO}_2} \) increases with increasing \( T \), and high \( v_{\text{CO}_2} \) results in lower postharvest life of a commodity. \( v_{\text{CO}_2} \) has been
expressed in terms of decreased O₂ levels consumed, \( m_{O₂,s} \) [mg O₂ consumed (kg dry matter)\(^{-1}\)], increased respired carbon dioxide levels, \( m_{CO₂,s} \) [mg CO₂ (kg dry matter)\(^{-1}\)], or DML (%). Monitoring respired CO₂ during grain storage is important in developing systems to reduce postharvest losses (Huang et al., 2013).

Since the 1940s, grain quality degradation research has been conducted based on respired CO₂, and consequently DML (Table 3.1). Respired CO₂ is measured typically using one of two types of systems, which can be described as either static or dynamic. The difference between these two systems is the availability of air or, specifically, O₂ for grain respiration. In S-GRMS (Figure 3.1a), grain is placed in a sealed chamber in which O₂ is depleted while products of respiration, CO₂ and water vapor, build up over time. The chamber is hermetically sealed, and an accurate measurement of CO₂ concentration (\( C_{CO₂} \)) is made with a gas chromatograph, infrared CO₂ analyzer, gas pressure sensor, or CO₂ absorbent material. Saltveit (2016) described the measurement process for \( v_{CO₂} \) in S- and D-GRMS. For S-GRMS, \( v_{CO₂} \) can be mathematically described as:

\[
v_{CO₂} = \frac{dm_{CO₂,s}}{dt} = \frac{\sum m_{CO₂,V}}{\Delta t} \frac{V_c}{m_{dm}}
\]

(3.2)

where \( \frac{dm_{CO₂,s}}{dt} \) is the specific mass change in CO₂ over time [mg CO₂ (kg dry beans h)\(^{-1}\)], \( \sum m_{CO₂,V} \) is the accumulated mass of respired CO₂ in the chamber (mg CO₂ m\(^{-3}\)), \( V_c \) is the container volume (m\(^3\)), \( \Delta t \) is the duration of time between the collected samples of \( C_{CO₂} \) (h), and \( m_{dm} \) is the dry matter mass of the commodity (kg dry beans).

![Figure 3.1. Schematic of a (a) static and (b) dynamic grain respiration measurements systems.](image-url)
While S-GRMS are easy to set up, the system never equilibrates, so reduction of O₂, accumulation of \( m_{CO_2,s} \), and presence of other gases (e.g., H₂O vapor) and \( T \) increase as a result of the exothermic respiration process. The exothermic respiration process can influence in \( v_{CO_2} \) (Kader & Saltveit, 2002a). Lacey et al. (1994) stated that an increase in \( C_{CO_2} \) can restrain aerobic respiration and enable other respiration pathways including anaerobic. Thus, these systems typically run for a short period of time and accuracy of \( v_{CO_2} \) measurement depends on quality of the hermetic seal, relative mass of respiring grain, and accuracy of instrumentation. Some researchers devised a way to replenish O₂ inside the chamber during respiration test, so that strictly speaking their results are not from a S-GRMS. For example, White et al. (1982b) opened the grain-filled flask after each gas sampling and flushed the flask with air for 2-5 min. Lacey et al. (1994) used a laboratory electrolytic respirometer developed by Tribe and Maynard (1989) that enabled O₂ to be replenished over time inside a sealed chamber. As O₂ was consumed by the grain, respired CO₂ was absorbed by an alkali solution. The decrease in gas pressure triggered an electrode to come into contact with an anode, and O₂ was generated at the anode as copper was deposited on the cathode.

By contrast, in a D-GRMS (Figure 3.1b), air flows continuously through the grain, delivering a constant supply of O₂ and extracting respiration products (Saltveit, 2016). Likewise, \( v_{CO_2} \) can be mathematically described by:

\[
v_{CO_2} = \frac{dm_{CO_2,s}}{dt} = \frac{\sum m_{CO_2,V}}{m_{dm}} Q
\]

where \( Q \) is the flow rate of air through the system (\( m^3 \) h\(^{-1}\)). Accuracy of \( v_{CO_2} \) measurements depends highly on accuracy and control of \( Q \). In this system, \( v_{CO_2} \) measurement will start after the system has come into equilibrium, measured, for example, as elapsed time it takes to displace five times the dead air volume of the measurement system. Such a system can be run for extended periods and the gas mixture of the air supply is conditioned typically to maintain constant \( T \), \( \phi \), and O₂ levels during the respiration test. Operating a D-GRMS requires constant monitoring of input and output airflow conditions with feedback into a control system so that equilibrium conditions are maintained during testing (Kader & Saltveit, 2002a). However, it is also possible to measure \( v_{CO_2} \) from a dynamic system using CO₂ absorption. In this case,
\[ v_{CO_2} = \frac{dm_{CO_2,s}}{dt} = \frac{\sum m_{CO_2}}{\Delta t \ m_{dm}} \]  

(3.4)

where \( \sum m_{CO_2} \) is the absorbed mass of CO\(_2\) (mg CO\(_2\)). This method is a direct gravimetric option that eliminates high-precision \( Q \) measurement. Such a system was documented in Rukunudin (1997), Sood (2015) and Trevisan (2017), and most studies that measured respired CO\(_2\) used a D-GRMS (Table 3.1).

In the literature, grain deterioration has been defined in terms of \( v_{CO_2} \) or \( v_{DML} \). Another metric in use since the 1960s is elapsed time to reach 0.5\% \( DML \) (\( t_{0.5} \)) as a quality indicator of grain storability. Steele (1967) proposed using a 0.5\% \( DML \) threshold as the maximum time to store shelled corn, since he observed this threshold coincided with corn losing one quality grade level (e.g., from U.S. Grade No. 1 to No. 2). Rukunudin (1997) analyzed total damaged seeds on preserved soybeans and found a similar grade reduction at \( t_{0.5} \).

From Table 3.1, there is a wide range of \( v_{CO_2} \) and \( t_{0.5} \) measurements reported in the literature, despite similarities in grain storage conditions. For example, shelled corn with 20 to 21\% m.c. stored at 25°C reached 0.5\% \( DML \) in 9.25 to 10.9 d, according to Friday et al. (1989) and Al-Yahya (1991), who used a D-GRMS in their respective studies. However, 21\% m.c. corn stored at 23°C for 10 d evolved 0.00062\% \( DML \) (Ubhi and Sadaka, 2015). For soybeans, Sorour and Uchino (2004) achieved 0.5\% \( DML \) after 21.62 d in a D-GRMS for 22\% m.c. beans at 25°C and Rukunudin et al. (2004) reached the same point in 11.5 d for 21\% m.c. beans at 26°C. Yet, like Ubhi and Sadaka (2015), Jian et al. (2014) did not achieve \( t_{0.5} \); after 30 d the maximum \( DML \) was 0.00064\% in a S-GRMS for 23\% m.c. soybeans at 25°C. Discrepancies in reported \( DML \) in Table 3.1 may be due to differences in intrinsic factors, or to the GRMS used.

In the first objective of this thesis, the effect of GRMS on \( v_{DML} \) estimates was assessed. Specifically, the objective of this study was to conduct side-by-side respiration tests of 18\% m.c. soybeans stored at 30°C using either a S-GRMS or D-GRMS and compare resulting \( v_{DML} \) estimates.
Table 3.1. Measurement methods and ranges of corn and soybean respiration rates reported at different storage conditions.

<table>
<thead>
<tr>
<th>Grain commodity</th>
<th>CO₂ measurement used in GRMS[a]</th>
<th>Storage conditions[b]</th>
<th>Grain deterioration[c]</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>w (%)</td>
<td>T (°C)</td>
<td>𝑣_{CO₂}</td>
</tr>
<tr>
<td></td>
<td>D Abs</td>
<td>20.5</td>
<td>26</td>
<td>20.3 – 26.6</td>
</tr>
<tr>
<td></td>
<td>D IR</td>
<td>18 – 22</td>
<td>20</td>
<td>9.8</td>
</tr>
<tr>
<td></td>
<td>D NDIR</td>
<td>23.5</td>
<td>20</td>
<td>7.6 – 9.2[d]</td>
</tr>
<tr>
<td></td>
<td>S GC</td>
<td>14 – 22</td>
<td>30</td>
<td>15 – 55</td>
</tr>
<tr>
<td></td>
<td>S NDIR</td>
<td>14 – 22.2</td>
<td>10 – 30</td>
<td>10 – 45</td>
</tr>
<tr>
<td></td>
<td>S PS</td>
<td>13 – 21</td>
<td>23 – 45</td>
<td>0 – 47.79</td>
</tr>
<tr>
<td>soybeans</td>
<td>D Abs</td>
<td>16.7–24.5</td>
<td>25.4–40.7</td>
<td>5 – 45.83</td>
</tr>
<tr>
<td></td>
<td>D GC</td>
<td>14 – 26</td>
<td>15 – 30</td>
<td>7.1 – 47.2</td>
</tr>
<tr>
<td></td>
<td>D Abs</td>
<td>12.1 – 12.5</td>
<td>25</td>
<td>0.0075–0.0240</td>
</tr>
<tr>
<td></td>
<td>S DM</td>
<td>12.5</td>
<td>21.4</td>
<td>64.1 – 111</td>
</tr>
</tbody>
</table>

[a]Grain respiration measurement systems (GRMS) were either be static (S) or dynamic (D) and used different instruments to measure respired CO₂: gravimetric using a CO₂ absorbent material (Abs), infrared spectrophotometer (IR), nondispersive infrared analyzer (NDIR), gas chromatograph (GC), pressure sensor (PS), or dry matter mass (DM).

[b]Grain moisture content (w) and storage temperature (T).

[c]Grain deterioration are typically reported as respiration rate (𝑣_{CO₂}) or time to reach 0.5% DML (𝑡_{0.5}). Values for dry matter loss rate (𝑣_{DML}) were estimated using one of the following equations: 𝑣_{DML} = [(𝑀_{C₆H₁₂O₆} / 6 M_{CO₂}) v_{CO₂} (24 h/d) 10⁻⁴] or 𝑣_{DML} = (0.5% DML/𝑡_{0.5}).

[d]Values have been converted to hourly respiration rate or in days to facilitate comparison.

[e]At a single set of grain w and storage T, grain deterioration was reported for different corn hybrids.

[f]Grain deterioration reported based on sets of grain dried at five different T (40 to 80°C in 10°C steps) to achieve the same w, then stored at the same T = 21.4°C.
3.2. Materials and Methods

3.2.1. Soybeans and sample preparation

Soybeans (P35T75X RR2X, DuPont Pioneer, Johnston, IA, USA) were harvested at 11.1% m.c. from the Crop Sciences Research and Education Farm of the University of Illinois at Urbana-Champaign on September 29, 2017. The beans were stored in plastic containers (68 L capacity) at 4°C until the start of each respiration test (Figure 3.2).

Before the start of each test, the soybeans were mixed manually in the container and a 3 kg sample ($m_{soy,0}$) was retrieved and cleaned using a sieve (Grainman 10/64” x 3/4”, Miami, FL, USA) to remove impurities and splits or damaged beans. The sample was acclimated at room temperature (22-23°C) for 30-40 min. Afterwards, its estimated initial moisture content ($\hat{w}_{soy,0}$)
was measured using a portable moisture meter (Model No SW16060, John Deere, Moline, IL, USA).

The soybean sample was rewetted to the desired 18% test moisture content ($\hat{w}_{soy,1}$), by adding a quantity of deionized water ($m_{H_2O}$) calculated as follows:

$$m_{H_2O} = m_{soy,0} \left( \frac{\hat{w}_{soy,1} - \hat{w}_{soy,0}}{100 - \hat{w}_{soy,1}} \right) 100$$  \hspace{1cm} (3.5)

The soybeans were poured into two 2 L capacity plastic bottles and placed in a roller mixer (Model No. MX-T6-S, Scilogex, Rocky Hill, CT, USA) at 60 rpm for 60 min. After every 5 min of mixing, small aliquots of deionized water were added until $m_{H_2O}$ was reached. In addition to $m_{H_2O}$, 10 mL of excess water was added to ensure complete hydration of the beans. Afterwards, the wet soybean sample was poured as a thin layer onto a metal tray and allowed to evaporate the excess external moisture at room temperature for 20-30 min. Every 5 min, $\hat{w}_{soy,1}$ was tested and air drying ceased once 18% was reached. The actual test moisture content ($w_{soy,1}$) of the sample was determined gravimetrically at 103°C for 72 h in triplicates (ASAE Standard S352, R2017):

$$w_{soy,1} = \bar{w}_{soy,r}, \quad w_{soy,r} = \left( \frac{m_{sub,w} - m_{sub,d}}{m_{sub,w}} \right) 100$$  \hspace{1cm} (3.6)

where, $\bar{w}_{soy,r}$ is the average moisture content of the 3 replicates, $w_{soy,r}$ is the moisture content for each replication, $m_{sub,w}$ is the rewetted subsample mass and $m_{sub,d}$ is the dried subsample mass. The rewetted sample was then placed in the respective GRMS. For every 3 kg of cleaned soybeans, four replications can be conducted in a S-GRMS and only one replication in a D-GRMS due the difference between the storage capacities of each respiration chamber (RC). The RC in the S-GRMS could hold 500 g of soybean sample, while the one in D-GRMS could hold 1800 g of soybean sample. At the end of each respiration test, the $w$ was again checked gravimetrically.
3.2.2. Respiration tests

Four replications of respiration tests were conducted in each S- and D-GRMS. Both systems conditioned 18% m.c. soybean samples in a controlled $T$ of 30°C. However, the respiration chamber (RC) from a S-GRMS stored 500 g of sample, while the one from a D-GRMS stored 1800 g.

3.2.2.1. Static grain respiration measurement system

A S-GRMS was set up using a hermetically sealed RC and a sensor package with an internal data logger and battery pack (Catalog No. K33-BLG, CO2Meter, Inc., Ormond Beach, FL, USA) placed on top of the grain (Figure 3.3). The RC used was a 10 L glass desiccator, and the sensor package was used to monitor $C_{CO_2}$ (% or m$^3$m$^{-3}$), $T$ (°C), and $\phi$ (%RH). A temperature-controlled incubator (Model No. 3033, Steri-Culti 200, Forma Scientific, Inc., Marjetta, OH, USA) that could hold up to four S-GRMS units was set at 30°C.

![Diagram of S-GRMS unit](image)

**Figure 3.3.** Static grain respiration measurement system (S-GRMS) unit included a 10 L desiccator for a respiration chamber capable of holding 500 g of soybean sample and a battery-operated sensor package to monitor temperature, relative humidity, and carbon dioxide levels inside the chamber. Temperature of the S-GRMS unit was controlled over time by placing it inside a thermoregulated incubator.

Prior to testing, four sensor packages were calibrated for $C_{CO_2}$ measurements using certified gas (100% nitrogen which is 0% CO$_2$ and CO$_2$-air gas mixtures, 0.15, 0.5, 1, 5, and 10% CO$_2$ v/v) (Airgas, Inc., Danville, IL, USA) using the sensor manufacturer’s DAS software (Data
Acquisition Software, CO2Meter, Inc., Ormond Beach, FL, USA) and a SenseAir cable using the UART communication protocol of the sensor. Each reference gas was certified as ± 0.03% of the labeled value on each cylinder calibration sheet. During calibration, the four sensor packages were placed in a sealed plastic bag (1 gal. capacity), into which the desired reference gas was introduced at 1 L min⁻¹ for 20 min. The sensors were set to record $C_{CO_2}$ every 20 s.

Measurements stabilized after about 5 min of introducing the reference CO₂ gas. Henceforth, after 15 min, measurements were averaged ($\overline{C_{CO_2}}$) and regressed against $C_{CO_2}$ reference values (Figure 3.4). Linear regressions were obtained from the Data Analysis ToolPak in MS Excel (Version 2016, Microsoft Corporation, Redmond, WA, USA) and they were used to correct respired $C_{CO_2}$ measurements. Additional information regarding the S-GRMS, test protocols, data analyses and calibration are included in Appendix A.

![Figure 3.4. Carbon dioxide concentration measurements over time and an example regression for one sensor package unit.](image)

Since the incubator was large enough to accommodate four desiccators or RCs, respiration tests with four replications were conducted simultaneously. The sensors were set to record $C_{CO_2,t}$ every 10 min. The desiccator lid was sealed with vacuum grease and set inside the incubator at 30°C for 20 d.
The beginning of each respiration test ($t_0$) was defined as the time when the $T$ inside the desiccator had reached 30°C ($\pm$1°C). For any time during the test, the accumulated respired $C_{CO_2,t}$ measurements were corrected, first, using the corresponding calibration equation obtained by inverting the regression equation, and then by subtracting $C_{CO_2,t<0}$, the average gas concentration prior to the start of a respiration test. The ideal gas law was used to convert this adjusted concentration measurement to a specific mass (Equation 3.7).

$$\sum m_{CO_2,s} = \frac{(C_{CO_2,t}) PV M_{CO_2}}{R T}$$

where $\sum m_{CO_2,s}$ is the accumulated specific mass of respired CO$_2$ per unit mass of dry matter of soybeans ($m_{dm}$), $P$ is the pressure (1 atm), $V$ the RC volume (10 L), $R$ the ideal gas constant (0.08205 L atm K$^{-1}$ mol$^{-1}$), $T$ the temperature (K), and $M_{CO_2}$ the molar mass of CO$_2$ (44 g mol$^{-1}$).

3.2.2.2. Dynamic grain respiration measurement system

For the dynamic respiration tests, two D-GRMS were set up to conduct two replications at 18% m.c. soybeans at 30°C simultaneously and repeated once for a total of four replications with each D-GRMS unit acting as a block. A full description of D-GRMS is described in Trevisan (2017) and the respiration test protocol is included in Appendix B.

Briefly, each D-GRMS (Figure 3.5) was supplied with a mixture of compressed air (Figure 3.5 – Item 1 and 2) at 0.5 L min$^{-1}$ controlled by a precision mass flow controller (Figure 3.5 – Item 3, Model No. GFC17A, Aalborg®, Orangeburg, NY, USA, accuracy ± 0.02 L min$^{-1}$). CO$_2$ present in the supplied air was removed by a scrubber of absorbent (Figure 3.5 – Item 4, Sodasorb®, Amron Int., Vista, CA, USA) and conditioned to desired test $T$ and equilibrium relative humidity ($\phi_e$) by bubbling it through a temperature-controlled glycerol-water solution (Figure 3.5 – Item 5 and 6), prepared following guidelines by Forney and Brandl (1992) for each test $w$. Conditioned air passed through grain-filled 30°C water-jacketed RC (Figure 3.5 – Item 8). Air exiting the top of the RC carried the initial humidified air and grain respiration products (CO$_2$ and H$_2$O vapor). This air was dehumidified using a desiccant unit (Figure 3.5 – Item 10, Catalog No. 26800 filled with indicating desiccant Catalog No. 23025, WA Hammond Drierite Co., Ltd., Xenia, OH, USA) and respired CO$_2$ was captured using a final CO$_2$ scrubber (Figure 3.5 – Item 11, Catalog No. 27070, WA Hammond Drierite Co., Ltd., Xenia, OH, USA filled with
Sodasorb® and indicating desiccant). In this RC CO₂ scrubber, ∑ₘ₇₆ was monitored five times per day (during business hours) for 20 d. At the end of a respiration test, ∑ₘ₇₆ was normalized to m₇₆ to yield ∑ₘ₇₆₆. Both D-GRMS were instrumented before and after the RC (Figure 3.5 – Item 7 and 12) to monitor air flow, T, φ, and CC₇₆. Sensors for T and φ (Model No. DHT11, WAVGAT, Caizhixing, China) were used to verify the test T of 30 ± 2°C, and φ of 88% RH for w of 18%. CC₇₆ were monitored using two CO₂ nondispersive infrared (NDIR) sensor probes and transmitters (Model Nos. GMP222 and GMPG0N0, Vaisala, Boulder, CO, USA) to ensure they remained below 20 ppm throughout each respiration test.

![Simplified schematic of dynamic grain respiration measurement system (D-GRMS).](image)

Figure 3.5. Simplified schematic of dynamic grain respiration measurement system (D-GRMS).

### 3.2.3. Conversion of respired CO₂ to dry matter loss rate

DML was estimated using the stoichiometric ratios from the respiration chemical reaction (Equation 3.1), wherein for every mole of C₆H₁₂O₆ consumed, six moles of CO₂ were respired:

\[
DML = \sum m_{CO_2} \left( \frac{1 \text{ mol}C_6H_{12}O_6}{6 \text{ mol}C\text{O}_2} \right) \left( \frac{M_{C_6H_{12}O_6}}{M_{\text{CO}_2}} \right) \times 100\% \tag{3.8}
\]

The elapsed Gregorian time (MM/DD/YYYY) was converted to Julian Date (JD):
where the last two digits of the Gregorian year are multiplied by 1000 and added to the total number of days since January 1 of the same year \((D_j)\) and the fraction of day.

\[ JD = (YY) \times 10^3 + D_j + \frac{hh:mm}{24 \, h^{-1}} + \frac{mm}{1440 \, min^{-1}} \]  

\((3.9)\)

\(DML\) estimates from both S- and D-GRMS showed an initial lag period before reaching a steady increase in \(DML\). Therefore, a threshold of 0.05\% \(DML\) was used to remove the lag period, i.e., data below this threshold value were not considered in subsequent analysis. \(v_{DML}\) was estimated by resetting the origin of \((DML, t)\) from \((0,0)\) to \((0.05, t_{0.05})\) (Figure 3.6) followed by a least squares linear regression using the Regression option of the Data Analysis ToolPak in MS Excel (Office 365, Microsoft Corporation, Redmond, WA, USA), and wherein the intercept was set to zero. The resulting slope was used as the estimate of \(v_{DML}\). The summary output of the regression was:

- regression statistics: coefficient of determination \((R^2)\), standard error of regression \((SE_{reg})\), and the number of observations \((n)\),
- analysis of variance table (ANOVA),
- estimates of slope and its standard error \((v_{DML} \pm SE_{v_{DML}})\),
- optional residuals and percentile plots.
3.2.4. Statistical analyses

3.2.4.1. Pooled standard deviation

The overall, or pooled, standard deviation of $v_{DML}$ was calculated from the mean weighted $SE_{v_{DML}}$ of each replicate slope:

$$
\left( \sigma_{v_{DML}} \right)_{p} \approx \sqrt{\frac{\sum_{i=1}^{k}(n_i - 1)(SE_{v_{DML}}^{i})^2}{\sum_{i=1}^{k}(n_i - 1)}}
$$

(3.10)

where $n$ is the number of observations from each replicated respiration test, $i$ denotes replication, and a total of $k = 4$ replications was used for each system.

3.2.4.2. Comparison of dry matter loss rates

An independent sample $t$-test assuming equal variance was used to compare four replications ($n$) of $v_{DML}$ from both respiration systems using the function PROC TTEST and the statements CLASS and VAR in SAS (2017 University Edition Software, SAS Institute, Inc., Cary, NC, USA). The $t$-test was calculated based on two independent populations ($A, B$), with
null hypothesis $H_0 (\mu_A = \mu_B)$ and alternative $H_a (\mu_A \neq \mu_B)$, degree of freedom equal to $(n_A - 1) + (n_B - 1)$, and $\alpha = 0.05$. Population $A$ and $B$ were $v_{DML}$ from S- and D-GRMS, respectively.

3.3. Results and Discussion

3.3.1. Dry matter loss estimates

At the start of each test, $DML$ was relatively low for about 4 d in both systems and increased exponentially during Day 4 to Day 11 in a S-GRMS and, on average, Day 5 to Day 8 in D-GRMS (Figure 3.7). This behavior was noted also by Rukunudin et al. (2004) in their experiments using 9 to 22% m.c. soybeans stored in a D-GRMS. However, in studies that used S-GRMS by Ochandio et al. (2012) and Jian et al., 2014 did not present high values for $DML$, and a lag period was not reported. The relatively steady increase after Day 4 to Day 8 was due to mold growth on the soybeans, which was also observed by Rukunudin et al. (2004). They reported visible mycelial growth after 4 to 13 days of storage, depending on whether their soybeans were combine-harvested or hand-harvested. Combine-harvested soybeans exhibited faster visible mold growth than hand-harvested beans and the dominant mold species were field fungi.

$DML$ estimates in a D-GRMS were about 1.4 times higher than those from a S-GRMS (Figure 3.7). The range in $DML$ was 0.15 to 0.23% for 18% m.c. soybeans stored in S-GRMS, while these estimates were 0.25 to 0.33% in D-GRMS for the same storage time of 20 d.

3.3.2. Comparison of estimated dry matter loss rates with static and dynamic systems

Estimates of $v_{DML}$ (Table 3.2) for 18% m.c. soybeans stored at 30°C in ranged from 0.0123 to 0.0175% d$^{-1}$ with a $\overline{v_{DML}}$ and $(\sigma_{v_{DML}})_p$ of 0.0157 ± 0.00001% d$^{-1}$, and 0.0165 to 0.0217% d$^{-1}$ with a $\overline{v_{DML}}$ and $(\sigma_{v_{DML}})_p$ of 0.0189 ± 0.00010% d$^{-1}$ for S-GRMS and D-GRMS, respectively. Comparing these estimates, the dynamic system had $v_{DML}$ values 1.2 times higher and $(\sigma_{v_{DML}})_p$ 10 times greater. However, these $DML$ rates were not different ($p = 0.09$).
Because DML is directly related to respired CO₂, the small numerical difference measured between the systems may be explained in part by the difference in long-term availability of O₂ for respiration. In a static system, O₂ becomes limiting over time, whereas in a dynamic system, O₂ levels are kept constant by a continuous flow of air through the grain bed. It may also be possible that elevated CO₂ concentration above 10% limits respiration, at least in a secondary manner (Kader and Saltveit, 2002b). Kader and Saltveit (2002b) also stated that υ_{CO₂}, ethylene production, compositional changes, and deterioration can be decreased by elevated levels of CO₂ or reduced levels of O₂. However, the υ_{DML} for S-GRMS was not significantly lower in these tests, when tested for significance at α = 0.05 level, which may be a confirmation that O₂ is not at levels in which limited respiration over 20 d test of a 500 g soybean sample. For example, in a test in which C_{CO₂} became approximately 8% (v/v) after 20.1 d, the O₂ concentration (C_{O₂}) can be estimated to be depleted by 8% (v/v), i.e. C_{O₂} from 21% (v/v) depleted to 13%, approximately. This depletion may not be sufficient to limit respiration, suggesting a longer-term measurement with a higher volume of sample or some other methods to reduce O₂ levels.
Table 3.2. Dry matter loss rates of 18% moisture soybeans stored at 30°C in static and dynamic grain respiration measurement systems.

<table>
<thead>
<tr>
<th>Replication</th>
<th>Dry matter loss rate and Std. Error, $v_{DML} \pm SE_{v_{DML}}$ (%) d$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Static</td>
</tr>
<tr>
<td>1</td>
<td>0.0175 ± 0.00001</td>
</tr>
<tr>
<td>2</td>
<td>0.0123 ± 0.00001</td>
</tr>
<tr>
<td>3</td>
<td>0.0168 ± 0.00002</td>
</tr>
<tr>
<td>4</td>
<td>0.0162 ± 0.00001</td>
</tr>
</tbody>
</table>

Mean and Pooled Std. Deviation, $\bar{v}_DML \pm (\sigma_{v_{DML}})_p$ [a]

- Static: 0.0157 ± 0.00001
- Dynamic: 0.0189 ± 0.00010

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

3.4. Conclusion

Overall dry matter loss rate ($v_{DML}$) estimates from independent samples of soybeans were found to be about 1.20 times lower (but not significant) when measured using a static grain respiration measurement system (S-GRMS) compared with a dynamic system (D-GRMS). The relatively small difference in $v_{DML}$ observed in this research using S and D-GRMS may have been because O$_2$ levels in S-GRMS were not sufficient to limit respiration, which reinforces the idea of the influence of the oxygen supply on soybean respiration rate. Mean $v_{DML}$ were 0.016 and 0.019% d$^{-1}$ with $(\sigma_{v_{DML}})_p$ 1 x 10$^{-5}$ and 1 x 10$^{-4}$ % d$^{-1}$ for S- and D-GRMS, respectively. Care should be taken when interpreting reported $v_{DML}$ values in the literature. When $v_{DML}$ are underestimated, resulting time to reach 0.5% DML ($t_{0.5}$) increases which in turn can lead to a safe storage time recommendation that exceeds the intended quality threshold.
CHAPTER 4. EFFECTS OF SPLIT BEANS CONTENT ON DRY MATTER LOSS RATES OF SOYBEANS MEASURED USING A STATIC GRAIN RESPIRATION MEASUREMENT SYSTEM

4.1. Introduction

Grain starts deteriorating from the time of harvest. During storage, two factors influence deterioration rates and, ultimately, grain dry matter and quality losses. The first is the presence of spoilage organisms, such as mites, molds, and insects. The second factor is unfavorable storage conditions, such as high $T$, $O_2$ levels, and high $w$ in the grain and surrounding air (Coker, 1994). However, even in the absence of spoilage organisms and favorable storage conditions, grains will continue to respire, albeit at rates so low that they are practically negligible, and the grains can be safely stored indefinitely. On the other hand, increases in storage $T$, $w$, or $D$ can dramatically decrease $t_s$; alternatively, increases in fungi resistance from either hybrid traits or fungicide application can increase $t_s$.

To estimate shelled corn storage time for 0.5% dry matter loss ($DML$), ASABE Standard D535 (R2014) provides an equation for $t_s$:

$$t_s = 9.583 M_T M_w M_D M_H M_F$$

(4.1)

where $M_T$, $M_w$, $M_D$, $M_H$, and $M_F$ are temperature, moisture, damage, hybrid, and fungicide multipliers, respectively. For different ranges of $T$, $w$, and $D$, empirical models of the multipliers have been developed based on respiration data for shelled corn (Steele et al., 1969; Thompson, 1972; Friday et al., 1989; Stroshine & Yang, 1990; Al-Yahya et al., 1993; Wickle et al., 1993; Bern et al., 2002). The synergistic effects of $w$ and $T$ on $t_s$ for a generic hybrid of shelled corn without fungicide treatment, but with 30% total $D$ from mechanical harvesting laid out in Table 1 of the Standard. From Table 1, these multipliers are all equal to unity and the corn stored at 15.6°C and 25% m.c. has an estimated $t_s$ of 9.583 d. However, the individual effects of $T$, $w$, and $D$ on $t_s$ are difficult to ascertain from looking at the empirical models of the multipliers or Table 1 alone but may be elucidated once the models are plotted against $T$, $w$, and $D$ (Figure 4.1).
Figure 4.1. Empirical models for multipliers ($M$) of allowable storage time for various factors – temperature ($T$, °C), moisture ($w$, % w.b.), and damaged kernels content ($M_D$, $D$, % w/w) – defined in ASABE Standard D535 (R2014) for shelled corn.

For example, using the shelled corn storage time table for 16% m.c. corn from Standard D535 (R2014) and visualizing the multiplier value on Figure 4.1, an increase in $T$ from 15.6°C to 26.7°C can decrease $t_s$ from 151 to 47 days, which represents a temperature multiplier of approximately 0.3. Increasing $w$ from 16% to 26% at a constant $T$ (16°C) decreases $t_s$ from 9.6 to 8 days, using the moisture multiplier of 0.83 in the same Standard. These $t_s$ values assume $M_D, M_H$, and $M_F$ are equal to unity.

The effect of damaged corn kernels on $t_s$ is not as dramatic. Corn harvesting may begin when the grain moisture content decreases to 30% m.c., but ideal harvest conditions are typically with 20-25% m.c. grain. Cracked or broken kernels result when the combine is poorly adjusted such that more beating, shearing, or pinching of the grain occurs. During drying, stress cracks can form on the kernels when the drying temperature is too high, or the grain is rapidly cooled after heating in the dryer (Steele, 1967; Fortes & Okos, 1980). Hence, ASABE Standard D535
(R2014) assumes a $D$ of 3% (w/w) for hand-shelled corn, 30% (w/w) for corn that has been mechanically harvested under typical conditions, and 40% for heavily damaged corn during harvest. These $D$ levels correspond to a $M_D$ of 2.0, 1.0, and 0.8, respectively (Figure 4.1) meaning hand-shelled corn can be safely stored twice as long as mechanically harvested corn at the same $T$ and $w$. Yet, a 10% increase in $D$ decreases $t_s$ by only 20%.

To develop $M_D$, Steele et al. (1969) defined mechanical damage for corn kernels as any visual ruptures or breaks in the seed coat. On the other hand, the U.S. standard for corn (USDA, 1996) defines damaged as kernels and pieces of corn that are badly ground-damaged, badly weather-damaged, diseased, frost-damaged, germ- damaged, heat-damaged, insect-bored, mold-damaged, sprout-damaged, or otherwise materially damaged. Corn graded as U.S. No. 5 has a maximum limit of total damaged kernels of 15% (w/w). Therefore, care should be taken when assuming a 30% (w/w) $D$ for mechanically harvest corn as defined in ASABE Standard D535 (R2014) due its peculiar damage definition.

The effect of damage is expected to be significant for soybeans and other legumes. Corn seed has one cotyledon which does not readily split, but soybean seed has a moderately thick seed coat that cracks, revealing two cotyledons that readily split and expose the endosperm and embryo to fungal attack and oxidation. At optimum harvest $w$ of 13-15% for soybeans for maximum weight and minimum field losses (Bern et al., 1999), the soybean seeds are prone to cracking after repeatedly hitting the metal surfaces of the combine and other seeds during harvest and handling (Paulsen et al., 1981). Weathering before harvest and mechanical damage to the seed coat during harvest, even when kept to a minimum, make soybeans inherently unstable during storage. Compared to other crops, soybean seed deteriorates faster (Priestley et al., 1983) and is more susceptible to hydrolysis of triglycerides and protein degradation, leading to elevated levels of FFA and decreased protein content during storage (Bern et al., 1999; Alencar et al., 2010; Kong & Chang, 2013). Elevated levels of FFA in split soybeans have been correlated to poor oil quality (Mustakas et al., 1969) and refining losses during soybean oil processing (Carr, 1976). Thus, while the effects of $T$ and $w$ on $t_s$ for corn and soybeans may be comparable, the effects of damaged and split beans content ($x_s$) on $t_s$ and dry matter loss rate ($v_{DML}$) are expected to be significant and must be quantified.
The objectives of this study were (1) to compare \( \nu_{DML} \) of 18% m.c. soybeans with 0, 4, 8, and 16% (w/w) split beans content and stored at 35°C, and (2) to use these results to develop a \( M_D \) for soybeans similar to that utilized in the ASABE Standard D535 for corn.

4.2. Materials and Methods

4.2.1. Soybeans and sample preparation

Soybeans (28T33R, DuPont Pioneer, Johnston, IA, USA) were harvested at 15% m.c. from the Crop Sciences Research and Education Farm of the University of Illinois at Urbana-Champaign in October 2016. The beans were dried to 12-13% and placed in a grain bin. On 19 January 2017, approximately 327 kg were removed from storage, placed in plastic containers (68 L capacity), and stored at 4°C until testing.

A batch of split soybeans was initially prepared by retrieving 3 kg from cold storage and screening them for large impurities and split and damaged beans (Sieve 1: Grainman 10/64” x 3/4”, Miami, FL, USA; Figure 4.2). The clean sample was passed through a custom-fabricated degerminator, screened using Sieve 1 and then a different size of sieve (Sieve 2: USA Std. Sieve No 8, Dual Manufacturing Co., Franklin Park, IL, USA), and separated into 700 g aliquots, which were placed in sealed plastic bags prior to storing at 4°C.

Before each respiration test, four glass desiccators (10 L capacity each) were placed in an incubator (Model No. 3033, Steri-Culti 200, Forma Scientific, Inc., Marietta, OH, USA) set at 35°C to acclimatize. A single aliquot of split soybeans was removed from cold storage and spread onto a tray to acclimate at room temperature (approximately 23°C) for 30-40 min. In the same fashion, a 3 kg sample of whole soybeans was removed from cold storage and acclimated onto a separate tray. Split and whole soybean \( w \) were estimated using a portable moisture meter (Model No. SW16060, John Deere, Moline, IL, USA), which were used to estimate the amounts of water to be added to reach the desired 18% m.c. for testing. Whole and split soybeans were placed in separate containers and rehydrated by adding the required amount of water (plus 10 ml of excess water) every 10 min. In between water additions, the containers were placed on roller mixers (Model No. MX-T6-S, Scilogex, Rocky Hill, CT, USA) set to 60 rpm. After 60 min of re-wetting, whole and split soybeans were spread into thin layers on separate trays to facilitate the evaporation of excess moisture at room temperature for 30-40 min. Every 5 min, \( w \) of both
batches were estimated using the portable moisture meter. Once 18% m.c. was reached, split beans were mixed with whole soybeans to yield four levels of $x_s$ (0, 4, 8, and 16% w/w), where 0% (w/w) splits served as a control sample. Each mixture weighed approximately 500 g. Three subsamples (30 g each) were set aside for gravimetric moisture content measurement following ASAE Standard S352 (R2017). Each mixed sample was placed in an acclimated desiccator outfitted with a sensor package with built-in data logger (Model K33-BLG, CO2Meter, Inc., Ormond Beach, FL, USA), sealed hermetically with vacuum grease, and ready for a respiration test (Figure 4.3). All filled desiccators were placed back in the 35°C incubator.

4.2.2. Respiration data collection and analysis

4.2.2.1. Respiration test

Each desiccator/sensor package was assigned to a single $x_s$ level and calibrated according to procedures described in da Silva et al. (2017) prior to a respiration test. The sensor packages’ built-in data loggers were set to record $T$ (°C), relative humidity ($\phi$, %RH), and carbon dioxide concentration ($C_{CO_2}$, %) inside each desiccator every 10 min for 10 days.

The experiment was a complete randomized design, with replicates in time. Each set of respiration tests consisted of one each of the four $x_s$ levels, randomly assigned to one of the four desiccators, and replicated five times over time with new soybean samples. Treatment levels were randomized among desiccator/sensor package combinations.

At the end of each respiration test, three subsamples (30 g each) from each desiccator were used to determine the gravimetric moisture content and the remaining mixed soybean sample was stored in a sealed plastic bag at -18°C for future soybean quality testing (quality data are not included in this paper).
Figure 4.2. Soybean sample preparation.
4.2.2.2. Conversion of respired CO₂ to DML estimates

The start of a respiration test (t₀) was designated as the time when the temperature in the desiccator reached 35 ± 1°C, about 6 h. CCO₂ readings were corrected using the appropriate calibration equation and by subtracting the CCO₂ at t₀. Using the ideal gas law, the corrected CCO₂ readings were converted to accumulated mass of respired CO₂ inside the desiccator (Equation 4.2), which, after normalizing to the dry matter content of the mixed soybean sample (mₘₐₜ), was subsequently converted to DML (Equation 4.4), according to the stoichiometric relationship between CO₂ and glucose during respiration (Equation 4.3):

\[ \sum m_{CO_2} = C_{CO_2} \left( \frac{PV_{CO_2}}{RT} \right) \]  

(4.2)

Respiration equation:  
\[ C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + 38 \text{ ATP} \]  

(4.3)

\[ DML = \left( \frac{\sum m_{CO_2}}{m_{dm}} \right) \left( \frac{1 \text{ mol } C_6H_{12}O_6}{6 \text{ mol } CO_2} \right) \left( \frac{M_{C_6H_{12}O_6}}{M_{CO_2}} \right) 100\% \]  

(4.4)

where \( \sum m_{CO_2} \) is the accumulated mass of respired CO₂ (g), \( P \) is the pressure inside the desiccator (1 atm), \( V \) is the desiccator volume (10 L), \( R \) is the ideal gas constant (0.08205 L atm K⁻¹ mol⁻¹), \( T \) is the temperature (K), and \( M_{C_6H_{12}O_6} \) and \( M_{CO_2} \) are the molar masses of glucose (180.16 g mol⁻¹) and carbon dioxide (44 g mol⁻¹), respectively.

4.2.2.3. Rate of dry matter loss

DML estimates showed an initial lag period until 0.05% DML has been reached followed by a steady increase in DML at a constant rate (Figure 4.4). This steady-state period (t₀.05) was considered the start time of grain DML. \( v_{DML} \) was estimated by taking the slope of the best-fit
line for the period following the lag using MS Excel (Data Analysis ToolPak, Office 365, Microsoft Corporation, Redmond, WA, USA). \( DML \) rates for mixed soybean samples (\( v_{DML,x_s} \)) were normalized to that of the control (\( v_{DML,x_s=0} \)) to yield a ratio, \( R_{x_s} \), which provided an initial measure of relative rates of \( DML \).

![Graph showing dry matter loss over time for different treatments.](image)

**Figure 4.4.** The rates of dry matter loss (\( v_{DML}, \%d^{-1} \)) were initially low and did not reach steady-state until after 0.05\% \( DML \) was reached. The rate of \( DML \) was estimated as the slope of the steady-state increase in \( DML \) after the lag period.

### 4.2.3. Statistical analyses

#### 4.2.3.1. ANOVA with Tukey’s range test

Two one-way analysis of variance (ANOVA) tests were conducted using the PROC ANOVA function with Tukey’s range test in SAS (2017 University Edition Software, SAS Institute, Inc., Cary, NC, USA). The first test used \( v_{DML} \) as the response variable across four treatment levels of \( x_s \) (0, 4, 8 and 16\% w/w) while the second test used \( R_{x_s} \) as the response variable across three treatment levels of \( x_s \) (4, 8 and 16\% w/w). Both were conducted to test for differences among treatment means at an alpha level of 0.05, and to quantify these differences.
4.2.3.2. Developing a damage multiplier ($M_D$) based on $R_{xs}$

By definition, storage time is inversely proportional to $v_{DML}$, so $t_s$ for soybean samples with 0% splits (i.e., control) will be reduced by a factor of $1/R_{xs}$ with increasing $x_s$:

$$v_{DML} = \frac{\Delta DML}{\Delta t} = \frac{\Delta DML}{t_s - 0} = \frac{\Delta DML}{t_s} \quad (4.5)$$

$$t_{s,xs} = \frac{t_{s,0}}{v_{DML,xs}} \quad (4.6)$$

$$t_{s,xs} = \left(\frac{v_{DML,0}}{v_{DML,xs}}\right) t_{s,0} = \frac{1}{R_{xs}} t_{s,0} = M_D t_{s,0} \quad (4.7)$$

Thus, $M_D$ for soybeans may be defined as $1/R_{xs}$ (Equation 4.7). Note that the soybeans used in the control and mixed samples were harvested from a single lot, so it was assumed that the degree of mechanical damage to the seed coat of the whole and split beans are the same and that the $M_D$ estimate includes the effects from both mechanical damage and $x_s$. The relationship between $M_D$ and $x_s$ was fitted with linear and exponential equations (Sigmaplot Version 13, Systat Software, San Jose, CA, USA). Regression results and the overall effect of the split soybeans on $t_s$ were compared to the $M_D$ equation for shelled corn in the ASABE Standard D535.

4.3. Results and Discussion

4.3.1. Dry matter loss estimates and rates

$DML$ estimates over time showed initial lag periods ranging from 2.82 to 7.21 d, which tended to decrease as $x_s$ increased (Figure 4.5). This lag period was observed by Rukunudin et al. (2004) and Trevisan (2017) in their studies, where grain respiration was measured in systems with a steady supply of airflow to the grain mass. However, Ochandio et al. (2012) and Jian et al. (2014) used hermetically sealed systems, similar to the ones used in this study, but they did not report an initial lag period, resulting in extremely low $DML$ values they observed.

Over a 10-day respiration test, $DML$ reached 0.09 to 0.16% for control ($x_s = 0\%$ splits) samples. These maximum $DML$ values tended to increase with increasing $x_s$: 0.10 to 0.21% for 4% splits, 0.14 to 0.27% for 8% splits, and 0.19 to 0.39% for 16% splits in mixed soybean
Figure 4.5. Dry matter loss tended to increase with increasing split beans content ($x_s$, % w/w). Each plot shows data from five replications.

Mean $v_{DML}$ was 0.0264 and 0.0405% d$^{-1}$ for 4% and 16% (w/w) split beans content in mixed soybean samples (Table 4.1).

ANOVA with Tukey’s range test results showed that the mean $v_{DML}$ for mixed samples with 16% split beans content was greater than that of the control mixed sample but was not different from those of mixed samples with 8% splits (Table 4.1). Likewise, the mean $v_{DML}$ for the control sample was not different from those of mixed samples with 4% splits. The variance ($\sigma^2$) of the mean $v_{DML}$ values increased with increasing $x_s$ and was large enough to result in
Table 4.1. Dry matter loss rates of mixed soybean samples at 18% moisture content with 0-16% (w/w) split beans content, stored at 35°C.

<table>
<thead>
<tr>
<th>$x_s$ (% w/w)</th>
<th>$v_{DML}$ (% d$^{-1}$)[a]</th>
<th>Mean and Std. Deviation $\bar{v}<em>{DML} \pm \sigma</em>{v_{DML}}$ (% d$^{-1}$)[b]</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.0245 0.0268 0.0222 0.0185 0.0266</td>
<td>0.0237 ± 0.0035 b</td>
</tr>
<tr>
<td>4</td>
<td>0.0240 0.0282 0.0281 0.0168 0.0350</td>
<td>0.0264 ± 0.0066 b</td>
</tr>
<tr>
<td>8</td>
<td>0.0215 0.0376 0.0372 0.0227 0.0300</td>
<td>0.0298 ± 0.0076 ab</td>
</tr>
<tr>
<td>16</td>
<td>0.0318 0.0450 0.0448 0.0283 0.0528</td>
<td>0.0405 ± 0.0101 a</td>
</tr>
</tbody>
</table>

[a]Standard errors of the rate estimates were less than 0.0001% d$^{-1}$.
[b]Means followed by the same letter were not different from each other ($p < 0.05$).

Table 4.2. Normalized rates of dry matter loss of mixed soybeans at 18% moisture content with 4-16% (w/w) split beans content, stored at 35°C.

<table>
<thead>
<tr>
<th>$x_s$ (% w/w)</th>
<th>$R_{x_s} = v_{DML,x_s}/v_{DML,0}$</th>
<th>Mean and Std. Deviation $\overline{R_{x_s}} \pm \sigma_R$ [a]</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>0.98 1.05 1.27 0.91 1.32</td>
<td>1.11 ± 0.18 b</td>
</tr>
<tr>
<td>8</td>
<td>0.88 1.40 1.68 1.23 1.13</td>
<td>1.26 ± 0.30 ab</td>
</tr>
<tr>
<td>16</td>
<td>1.30 1.68 2.02 1.53 1.98</td>
<td>1.70 ± 0.31 a</td>
</tr>
</tbody>
</table>

[a]Means followed by the same letter were not different from each other ($p < 0.05$).

overlapping treatment means. Similarly, ANOVA results of normalized $v_{DML,x_s}$ to those of the control showed a difference between $R_4$ and $R_{16}$, but neither values were different from $R_8$ (Table 4.2), which is attributed to large $\sigma_R$ at 8 and 16% split beans content.

4.3.2. Damage multiplier

From ASABE Standards D535 (R2014), $M_D$ for shelled corn ranged from 2.08 to 0.8 for mechanically harvested corn as damaged kernel content increased from 0 to 40% (w/w). $M_D$ for damaged corn kernels at 16% (w/w) is 1.42, a decrease of about 32% when compared to 0% (w/w) damage. Assuming $M_D$ is inversely proportional to $R_{x_s}$, mean $M_D$ ranged from 1.0 to 0.6 as $x_s$ increased from 0 to 16% (w/w) (Figure 4.6), a decrease of 40%. Thus, for the same level of damaged corn kernels and split beans, the decrease in $M_D$ is 1.25 greater in split beans than damaged corn kernels. Therefore, it appears that $M_D$ is more sensitive to bean damage than in corn kernels. Since $t_s$ is directly proportional to $M_D$, $t_s$ of soybeans is expected to be shorter than that for corn with a comparable level of mechanical damage. Based on the limited tests
conducted thus far, the relationship between $M_D$ and $x_s$ for soybeans can be described using a linear or exponential model, with a lower standard error ($SE$) obtained with the linear model.

4.4. Conclusion

Soybean DML rate ($v_{DML}$) and variability increased modestly with increasing split beans content ($x_s$), with a four-fold increase in split beans (from 4% to 16% w/w) resulting in 1.53 times greater $v_{DML}$. No significant difference in $v_{DML}$ was detected between samples with 8% and 16% split beans. The same trend held true when $v_{DML}$ was normalized to that of control samples to yield a split content ratio ($R_{x_s}$). The inverse relationship between $R_{x_s}$ and damage multiplier ($M_D$) showed that the effect of damage from 0 to 16% (w/w) content was 1.25 greater for soybeans than for corn, leading to reduced storage time if all other factors are constant. This experiment was useful in understanding the effects of damaged beans on safe storage time of 18% m.c. content soybeans at 35°C under hermetic conditions and could be expanded in the future to cover a wider range of moisture content, storage temperature, and non-hermetic conditions.
CHAPTER 5. DRY MATTER LOSS AND CHEMICAL CHANGES TO SOYBEANS AT 14, 18, AND 22% MOISTURE CONTENT AND 30°C MEASURED IN A DYNAMIC GRAIN RESPIRATION SYSTEM

5.1. Introduction

Soybean is one of the most valuable crops in the world, being used as protein source for billions of livestock and poultry (Nwokolo, 1996), and as oil source for vegetable oil and biodiesel. Soybeans are valued for high protein and oil contents, about 40 and 20% (d.b.), respectively (Asbridge, 1995). However, soybean quality during storage is affected by a range of factors T, w and t. Elevated levels of these factors increase the rate of grain deterioration by increasing $v_{DML}$, which can be estimated by measuring grain respiration (Equation 5.1); for every mole of $C_6H_{12}O_6$ (180 g mol$^{-1}$) reacted with respired $O_2$, six moles of $CO_2$ ($6 \times 44$ g mol$^{-1}$) are produced. This reaction also produces energy in form of ATP and kcal and requires intermediate molecules to occur, which are $ADP$ and $P_i$.

$$C_6H_{12}O_6 + 6O_2 + 38ADP + 38P_i \rightarrow 6CO_2 + 6H_2O + 38ATP + 686\text{ kcal} \quad (5.1)$$

Several authors have reported effects of storage conditions on $v_{DML}$ of soybeans (Table 5.1) in hopes of developing a set of safe t guidelines, similar to ASABE Standard D535 for shelled corn (R2014). Ramstad and Geddes (1942), Sorour and Uchino (2004), and Rukunudin et al. (2004) reported $v_{DML}$ within range of each other and in the order of 0.01 to 0.1% d$^{-1}$. They each used a D-GRMS, in which a continuous air or $O_2$ gas supply promoted continued grain respiration throughout a system. By contrast, studies that used S-GRMS reported $v_{DML}$ 0.0001 to 0.01% d$^{-1}$, which are several orders of magnitude lower than those for D-GRMS. This discrepancy results from having a limited $O_2$ supply in S-GRMS, which limits grain respiration during testing. Hence, when comparing one grain respiration study to another, the type of GRMS used must be taken into account. In addition, most soybean respiration studies, so far, have focused on grain storage in temperate climates. There is a dearth of respiration data, especially those collected in a D-GRMS, for grain storage conditions (e.g., high T, high $\phi$, $w_e$) in other large soybean-producing countries, such as Brazil, Argentina, India, Paraguay, Bolivia, and
Table 5.1. Measurement methods and ranges of soybean dry matter loss rates reported at different storage conditions.

<table>
<thead>
<tr>
<th>Grain respiration measurement systems (GRMS)[a]</th>
<th>Storage conditions</th>
<th>Dry matter loss rate</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dynamic</td>
<td>w (%)</td>
<td>T (°C)</td>
<td>t (d)</td>
</tr>
<tr>
<td>Dynamic</td>
<td>16.7-24.5</td>
<td>25.4-40.7</td>
<td>7-34</td>
</tr>
<tr>
<td>Static</td>
<td>11-15.2</td>
<td>10-29</td>
<td>180</td>
</tr>
<tr>
<td>Static</td>
<td>12.5</td>
<td>40</td>
<td>180</td>
</tr>
</tbody>
</table>

[a]GRMS are classified as static or dynamic based on the availability of oxygen (O_2) gas for grain respiration. Chapter 3 provides further discussion and comparison of these systems.

[b]Estimated values of DML rates based on respiration rates.

\[ v_{DML} = v_{CO_2} (g \text{ kg}^{-1} \text{ d}^{-1}) 24 \text{ h} \text{ d}^{-1} M_{C_6 H_{12} O_6} (g \text{ mol}^{-1}) / 6 M_{CO_2} (g \text{ mol}^{-1}) 10^{-4} \]

[c]Estimated values of DML rates, \( v_{DML} = DML \text{ (%)}/t \text{ (d)} \).

[d]Estimated values of DML rates (\( v_{DML} \)) based on CO_2 concentration (%).

Uruguay. More respiration data collected in a S-GRMS are needed for a wider range of \( w \), given increased use of silo bags for temporary storage.

Aside from storage conditions affecting \( v_{DML} \), changes in physical and mechanical properties of soybeans can occur. These properties include, but are not limited to, color, density decrease, alterations in hull and cotyledon structures, increase in water sorption ability, increase in hardness during cooking, and loss of seed vigor (Parrish & Leopold, 1978; Narayan et al., 1988; Cárabec-Trejo et al., 1989; Paredes-López et al., 1991; Braccini et al., 1999; Kong et al., 2008; Mendes et al., 2009; Kamizake et al., 2013). Chemical composition may also change. For soybeans, oil or lipids can undergo hydrolysis and oxidation, while proteins degrade (Bern et al., 1999; Alencar et al., 2010; Kong and Chang, 2013; Yang et al., 2014). Drying and storage \( T \) and \( t \) also impact germination rates, mold growth, and lipid quality based on FFA measurement (White et al., 1976).

There are simultaneous alternate pathways in which lipid oxidation occurs, and the balance among these pathways shifts with different conditions. Therefore, in order to characterize the rate and degree of lipid oxidation, multiple products of oxidation must be estimated (Schaich, 2016). Some of these pathways includes the continuously formation of
hydroperoxides as primary oxidation products followed by a production of a variety of nonvolatile and volatile secondary oxidation compounds, which are formed by subsequent reactions of hydroperoxides (Dobarganes & Velasco, 2002). PV is the main method used to measure hydroperoxides, while TBA value is widely used for evaluating secondary oxidation compounds (Fennema, 1996). Although these are the main methods used to evaluate lipid oxidation, most grain storage studies reported lipid degradation in the form of FFA, which correlates to lipid hydrolysis that leads to increased lipid oxidation (Table 5.2).

<table>
<thead>
<tr>
<th>Storage conditions</th>
<th>Chemical analysis</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>w (%) w.b.</td>
<td>T (°C)</td>
<td>t (d)</td>
</tr>
<tr>
<td>9-21</td>
<td>room</td>
<td>365</td>
</tr>
<tr>
<td>12-17</td>
<td>10-32</td>
<td>180</td>
</tr>
<tr>
<td>10-20[b]</td>
<td>room[c]</td>
<td>365-730</td>
</tr>
<tr>
<td>9-22</td>
<td>26</td>
<td>[d]</td>
</tr>
<tr>
<td>22</td>
<td>26</td>
<td>0-41</td>
</tr>
<tr>
<td>15</td>
<td>20-40</td>
<td>180</td>
</tr>
<tr>
<td>11-15</td>
<td>20-40</td>
<td>180</td>
</tr>
<tr>
<td>12-16[b]</td>
<td>22-40</td>
<td>365</td>
</tr>
<tr>
<td>12.5</td>
<td>40</td>
<td>0-180</td>
</tr>
</tbody>
</table>

[a] PV unit equal to milliequivalent per kilogram of lipid.
[b] Equilibrium moisture content (w_e) based on T and ϕ of storage conditions provided by authors. The soybean moisture sorption isotherms used were based on ASAE Standard D245.6 (R2012) cited in Sood (2015). Narayan et al. (1988) reported room ϕ of 50 to 90%RH.
[c] Room temperature ranged from 16 to 40°C.
[d] Author mention only achieved DML when sample was collected without reporting t, on average DML were 1.62% for 9% m.c. and 1.70% for w of 22%.

Increase in respiration and DML tend to increase FFA (Bern et al., 1999). Three soybean varieties adapted to Central Iowa stored at 26°C and 22% m.c. for 22 d had DML estimates of 0.56, 0.48, and 0.54% and FFA means of 0.38, 0.24, and 0.41%, respectively (Bern et al., 1999). After 41.5 days, DML increased to 1.28, 1.02, and 1.23% and FFA of 0.68, 0.56, and 1.2%, respectively. For all three varieties, DML and FFA increased over time. Similarly, Rukunudin (1997) found the same correlation between DML and FFA increases. Before respiration test, fresh soybeans with 9% and 22% m.c. initially had FFA means of 0.15 and 0.46%, respectively.
After a storage test at 26°C, 9% m.c. beans reached 1.62% DML and increased its FFA to 1.13% while beans at 22% m.c. reached 1.74% FFA. These two reported studies are the only ones which address a comparison between DML and FFA, the remaining references from table 5.2 only characterized lipid degradation at specific t without evaluating DML.

From reported studies (Table 5.2), it is possible to see that t, w and T are factors that increase lipid degradation. A correlation between increased t, FFA and PV was observed by Narayan et al. (1988). At room temperature storage conditions, soybeans stored for 365 d presented FFA of 0.16% and PV of 18 mEquiv per kg lipid, and after 730 d of storage FFA and PV increased to 4.31% and 40 mEquiv per kg lipid. On the other hand, Alencar et al. (2010) observed this increase in FFA and PV by increasing w and T. Soybeans stored for 180 d at 13% w and 20°C presented FFA and PV of 0.2% and 2 mEquiv per kg lipid, increasing storage T to 40°C, these values increased to 1% and 7 mEquiv per kg lipid. Likewise, 15% m.c. beans stored at 20°C had FFA and PV of 1% and 6 mEquiv per kg lipid, and at 40°C values of 12% and 14 mEquiv per kg lipid. It is possible to see that both factors, w and T, contributed to increase FFA and PV.

According to the summary of literature in Tables 5.1 and 5.2, few studies have measured the effects of storage conditions on DML, chemical composition, and lipid oxidation. Relatively speaking, DML is easier to measure than conducting chemical analyses, but soybeans are valued for their protein and oil contents and qualities. Hence, safe storage time (tₚ) guidelines should be based on chemical properties and knowing the correlation (if any) of DML measurements to these properties becomes paramount. Therefore, the objectives of this study were (1) to measure ν₁DML of soybeans at 14, 18, and 22% m.c. and 30°C, (2) to estimate safe storage time for soybeans stored at the measured storage conditions, and (3) to measure compositional and lipid quality changes at 30°C correlating to DML. Storage conditions chosen for this study are typical for Mato Grosso, Brazil where w during soybean harvest can range from 14-22% m.c. and T can be as high as 30°C (Danao et al., 2015).
5.2. Materials and Methods

5.2.1. Soybeans and sample preparation

Soybeans (P35T75X RR2X, DuPont Pioneer, Johnston, IA, USA) were harvested at 11.1% m.c. from the Crop Sciences Research and Education Farm of the University of Illinois at Urbana-Champaign in 2017. The beans were stored in plastic containers (68 L capacity) at 4°C until testing.

Prior to a respiration test, a 3 kg sample of soybeans was retrieved from cold storage and cleaned to remove large impurities, split and damaged beans using a sieve (Grainman 10/64” x 3/4”, Miami, FL, USA, Figure 5.1). They were acclimated inside an incubator at 30°C for 5 d. Using a portable moisture meter (Model No. SW16060, John Deere, Moline, IL, USA), \( w \) was estimated to determine the minimum amount of deionized water needed to reach the test \( w \) (14, 18 or 22%). The sample was subdivided and placed into 2 L containers. The containers were placed on roller mixers (Model No. MX-T6-S, Scilogex, Rocky Hill, CT, USA) set to 60 rpm for 60 min. Small aliquots of deionized water were added every 10 min until one half of the minimum amount of water plus 10 mL was added to each container.

After rewetting, samples were combined and spread into a thin layer onto metal trays at room temperature for 30-40 min to evaporate excess of moisture. During this step, \( w \) was measured with the moisture meter every 5 min until the test \( w \) was attained. Afterwards, approximately 1800 g of sample was placed inside the respiration chamber of D-GRMS (Figure 5.2) to initiate the respiration test. From the remaining soybeans, three subsamples (30 g each) were reserved to determine \( w \) gravimetrically at 103°C for 72 h in triplicates, following ASAE Standard S352 (R2017), and 500 g was poured into a plastic bag, sealed, and stored at -18°C for subsequent chemical analyses. After the respiration test, \( w \) was also measured gravimetrically following the same standard and 500 g of sample was saved in the same manner as before the respiration test for chemical analyses.
5.2.2. Respiration data collection and analysis

5.2.2.1. Respiration test

Two D-GRMS were used to conduct simultaneous measurements. Respiration tests (1 T x 3 w) with four replications were conducted in a randomized complete block design, using each D-GRMS as a block. A full description of D-GRMS and respiration test protocol is described in Trevisan (2017) and Appendix B. Briefly, each D-GRMS was supplied with a mixture of...
compressed air (Figure 5.2 – Item 1 and 2) at 0.5 L min$^{-1}$ controlled by a precision mass flow controller (Figure 5.2 – Item 3, Model No. GFC17A, Aalborg®, Orangeburg, NY, USA, accuracy ± 0.02 L min$^{-1}$). CO$_2$ present in the supplied air was removed by a scrubber of absorbent (Figure 5.2 – Item 4, Sodasorb®, Amron Int., Vista, CA, USA) and conditioned to desired test $T$ and equilibrium relative humidity ($\phi_e$) by bubbling it through a temperature controlled glycerol-water solution (Figure 5.2 – Item 5 and 6), prepared following guidelines by Forney and Brandl (1992) for each test $w$. Conditioned air passed through grain-filled 30°C water-jacketed respiration chamber ($RC$, Figure 5.2 – Item 8). Air exited the top of the $RC$ carries with its initial humidified air and grain respiration products. This air was dehumidified by a desiccant unit (Figure 5.2 – Item 10, Catalog No. 26800 filled with indicating desiccant Catalog No. 23025, WA Hammond Drierite Co., Ltd., Xenia, OH, USA) and respired CO$_2$ was captured using a final CO$_2$ scrubber (Figure 5.2 – Item 11, Catalog No. 27070, WA Hammond Drierite Co., Ltd., Xenia, OH, USA filled with Sodasorb® and indicating desiccant).

Accumulated mass of respired CO$_2$ ($\Sigma m_{CO_2}$) in this $RC$ CO$_2$ scrubber was monitored five times per day (during business hours) for either 20 days or when 1.0% $DML$ had been reached. Both D-GRMS were instrumented before and after the $RC$ (Figure 5.2 – Item 7 and 12) to monitor air flow, $T$, $\phi$, and CO$_2$ levels. Sensors for $T$ and $\phi$ (Model No. DHT11, WAVGAT, Caizhixing, China) were used to verify the test $T$ of 30 ± 2°C, and $\phi$ of 77, 88 and 96% RH for 14, 18, and 22% m.c., respectively. CO$_2$ levels were monitored using two CO$_2$ nondispersive infrared (NDIR) sensor probes and transmitters (Model Nos. GMP222 and GMPG0N0, Vaisala, Boulder, CO, USA) to ensure they remained below 20 ppm throughout each respiration test.

5.2.2.2. Conversion of respired CO$_2$ to DML rates and safe storage time

Accumulated CO$_2$ was normalized to the dry matter content of the tested soybean sample and converted to $DML$ (%) (Equation 5.2), according to the stoichiometric relationship between CO$_2$ and glucose during respiration (Equation 5.1).

$$DML = \left( \frac{\Sigma m_{CO_2}}{m_{dm}} \right) \left( \frac{1 \text{ mol } C_6H_{12}O_6}{6 \text{ mol CO}_2} \right) \left( \frac{M_{C_6H_{12}O_6}}{M_{CO_2}} \right) 100\%$$

(5.2)

where $\Sigma m_{CO_2}$ is accumulated mass of respired CO$_2$ (g), $m_{dm}$ is dry matter content of the soybean sample (g), and $M_{C_6H_{12}O_6}$ and $M_{CO_2}$ are molar masses of glucose (180.16 g mol$^{-1}$) and carbon dioxide (44 g mol$^{-1}$), respectively.
Figure 5.2. Simplified schematic of dynamic grain respiration measurement system (D-GRMS).

DML estimates were plotted over time. Following Trevisan (2017), initial lag periods were removed by setting a threshold of 0.05% DML; data below this threshold was not included in subsequent analysis (Figure 5.3). An upper threshold DML was based on either 20 d since start of experiment, or 1% DML, depending on w, with 14 and 18% m.c. soybeans held for 20 d and the 22% m.c. held until 1% DML was achieved. To estimate $v_{DML}$, the origin of the plot was re-set to $(t_{0.05}, 0.05)$, followed by a least squares linear regression of the data (Data Analysis ToolPak in MS Excel, Office 365, Microsoft Corporation, Redmond, WA, USA). The slope of the best fit line was the estimate of $v_{DML}$.

Safe storage time based on DML ($t_{DML}$) was estimated using 0.5% DML as a threshold, which is equivalent to corn and soybeans losing one USDA market grade level (Steele, 1967; Rukunudin, 1997).
5.2.3. Chemical analyses

5.2.3.1. Proximate analysis

A pair of samples (initial, final) from the four replications of each respiration test treatment (14, 18, and 22% m.c.) were sent to the Food Processing Center (University of Nebraska, Lincoln, NE, USA) for analysis. Moisture content, ash, protein, total fat and carbohydrates from subsamples of the frozen samples collected at the beginning and end of each respiration test were determined. Each sample was 70 g in size and was cryogenically ground prior to analysis. The proximate analysis was done in triplicates.

Moisture content ($w$, % w.b.) was obtained gravimetrically from 1 g sample at 103°C for 24 h. Ash ($A$, % w.b.) was determined by incinerating a 2 g sample at 525°C for 24 h. Protein content ($P$, % w.b.) was calculated from nitrogen content ($N$, % w.b.) measured using a nitrogen analyzer (Model No. TruMac N®, LECO Corporation, Saint Joseph, MI, USA) and by multiplying $N$ by a 6.25 factor. For this analysis, 0.3 g sample was burned at 1100°C. Total fat content ($F$, % w.b.) was determined according to AOAC Method 922.06 (2012) by extracting fat from a 2 g sample and 90 mL hexane (ACS grade) in a Soxtec extractor (Model No. ST 255.
Carbohydrates ($C$, % w.b.) were calculated by subtraction:

$$C = 100 - (w + A + P + F)$$

Prior to comparing the proximate analysis results, $A$, $P$, $F$ and $C$ were converted to dry basis (d.b.) by multiplication with the factor $100/(100 - w)$.

The changes in $C$ ($\Delta C$, % d.b.) corresponding to before and after respiration test samples were calculated from the difference between the final ($C_f$) and initial ($C_i$) adjusted values:

$$\Delta C = C_f - C_i$$

The changes in carbohydrates content were then compared to the reached DML.

5.2.3.2. Lipid oxidation tests

A pair of samples (initial, final) from the first replication for each of the 14 and 18% m.c. respiration tests and one pair from each of the first two replications at 22% m.c. were tested for products of lipid oxidation by the Schlegel Laboratory (Department of Food Science and Technology, University of Nebraska, Lincoln, NE, USA). $PV$ (mEquiv per kg sample) was measured according to the method in Li et al. (2001). For this analysis, fat was extracted with a 2:1 chloroform: methanol (v/v). 2-Thiobarbituric Acid ($TBA$, mmol TBARS per g of sample) value was measured according to AOCS Method CD 19-90 (2004).

The changes in $PV$ ($\Delta PV$, mEquiv per kg sample) and in $TBA$ ($\Delta TBA$, mmol TBARS per g of sample) corresponding to before and after respiration test samples were also calculated from the difference between the final ($PV_f$ and $TBA_f$) and initial ($PV_i$ and $TBA_i$) adjusted values:

$$\Delta PV = PV_f - PV_i$$
$$\Delta TBA = TBA_f - TBA_i$$

Then, the changes in $PV$ and $TBA$ were also compared to the reached DML.

5.2.4. Statistical analysis

Values of $v_{DML}$ from 30°C, and of initial and final sampling in $A$, $P$, $F$ and $C$ were individually analyzed with a one-way analysis of variance (ANOVA) test with three levels of $w$.
as the main effect for $v_{DML}$ and two level of sampling (initial and final). ANOVA was conducted using the PROC ANOVA function with Tukey’s range test in SAS (2017 University Edition Software, SAS Institute, Inc., Cary, NC, USA). To evaluate the block effect between the two systems and their respective $v_{DML}$ at 14, 18, and 22% m.c., the PROC ANOVA function was used with block (system 1 and 2) and moisture as CLASS for main effects, and among treatment means, were considered significant at $\alpha = 0.05$. Paired t-test were conducted to compare individually initial ($i$) and final ($f$) values from proximate analysis in SAS (2017 University Edition Software, SAS Institute, Inc., Cary, NC, USA). The t-test was calculated based on the two populations ($i, f$) with $\alpha = 0.05$ for each $A$, $P$, $F$, and $C$ content. Pearson product-moment correlations were computed to estimate a parametric measure of a linear relationship between two variables, $DML$ and $\Delta C$, $\Delta PV$ or $\Delta TBA$ using the CORR function in SAS (2017 University Edition Software, SAS Institute, Inc., Cary, NC, USA).

5.3. Results and Discussion

5.3.1. Dry matter loss estimates and rates

An initial lag period of $DML$ was observed on the 18 and 22% m.c. treatments (Figure 5.4a). The lag time to reach 0.05% $DML$ ($t_{0.05}$) ranged from 7.6 to 18.7 d for 14% m.c., 6.9 to 8.1 d for 18%, and 0.8 to 2 d for 22% m.c. even after an acclimation period of 5 d in an incubator. At 14% m.c., at such low observed $DML$, data prior to reaching $t_{0.05}$ were discarded despite having no significant difference ($p < 0.05$) between $v_{DML}$ computed with and without data from below the threshold, thus, the data could have been considered in the regression analysis. Rukunudin (1997) and Trevisan (2017) also reported this lag period, but neither reported a consistent acclimation period to minimize lag time during a respiration test. Rukunudin (1997) reported $t_{0.05}$ of 2.61 d for machine-harvested soybeans previous stored cold and tested at 26°C and 21% m.c.. Trevisan (2017) observed soybeans stored at 35°C had an average $t_{0.05}$ of 4.34 d and 3.65 d for 14% and 18% m.c., respectively.

As others have reported, $DML$ increased with $w$ and $T$. $DML$ reached 0.06 to 0.10% for 14% m.c. soybeans in 20 d. By contrast, 18% m.c. soybeans reached 0.25 to 0.28% $DML$ in 20 d, while 22% m.c. soybeans exceeded 1% $DML$ within 15 d. $DML$ estimates by Rukunudin (1997) ranged from 0.78 to 0.86% for machine-harvested soybeans stored at 26°C and 21% m.c. after
Figure 5.4. Dry matter loss (DML, %) estimates tended to increase with increasing moisture content and time (t, d) to reach 0.05% DML (t_{0.05}) decreases with increasing moisture content (a). DML and t were adjusted (DML', t') to remove the lag period to reach t_{0.05} (b).

about 15 d, while Trevisan (2017) reported 1.52 to 1.77% DML for soybeans stored at 35°C and 22% m.c. after about 5.8 to 7.5 d.

During one respiration test at 30°C and 18% m.c., v_{DML} was significantly higher than the 95% confidence interval for the mean v_{DML} from all other replicates. This replication had visually higher mold growth, which could be explained by the visual mold growth observed in the glycerol solution. Therefore, this replication was not used and another respiration test at the same conditions was conducted to complete the four replications needed.

Adjusted DML and time data from this study are represented in Figure 5.4b, and respective v_{DML} are summarized at Table 5.3. The standard errors for each v_{DML} are not reported in Table 5.3. However, v_{DML} at 30°C had standard errors less than 0.0003% d^{-1}, except for replications 3 and 4 at 22% m.c. where the standard errors were 0.0012, and 0.0014% d^{-1}, respectively.

A one-way ANOVA with Tukey’s range test of v_{DML} at 30°C indicated a significant effect of w, with \bar{v}_{DML} increasing with w. Rates of 0.0047, 0.0189 and 0.0645 % d^{-1} for 14, 18 and 22% m.c. represented a 4-fold increase in v_{DML} for 14 to 18% m.c. and 14 times increase for 22% m.c. compared to 14%.
5.3. Dry matter loss rates of soybean samples at 14, 18, and 22% moisture content stored at 30°C.

<table>
<thead>
<tr>
<th>Moisture (w, % w.b.)</th>
<th>Dry matter loss rate ($v_{DML}$, % d⁻¹)</th>
<th>Mean and Std. Deviation ($\overline{v}<em>{DML} \pm \sigma</em>{v_{DML}}$, % d⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rep 1</td>
<td>Rep 2</td>
<td>Rep 3</td>
</tr>
<tr>
<td>14</td>
<td>0.0043</td>
<td>0.0041</td>
</tr>
<tr>
<td>18</td>
<td>0.0184</td>
<td>0.0217</td>
</tr>
<tr>
<td>22</td>
<td>0.0576</td>
<td>0.0609</td>
</tr>
</tbody>
</table>

[a] Means followed by the same letter were not different from each other ($p < 0.05$).
[b] Block effect between the two systems was not significant ($p < 0.0001$).

5.3.2. Safe storage time estimation

Table 5.3 lists $\overline{v}_{DML}$ which were used to estimate $t_s$ (Table 5.5) corresponding to the time to reach 0.5% DML ($t_{0.5}$). At 30°C, clean soybeans at 14% m.c. may be safely stored for 106 d until losing one grade level; time decreased to 26 d for 18% m.c. soybeans and to 8 d for 22% m.c. These are upper estimates for clean beans with no other preliminary varying storage conditions.

ASABE Standard D535 (R2014) provides safe storage time for shelled corn also based on $t_{0.5}$. This standard was developed nearly 50 years ago (Steele et al., 1969) based on assumed 30% D and $15 \leq T \leq 26°C$ and it is sometimes used directly for soybeans. Table 1 in this standard estimates safe storage time of corn at 29.4°C to be 35, 14 and 5 d for 16, 18 and 22% m.c., respectively (Table 5.4). Hellevang (2014) developed an approximate allowable storage time table for soybeans based on assumptions of water activity ($a_w$) using the ASABE Standard D535 (R2014) for shelled corn without measuring CO₂ data of soybeans. For a storage $T$ of 26°C, Hellevang (2014) estimated safe storage time of 20, 7, and 2 d for soybeans at 14, 17, and 21% m.c., respectively. These values of $t_{0.5}$ are 4 to 5 times greater than the estimates found in this study, even with an increase in $T$, which would decrease $t_s$. However, the safe storage time at 0.5% DML from this study neglected the initial lag period up to 0.05% DML (15, 8, and 1 d for 14, 18, and 22% m.c., respectively). If these mean values are subtracted from $t_{0.5}$, the adjusted storage time is reduced to 91, 18, and 7 d for 14, 18, and 22% m.c., respectively (Table 5.4). While there is a discrepancy between the estimates from literature and those from this...
Table 5.4. Safe storage time estimation for soybean samples at 14, 18, and 22% moisture content stored at 30°C and reported literature.

<table>
<thead>
<tr>
<th>Grain commodity</th>
<th>Temperature (°C)</th>
<th>Moisture (w, % w.b.)</th>
<th>Safe storage time (t₀.₅, d)</th>
<th>Adjusted storage time (t₀.₅ − t₀.₀5, d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soybeans</td>
<td>30</td>
<td>14</td>
<td>106</td>
<td>91</td>
</tr>
<tr>
<td></td>
<td></td>
<td>18</td>
<td>26</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td></td>
<td>22</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>26</td>
<td>14</td>
<td>20</td>
<td>-</td>
</tr>
<tr>
<td>Hellevang (2014)</td>
<td></td>
<td>17</td>
<td>7</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>21</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>Corn</td>
<td>29.4</td>
<td>16</td>
<td>35</td>
<td>-</td>
</tr>
<tr>
<td>(ASABE Standard D535, R2014)</td>
<td></td>
<td>18</td>
<td>14</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>22</td>
<td>5</td>
<td>-</td>
</tr>
</tbody>
</table>

[a] Soybeans samples were cleaned to remove any impurities and splits.
[b] Based on equivalent soybean αₜ from corn isotherms in ASABE Standard D535 (R2014).
[c] Data from Table 1 in ASABE Standard D535 (R2014) based on extrapolated data collected between 15 to 26°C.

study, the adjusted time presented is based on direct measurement of DML of clean soybeans at the storage conditions noted in Table 5.4. Yet, before applying these results, effects of other factors should be quantified, e.g. total damage, foreign material, and splits content.

5.3.3. Chemical analyses

Table 5.5 represents the proximate analysis results for all respiration tests based on values of w, A, P, F, and C. There was no effect of w or the respiration test (change between initial and final) on A, F, P and C, except for C estimates of final samples at different w levels. ΔC values generally confirm a numerical (but not significant) decrease in C of 1.16% to 3.25% for 14 to 22% m.c. (Table 5.6 and Figure 5.5). However, the correlation coefficient between DML and ΔC was -0.40 (p = 0.18), for all data combined (n =13). The correlation coefficients between DML and ΔC by w were 0.79 (p = 0.21), -0.83 (p = 0.08), and 0.47 (p = 0.53), for 14, 18, and 22% m.c., respectively. None of the correlations were significantly different from zero.
Table 5.5. Proximate analysis of soybean samples before and after respiration tests at 14, 18, and 22% moisture content stored at 30°C. (Values in percentage, based on three subsamples).

<table>
<thead>
<tr>
<th>Moisture content, (w, %) w.b.</th>
<th>Ash ± Std. Deviation, (A ± \sigma_A, %) d.b.</th>
<th>Protein ± Std. Deviation, (P' ± \sigma_P, %) d.b.</th>
<th>Fat ± Std. Deviation, (F' ± \sigma_F, %) d.b.</th>
<th>Carbohydrates ± Std. Deviation, (C' ± \sigma_C, %) d.b.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>initial</td>
<td>final</td>
<td>initial</td>
<td>final</td>
</tr>
<tr>
<td>14</td>
<td>5.02 ± 0.03</td>
<td>5.18 ± 0.07</td>
<td>40.62 ± 0.47</td>
<td>39.98 ± 0.24</td>
</tr>
<tr>
<td></td>
<td>4.96 ± 0.02</td>
<td>4.93 ± 0.04</td>
<td>34.48 ± 1.47</td>
<td>35.43 ± 0.78</td>
</tr>
<tr>
<td></td>
<td>4.94 ± 0.01</td>
<td>4.90 ± 0.01</td>
<td>35.69 ± 0.47</td>
<td>36.19 ± 0.59</td>
</tr>
<tr>
<td></td>
<td>5.00 ± 0.02</td>
<td>4.91 ± 0.02</td>
<td>36.61 ± 0.29</td>
<td>35.25 ± 0.68</td>
</tr>
</tbody>
</table>

Initial vs. final\(^[a]\) ns ns ns ns

<table>
<thead>
<tr>
<th>Mean ± Std. Dev.(^[b])</th>
<th>initial</th>
<th>final</th>
<th>initial</th>
<th>final</th>
<th>initial</th>
<th>final</th>
<th>initial</th>
<th>final</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>4.98 ± 0.04a</td>
<td>4.98 ± 0.13a</td>
<td>36.85 ± 2.66a</td>
<td>36.71 ± 2.22a</td>
<td>10.43 ± 1.67a</td>
<td>11.65 ± 3.36a</td>
<td>48.14 ± 1.47a</td>
<td>46.66 ± 1.87a</td>
</tr>
<tr>
<td>18</td>
<td>4.92 ± 0.05</td>
<td>5.00 ± 0.07</td>
<td>39.83 ± 0.31</td>
<td>40.21 ± 0.14</td>
<td>6.22 ± 0.23</td>
<td>8.79 ± 0.51</td>
<td>49.04 ± 0.46</td>
<td>46.60 ± 0.41</td>
</tr>
<tr>
<td>22</td>
<td>5.09 ± 0.05</td>
<td>5.11 ± 0.06</td>
<td>41.56 ± 0.78</td>
<td>40.74 ± 0.46</td>
<td>11.22 ± 0.37</td>
<td>13.23 ± 0.36</td>
<td>42.13 ± 1.08</td>
<td>40.91 ± 0.52</td>
</tr>
</tbody>
</table>

Initial vs. final\(^[a]\) ns ns ns

<table>
<thead>
<tr>
<th>Mean ± Std. Dev.(^[b])</th>
<th>initial</th>
<th>final</th>
<th>initial</th>
<th>final</th>
<th>initial</th>
<th>final</th>
<th>initial</th>
<th>final</th>
</tr>
</thead>
<tbody>
<tr>
<td>18</td>
<td>4.97 ± 0.06a</td>
<td>5.00 ± 0.05a</td>
<td>37.05 ± 1.79a</td>
<td>37.13 ± 1.76a</td>
<td>13.27 ± 4.48a</td>
<td>14.33 ± 3.46a</td>
<td>44.71 ± 3.13a</td>
<td>43.55 ± 1.85ab</td>
</tr>
<tr>
<td>22</td>
<td>5.05 ± 0.08a</td>
<td>5.05 ± 0.04a</td>
<td>39.20 ± 2.50a</td>
<td>39.12 ± 2.45a</td>
<td>12.70 ± 3.42a</td>
<td>16.03 ± 3.44a</td>
<td>43.05 ± 2.80a</td>
<td>39.80 ± 2.25b</td>
</tr>
</tbody>
</table>

\(^[a]\) Results of paired t-test for initial versus final content of each component (ns = not significant at \(p < 0.05\)).

\(^[b]\) Means followed by the same letter in a column were not different from each other \((p < 0.05)\) per individual proximate analysis \((A, P, F, \text{ or } C)\) by Tukey test.
Table 5.6. Changes in carbohydrates compared to reached DML of soybeans with 14, 18, and 22% moisture content stored at 30°C.

<table>
<thead>
<tr>
<th>Moisture content, (w, % w.b.)</th>
<th>Dry matter loss (DML, %)[a]</th>
<th>Change in carbohydrates (ΔC, % d.b.)[b]</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>0.16</td>
<td>0.80</td>
</tr>
<tr>
<td></td>
<td>0.06</td>
<td>-1.54</td>
</tr>
<tr>
<td></td>
<td>0.08</td>
<td>-0.42</td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>-4.77</td>
</tr>
<tr>
<td>Mean ± Std. Deviation[c]</td>
<td>0.09 ± 0.05</td>
<td>-1.48 ± 2.39</td>
</tr>
<tr>
<td>18</td>
<td>0.53</td>
<td>-3.04</td>
</tr>
<tr>
<td></td>
<td>0.33</td>
<td>-1.80</td>
</tr>
<tr>
<td></td>
<td>0.60</td>
<td>-2.24</td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>0.38</td>
</tr>
<tr>
<td></td>
<td>0.28</td>
<td>0.91</td>
</tr>
<tr>
<td>Mean ± Std. Deviation[c]</td>
<td>0.40 ± 0.16</td>
<td>-1.16 ± 1.56</td>
</tr>
<tr>
<td>22</td>
<td>1.35</td>
<td>-1.22</td>
</tr>
<tr>
<td></td>
<td>1.04</td>
<td>-3.36</td>
</tr>
<tr>
<td></td>
<td>1.03</td>
<td>-1.95</td>
</tr>
<tr>
<td></td>
<td>1.04</td>
<td>-9.86</td>
</tr>
<tr>
<td>Mean ± Std. Deviation[c]</td>
<td>1.12 ± 0.16</td>
<td>-3.25 ± 5.00</td>
</tr>
</tbody>
</table>

[a] DML achieved after established time of respiration test (20 d for 14 and 18% m.c.; 1% DML for 22% m.c.).
[b] Final minus initial carbohydrates concentration, dry basis.
[c] Means ΔC values represented in Figure 5.5 as Δ14, Δ18, and Δ22.

Figure 5.5. Comparison between average increase in dry matter loss (DML, %) and changes in carbohydrates (ΔC) before and after respiration test for soybeans at 14, 18, and 22% m.c. (Δ14, Δ18, and Δ22, respectively).
Overall, the preliminary lipid oxidation tests did not result in an apparent trend comparing soybeans samples before and after respiration tests at 30°C (Table 5.7 and Figure 5.6). Final $PV$ was 7.5 mEquiv per kg sample for 14% m.c. at 0.16% $DML$, 12.7 for 18% m.c. at 0.53% $DML$, and an average of 21.23 mEquiv per kg sample for 22% m.c. at mean $DML$ of 1.2%. A $PV$ threshold of 10 mEquiv per kg lipid for refined oil was established as a quality indicator by the Joint FAO/WHO Codex Alimentarius Commission (2009), based on correlation with rancid off-flavors (Patterson, 2011). Al-Kahtani (1989) and Anwar et al. (2016) reported crude oil $PV$ for soybeans with 5.73 to 10.20% m.c. between 1.80 to 5.40 mEquiv per kg lipid. The $PV$ values from literature cannot be directly compared to the values in this study, however. The correlation coefficient between $DML$ and $∆PV$ from Table 5.8 was 0.35 ($p = 0.65$). This lack of correlation may be explained by the fact that $PV$ oscillates during the oxidation pathway (Fennema, 1996).

In terms of secondary compounds, values were between 12 to 27 mmol TBARS per g of sample (Table 5.7 and Figure 5.6). TBA values increased by 9 mmol TBARS per g of sample between initial and final respiration test samples at 14% m.c. for 0.16% $DML$. TBA values only increased 2 mmol at 18% m.c. for 0.53% $DML$, and 5 mmol TBARS per g of sample at 22% m.c. for 1.35 and 1.04% $DML$. This increase in $TBA$ after respiration tests shows that the lipid of these samples went through secondary oxidation stages. The correlation coefficient between $DML$ and $∆TBA$ from Table 5.8 was -0.38 ($p = 0.62$). This lack of correlation may also be explained by the oscillatory production of hydroperoxides over time and their fast decomposition (Fennema, 1996), which directly affects the production of secondary compounds over time. Therefore, to improve the comparison between primary and secondary lipid oxidation products it would be of interest to run those tests at several time points; however, the D-GRMS was not designed to allow a collection of samples during the respiration test. Therefore, for the remaining samples from the other respiration test replications were not send for lipid oxidation tests.
Table 5.7. Products of lipid oxidation for soybeans samples before and after respiration tests at 14, 18, and 22% moisture content stored at 30°C.

<table>
<thead>
<tr>
<th>Moisture content (w, % w.b.)</th>
<th>Peroxide Value (mEquiv per kg sample ± Std. Dev.)</th>
<th>Thiobarbituric acid (mmol TBARS per g sample ± Std. Dev.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>initial final</td>
<td>initial final</td>
</tr>
<tr>
<td>14</td>
<td>18.17 ± 2.93 7.47 ± 0.84</td>
<td>12 ± 1.5 21 ± 1.5</td>
</tr>
<tr>
<td>18</td>
<td>11.51 ± 2.49 12.70 ± 0.09</td>
<td>19 ± 0.5 21 ± 0.5</td>
</tr>
<tr>
<td>22</td>
<td>23.98 ± 3.91 16.94 ± 1.82</td>
<td>22 ± 0.5 27 ± 1.5</td>
</tr>
<tr>
<td>22</td>
<td>4.71 ± 0.75 25.52 ± 3.36</td>
<td>18 ± 2.0 23 ± 1.0</td>
</tr>
</tbody>
</table>

Figure 5.6. Comparison between increase in dry matter loss (DML, %) and lipid oxidation products before and after respiration test based on peroxide value (PV, mEquiv per kg sample) and thiobarbituric acid (TBA, mmol TBARS per g of sample).

Table 5.8. Change in peroxide value and thiobarbituric acid compared to reached DML of soybeans with 14, 18, and 22% moisture content stored at 30°C.

<table>
<thead>
<tr>
<th>Moisture content (w, % w.b.)</th>
<th>Dry matter loss (DML, %)</th>
<th>Change in peroxide value (ΔPV, mEquiv per kg sample)</th>
<th>Change in thiobarbituric acid (ΔTBA, mmol TBARS per g sample)</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>0.16</td>
<td>-10.7</td>
<td>9</td>
</tr>
<tr>
<td>18</td>
<td>0.53</td>
<td>1.19</td>
<td>2</td>
</tr>
<tr>
<td>22</td>
<td>1.35</td>
<td>-7.04</td>
<td>5</td>
</tr>
<tr>
<td>22</td>
<td>1.04</td>
<td>20.81</td>
<td>5</td>
</tr>
</tbody>
</table>
5.4. Conclusion

*DM* rates ($v_{DML}$) increased with increasing moisture content (14, 18, and 22%) for soybeans stored at 30°C in a dynamic grain respiration measurement system (D-GRMS). $v_{DML}$ increased 4-fold for soybeans at 18% m.c. compared to 14% and 14 times for beans at 22% m.c. compared to 14%. Estimated safe storage time from this study is 2.5 to 4 times greater than reported values based on corn isotherms and equivalent water activity. Based on proximate analysis, carbohydrate concentration loss during respiration was not correlated with *DML* ($r = -0.40$ and $p = 0.18$) to *DML*. Lipid oxidation tests were only able to indicate that the first and second stages of oxidation were present during the respiration tests and that the products had been accumulating over time.
CHAPTER 6. SUMMARY, CONCLUSIONS AND FUTURE WORK

The main purpose of this thesis was to compare the effect of different storage conditions on dry matter loss ($DML$) of soybeans by measuring respiration. The two grain respiration measurement methods developed and used were static grain respiration measurement system (S-GRMS) and dynamic grain respiration measurement system (D-GRMS), which simulate hermetic and aerated storages, respectively. The static respiration measurements were based on soybeans with 18% moisture content (m.c.) stored at temperature ($T$) of 30 and 35°C with four split beans contents ($x_s$); the dynamic respiration measurements were based on three levels of $w$ and 30°C. Chapter 3 provides a comparison of dry matter loss rates ($v_{DML}$) of 18% m.c. soybeans stored at 30°C from both static and dynamic grain respiration measurement system. Chapter 4 provides $v_{DML}$ of 18% m.c. soybeans with 0, 4, 8, and 16% (w/w) $x_s$ stored at 35°C in S-GRMS to develop a damage multiplier ($M_D$). The last chapter (Chapter 5) provides estimates of $v_{DML}$ for $DML$ measurements on soybeans at 14, 18, and 22% m.c. and 30°C in D-GRMS. Samples before and after each respiration test from this chapter were analyzed for compound partitioning with proximate analysis, and lipid oxidation with peroxide value ($PV$) and thiobarbituric acid ($TBA$) value. Based on $v_{DML}$ from D-GRMS at 30°C, safe storage time table was estimated based on time to reach 0.5% $DML$ ($t_{0.5}$).

The main objective of this thesis was to better understand the effects of GRMS, damage, and $w$ on $v_{DML}$ of soybeans at elevated temperatures. The major findings of the research were:

- $v_{DML}$ estimates of 18% m.c. soybeans at 30°C were 1.20 times lower (but not significant) when using a S-GRMS than a D-GRMS.
- $v_{DML}$ increased 1.10 to 1.70-fold as $x_s$ increased from 0% splits to 4 and 16%, respectively, but no significant difference in $v_{DML}$ was found between 8 and 16% splits. Mean $M_D$ ranged from 1.0 to 0.6 as $x_s$ increased from 0 to 16% (w/w) and was found to be 1.25 times more sensitive to $x_s$ than $M_D$ of corn.
- Soybeans stored at 30°C in a D-GRMS presented a significant increase in $v_{DML}$ with increasing $w$. Mean rates of 0.0047, 0.0189 and 0.0645% d$^{-1}$ for 14, 18 and 22% m.c. represents 4-fold increase in $v_{DML}$ for 18% m.c. compared to 14% and 14 times greater for 22% m.c. compared to 14%.
In addition, decreases in carbohydrates content of the soybeans after respiration testing did not corroborate increasing $DML$ ($p = 0.18$) and results from lipid oxidation tests showed that oxidation did occur during respiration testing, but single measurements of $PV$ and $TBARS$ were not directly correlated to final $DML$ ($p = 0.65$ and $0.62$, respectively). Estimates of safe storage time, adjusted for initial lag time ($t_{0.5} - t_{0.05}$), from the $v_{DML}$ at $30^\circ C$ showed that clean, undamaged, whole soybeans at 14 to 22% m.c. could be stored for 91 to 7 d, respectively.

For future work, in order to complete a MAST guideline for both static and dynamic storage systems, more data need to be collected for a wide range of $w$, $T$ and $x_s$. Given the large differences between $v_{DML}$ at $30^\circ C$ in this study and $35^\circ C$ found by Trevisan (2017), it is recommended that the next respiration test $T$ in a D-GRMS be between this range with the same levels of $w$ (14, 18, and 22%), for example, $32^\circ C$. After these high temperature storage conditions, it would be of interest to conduct several tests at lower $T$, such as $20^\circ C$ and $25^\circ C$. However, it is unclear if the D-GRMS methodology will be able to measure such low levels of respiration rates because of complications with mold growth and time to achieve $0.05\% DML$.

During respiration tests in a D-GRMS, 5 daily measurements are not needed if the dehumidifier and CO$_2$ scrubbers are checked frequently for saturation. With enough data for different $T$ and $w$, then it would be possible to develop multipliers for both conditions ($M_T$ and $M_w$) which accommodates their interactions. To evaluate the split beans content ($x_s$) effect on safe storage time ($t_s$), it will be important to run a few respiration tests in D-GRMS to see if it is possible to establish a relationship with the damage multiplier ($M_D$) found in this thesis using a S-GRMS. Beyond $x_s$ reported in this study, it would be interested to test contents equivalent to those at the USDA standard (10, 20, 30, and 40%). It would be also important to conduct respiration tests on different hybrids of soybeans, and soybeans with and without fungicide treatment, to develop two other multiplier ($M_H$ and $M_F$) contributors to estimate $t_s$. It is recommended to use the S-GRMS to develop these multipliers because four replications can be run at the same time. Then, like the $M_D$, a factor can be established between the static and dynamic systems. After finding the five multipliers, a mathematical model can be developed to estimate $t_s$ for soybeans stored at any $T$, $w$, $x_s$, hybrid and treated or not with fungicide and then develop a corresponded MAST guideline. This could be a useful contribution to the soybean industry.
Besides $t_s$, the quality degradation of stored soybeans is an important characteristic to be measured. The attempts in this thesis to evaluate lipid oxidation based on $PV$ and $TBA$ showed that these tests can quantify the oxidation level only if samples are collected over time during each respiration test. However, the D-GRMS was not designed for repeated sampling during a test. Therefore, it would be interesting to determine free fatty acids (FFA) of the samples before and after each respiration test to assign lipid degradation in terms of basic lipid hydrolysis. This is because FFA also oxidizes to form secondary oxidation compounds. The price for FFA test is not as high as the $PV$ and $TBA$ tests. It would be important to evaluate mold and microorganism growth in the samples, because part of the respiration rate is contributed by this microbial growth. This evaluation can be done by using test kits that are not expensive. Because soybeans are also the main source of feed protein, some analysis to evaluate protein degradation could improve the quality analysis. One possible method to analyze protein degradation would be the sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), which evaluates the molecular mass of the different proteins.

With a complete MAST guideline for soybeans based also on quality characteristics, it would be interest to contact ASABE and offer the data to create a standard of soybeans storage time for 0.5% $DML$. This way, the access of this standard would be open for professionals from the field. The next steps after concluding a MAST guideline that includes different quality parameters for safe storage of soybeans, it would be to use the protocols from both S- and D-GRMS to conduct respiration tests from other commodities, such as wheat or coffee.
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Steele, J. L. (1967). *Deterioration of damaged shelled corn as measured by carbon dioxide production.* Ph.D. diss. Ames, IA, USA: Iowa State University, Department of Agricultural Engineering.


APPENDIX A. OVERVIEW OF DESIGN AND OPERATION: STATIC GRAIN RESPIRATION MEASUREMENT SYSTEM (S-GRMS)

A.1. S-GRMS design, calibration, and CO₂ stratification

A.1.1. Overview of the system design

Each S-GRMS experimental unit (Figure A.1) consists of a temperature-controlled hermetically sealed respiration chamber (RC) housing a sensor package with an internal data logger (Catalog No. K33-BLG, CO2Meter, Inc., Ormond Beach, FL, USA) suspended on top of 500 g grain sample. The RC was a 10 L glass desiccator (Figure A.2a), and the sensor package (Figure A.2c) monitored CO₂ concentration (C_{CO₂}, \%), temperature (T, °C) and relative humidity (ϕ, %RH) of the grain sample (Figure A.2b). The experimental unit was acclimated inside a temperature-controlled incubator (Figure A.3, Model No. 3033, Steri-Culti 200, Forma Scientific, Inc., Marietta, OH, USA) at 35°C.

![Figure A.1. Grain respiration measurement system (S-GRMS) experimental unit.](image-url)
Figure A.2. Components of S-GRMS experimental unit: desiccator (a), soybean sample (b) and sensor package (c).

Figure A.3. Temperature-controlled incubator located at room 102 Burnside Research Laboratory.
A.1.2. Calibration of sensors

This is a brief description of the calibration of sensors used in S-GRMS (Appendix A of ASABE paper number 1700075). For a full description of the calibration protocol and procedure please refer to Document No. S-GRMS-001 from the Appendix A.2.

Materials

To develop a calibration curve for each of the four sensor packages with an internal data logger (CO2Engine™ Model No. K33-BLG, CO2Meter Inc, Ormond Beach, FL, USA), the following materials are necessary:

- Certified CO₂ calibration gas at different concentrations Compressed gas regulator
- Nitrogen gas for zero
- Flow meter
- Tubing (1/4 in ID)
- Resealable plastic bag
- Zip tie
- Standard SenseAir cable
- DAS (Data Acquisition Software, CO2 Meter Inc, Ormond Beach, FL, USA)

The first calibration curve was developed with five certified standard CO₂ compressed gas cylinders and the Nitrogen cylinder was used to calibrate to zero concentration. The six certified calibration gases, all from Airgas®, St. Louis, MO, USA, were:

1. 10% CO₂ concentration gas cylinder (uncertainty ± 2%, actual concentration 9.972%);
2. 5% CO₂ concentration gas cylinder (uncertainty ± 2%, actual concentration 5.017%);
3. 1% CO₂ concentration gas cylinder (uncertainty ± 2%, actual concentration 0.999%);
4. 0.5% CO₂ concentration gas cylinder (uncertainty ± 2%, actual concentration 0.5103%);
5. 0.15% CO₂ concentration gas cylinder (uncertainty ± 2%, actual concentration 0.1487%);
6. 0% CO₂ concentration (100% N₂ gas).

The second calibration curve was also developed with five gas cylinders and Nitrogen, however, the only change on gas cylinders was for 0.15% and 10% CO₂ concentration with actual concentration of 0.1493% and 9.980%, respectively.

Methods

A reference gas flow rate of 1 L/min for 20 min was applied to all four K33-BLG sensors placed in sealed plastic bag. \( C_{CO_2} \) measurements were recorded every 20 s and the mean of the values during the “stabilized period” (from 8 min and 20 s to 9 min and 20 s) was calculated and used to build a calibration curve. A simple linear regression model for each sensor was determined using the Data Analysis ToolPak in MS Excel (Version 2016, Microsoft Corp., Redmond, WA) where \( y \) was the averaged CO₂ measured values and \( x \) the certified CO₂ concentration.

Results

The table A.1 and A.2 summarizes the calibration curve equations for the range of 0-5% and 0-10% of the four sensors.
Table A.1. First calibration curve equations for four K33-BLG sensors used at 35°C. Units for intercept and standard error (SE) are percent CO₂, and slope is dimensionless.

<table>
<thead>
<tr>
<th>Calibration curve (0-5%)</th>
<th>Calibration curve (0-10%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slope</td>
<td>Intercept</td>
</tr>
<tr>
<td>Sensor A</td>
<td>0.9601</td>
</tr>
<tr>
<td>Sensor C</td>
<td>0.9025</td>
</tr>
<tr>
<td>Sensor D</td>
<td>1.0089</td>
</tr>
<tr>
<td>Sensor E</td>
<td>1.0051</td>
</tr>
</tbody>
</table>

[^a]Standard error of regression.

Table A.2. Second calibration curve equations for four K33-BLG sensors used in chapter 3 at 30°C. Units for intercept and standard error (SE) are percent CO₂, and slope is dimensionless.

<table>
<thead>
<tr>
<th>Calibration curve (0-5%)</th>
<th>Calibration curve (0-10%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slope</td>
<td>Intercept</td>
</tr>
<tr>
<td>Sensor A</td>
<td>0.9800</td>
</tr>
<tr>
<td>Sensor C</td>
<td>0.9719</td>
</tr>
<tr>
<td>Sensor D</td>
<td>0.8729</td>
</tr>
<tr>
<td>Sensor E</td>
<td>1.0629</td>
</tr>
</tbody>
</table>

[^a]Standard error of regression.

A.1.3. Stratification of CO₂ inside the RC in a S-GRMS

A respiration test was conducted using two sensors inside a desiccator – one suspended above the soybean sample and the other placed in the plenum, below the grain where CO₂ was assumed to accumulate. At this desiccator, the sensor above the grain was connected to a 0.5 L min⁻¹ diaphragm pump (Catalog No. CM-0111, CO2Meter, Inc., Ormond Beach, FL, USA), whose inlet was at the bottom, which mixed air inside the desiccator.

Results

Results from the test showed that there was no stratification of CO₂ inside the chamber when using the diaphragm pump. DML estimates from the sensors positioned above and below the grain showed no difference (Figure A.4). The sensor placed above the soybean sample had \( v_{DML} \) of 0.0198% d⁻¹, while the rate for sensor below was 0.0197% d⁻¹. The same results were found at the test without a pump. The shape of the DML vs. t curves and magnitudes were
similar to those from the first test. Hence, the static system and test protocol used during the first test was adequate and may be used for future static respiration tests.

Figure A.4. Dry matter loss estimates (DML, %) above and below soybean sample placed in a S-GRMS respiration chamber.
A.2. *Static respiration test at 18% moisture content and 35°C.*

Five replicated respiration tests were conducted in each S-GRMS experimental unit. The system conditioned 18% w soybean samples in a controlled $T$ of 35°C. The $RC$ from a S-GRMS experimental unit stored 500 g of sample.

At the start of each test, $DML$ was relatively low for about 4 d and increased exponentially between 4 to 6 d (Figure A.5). This behavior was noted also by Rukunudin et al. (2004) on 9 to 22% w soybeans stored in a D-GRMS. However, studies in different static systems from Ochandio et al. (2012) and Jian et al., 2014 did not present high values for $DML$, and a lag period was not reported. The relatively steady increase after 5 to 6 d was due to microbial, specifically mold, growth on the grain, which was also observed by Rukunudin et al. (2004). They reported visible mycelial growth after 4 to 13 days of storage, depending on whether their soybeans were combine-harvested or hand-harvested. Combine-harvested soybeans exhibited faster visible mold growth than hand-harvested beans and the dominant mold species were field fungi. The range in $DML$ was 0.10 to 0.17% for 18% w soybeans stored in the static system for 10 days.

![Figure A.5. Dry matter loss estimates ($DML$, %) over time ($t$, d) of 18% m.c. soybeans at 35°C in static grain respiration measurement systems (S-GRMS).](image_url)
After \( t_{0.05} \), estimates of \( v_{\text{DML}} \) (Table A.3) for 18% moisture soybeans stored in S-GRMS ranged from 19.6 to 28.5 (10\(^{-3}\) % d\(^{-1}\)) with a \( \overline{v_{\text{DML}}} \) and \( (\sigma_{v_{\text{DML}}})_p \) of 24.1 ± 0.05 (10\(^{-3}\) % d\(^{-1}\)).

Table A.3. Dry matter loss rates of 18% moisture soybeans stored at 35°C in static and dynamic grain respiration measurement systems.

<table>
<thead>
<tr>
<th>Replication</th>
<th>Dry matter loss rate and Std. Error, ( v_{\text{DML}} \pm SE_{v_{\text{DML}}} ) (% d(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.0237 ± 0.00004</td>
</tr>
<tr>
<td>2</td>
<td>0.0285 ± 0.00006</td>
</tr>
<tr>
<td>3</td>
<td>0.0256 ± 0.00003</td>
</tr>
<tr>
<td>4</td>
<td>0.0230 ± 0.00005</td>
</tr>
<tr>
<td>5</td>
<td>0.0196 ± 0.00004</td>
</tr>
</tbody>
</table>

Mean and Pooled Std. Deviation, \( \overline{v_{\text{DML}}} \pm (\sigma_{v_{\text{DML}}})_p \) 0.0241 ± 0.00005
A.3. Standard Operating Procedures (SOPs)

<table>
<thead>
<tr>
<th>University of Illinois at Urbana-Champaign</th>
<th>Title: Calibrating K33-BLG sensor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effective date: 09 October 2018</td>
<td>Document No. S-GRMS-001</td>
</tr>
<tr>
<td>Written by: A. B. P. da Silva</td>
<td>Approved by: R.S. Gates (supervisor)</td>
</tr>
</tbody>
</table>

1.0. PURPOSE

This SOP explains the protocol for calibrating the sensor package (Model K33-BLG, CO2Meter, Inc., Ormond Beach, FL, USA) prior a S-GRMS.

2.0. SCOPE

This SOP describes how to calibrate the sensor package with built-in data logger via DAS (Data Acquisition Software, CO2 Meter Inc, Ormond Beach, FL, USA) and create its calibration curve to adjust the measured values with their reference gas.

3.0. RESPONSIBILITY

The supervisor will be responsible for training the personnel on proper procedures to calibrate the K33-BLG sensor and to implement this procedure.

4.0. MATERIALS AND EQUIPMENT

4.2. Different CO₂ concentrations certified gas cylinders, all from Airgas®, St. Louis, MO, USA:
   - 10% CO₂ concentration gas cylinder (uncertainty ± 2%, actual concentration 9.972%);
   - 5% CO₂ concentration gas cylinder (uncertainty ± 2%, actual concentration 5.017%);
   - 1% CO₂ concentration gas cylinder (uncertainty ± 2%, actual concentration 0.999%);
   - 0.5% CO₂ concentration gas cylinder (uncertainty ± 2%, actual concentration 0.5103%);
   - 0.1% CO₂ concentration gas cylinder (uncertainty ± 2%, actual concentration 0.1487%);
   - 0% CO₂ concentration gas cylinder (100% N₂ gas).
4.3. Compressed gas regulator (Model HPT270-125-580-DK2S, UL® Listed, USA).
4.4. Flowmeter (Model No. RMA-13-SSV, Dwyer®, Michigan City, IN, USA).
4.5. Vincon Flexible PVC tubing – 6.35 mm (0.25 in) ID (Part No. ABH02017, Saint-Gobain, Akron, OH, USA).
4.6. Resealable plastic bag (1 gallon).
4.7. Zip ties.
4.9. DAS (Data Acquisition Software, CO2 Meter Inc, Ormond Beach, FL, USA).

5.0. PROCEDURES: ZERO CALIBRATION

5.1. Assemble the 0% CO₂ gas cylinder to the compressed gas regulator (Figure A.6) and connect it to the flow meter with PVC tubing (Figure A.7).

Figure A.6. Assembling the gas cylinder (a) with a gas regulator installed (b).

Figure A.7. Flowmeter inlet (a) connects to the gas regulator and outlet (b) to be used in the calibration.
5.2. The zero calibration needs to be done individually for each sensor. So, connect one powered sensor to the SenseAir cable and connect the cable to a USB port in the computer running the DAS software installed (Figure A.8).

5.3. The DAS software detects the sensor when the sensor is connected. To start the communication with the sensor, double-click on the device name at the devices box (Figure A.9).

5.4. Click on Configure Sensor to open the main tab in the settings panel (Figure A.10) and make sure the calibration box is set to Nitrogen Source instead of 400 ppm Air Source.
5.5. Place the connected and powered sensor and the flowmeter outlet inside a resealable plastic bag. Close the plastic bag and zip tie the tubing and cable (Figure A.11a). Leave a small opening for the gas exit the bag. Afterwards, open the compressed gas regulator filling the bag with Nitrogen (Figure A.11b). Set the flowmeter to 10cc/min x100 after filling the bag.

Figure A.11. Powered sensor and flowmeter outlet placed inside the resealable plastic bag (a) tied with a zip tie and bag filled with Nitrogen (b).
5.6. Back to the settings panel click on **Initiate Test Measurement** and let the gas flow for 20 minutes inside the bag.

5.7. When the CO₂ reading value is varying only about ± 0.02%vol CO₂ (i.e. 200 ppm) it is time to click **Calibrate** from the calibration box.

5.8. The sensor will be calibrated, so close the gas regulator, open the bag and repeat the Steps 5.2 to 5.6 for every sensor that will be calibrated.

6.0. **PROCEDURE: CALIBRATION CURVE**

6.1. Data from each gas cylinder needs to be recorded to build the calibration curve of each sensor. Therefore, the recording will need to be individual by gas but with as many sensors as you want to create the curves.

6.2. First, repeat the Steps 5.2 to 5.4 to connect a sensor.

6.3. Set each sensor log interval to 20 s by selecting the sensor logs tab from the settings panel filling the log interval with 20 s and clicking “Set” (Figure A.12a). Now synchronize the sensor time by clicking “Sync” (Figure A.12b). Then click in “Clear/Reset Log Memory” (Figure A.12c) to delete any old data saved on the memory.

![Figure A.12. Settings panel Sensor Logs tab commands to log interval (a), sensor time (b), and clear/reset log memory (c).](image)

6.4. Repeat Steps 6.2 to 6.4 to every sensor for which a calibration curve will be performed.

6.5. Repeat Step 5.1 to assemble the desired gas cylinder to initiate data logging.

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6.6. Set the jumper (Figure A.13) of all the configured sensors. The heartbeat LED of the sensor will light up to show that the sensor is powered and that the data collection has started.

![Image of sensor with jumper set](image)

**Figure A.13. Setting the jumper of a sensor to initiate data collection.**

6.7. Place all the sensors inside the resealable plastic bag with the flowmeter tubing outlet, close the bag and tie with a zip tie the tubing (Figure A.14a).

6.8. Open the gas regulator until the bag is completely inflated with gas (Figure A.14b), then set the flow to 1 L/min.

![Figure A.14(a) and A.14(b)](image)

**Figure A.14. Powered sensors and flowmeter tubing outlet placed and tied in a sealed plastic bag (a) inflated afterwards with desired gas (b).**

6.9. After 25 minutes data logging, close the compressed gas regulator, open the bag and change the gas cylinder to start recording a new CO₂ concentration.

6.10. Repeat Steps 6.5 to 6.9 for every CO₂ concentrated gas cylinder a calibration curve will be based on.

6.11. Connect one sensor at time on the computer repeating Steps 5.2 and 5.3 and click [Manage and Download Logs](#).
6.12. Then at the download panel, the logs by date and time can be selected and saved by clicking.

6.13. The data will be saved on the DAS Logs folder inside the Documents folder in the computer. It is important to double check that the data were saved inside the folder as a “.das” data file.

6.14. To convert the data from “.das” to “.csv” go back to the DAS window, click on (Open Data File) and sequentially click on (Export to Spreadsheet button). Repeat this step for every file saved.

6.15. Repeat Steps 6.11 to 6.13 for every sensor for which data were logged.

6.16. Open an Excel workbook and save the data of each sensor in a different tab. Plot the recorded data and check the time that the measurements started to be stable, generally after about 5 min.

6.17. Add a new tab worksheet to calculate the mean of the concentration value recorded by each sensor after the stable time interval ($\overline{C_{CO_2}}$ measured). Add a new column with the reference value of CO$_2$ concentration from the certified gas cylinder ($C_{CO_2}$ reference value). The table below is an example for one sensor.

**Table A.4. Example of calibration curve points of sensor A.**

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>$\overline{C_{CO_2}}$ measured (%)</th>
<th>$C_{CO_2}$ reference value (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 to 21.67</td>
<td>9.818</td>
<td>9.972</td>
</tr>
<tr>
<td>5 to 21.67</td>
<td>4.862</td>
<td>5.017</td>
</tr>
<tr>
<td>5 to 21.67</td>
<td>0.999</td>
<td>0.999</td>
</tr>
<tr>
<td>5 to 21.67</td>
<td>0.524</td>
<td>0.5103</td>
</tr>
<tr>
<td>5 to 21.67</td>
<td>0.189</td>
<td>0.1487</td>
</tr>
<tr>
<td>5 to 21.67</td>
<td>0.038</td>
<td>0</td>
</tr>
</tbody>
</table>

6.18. Create a scatterplot with y data as $\overline{C_{CO_2}}$ measured and x data as $C_{CO_2}$ reference value and add the regression line and equation (Figure A.2.10) of the data. The calibration equation will be used to adjust the data collected by the sensor during S-GRMS.

6.19. For each regression equation, the measured value is going to be corrected by inverting the regression in the electronic datasheet as:

$$C_{CO_2} = \left(\frac{C_{CO_2} measured - slope}{intercept}\right)$$

6.20. Repeat Steps 6.16 and 6.17 for every sensor that a calibration curve is desired to be used in S-GRMS.
7.0. CORRECTIVE ACTION

7.1. Pay attention to sensor heartbeat LED when the jumper is set or when the sensor is connected to the computer if the light is not flashing something is wrong with the data logger. If the heartbeat LED is not working properly notify supervisor immediately.

7.2. Pay attention to each sensor package’s data logger data. If the data are showing a different behavior while collecting data notify supervisor immediately and start checking if any connection was wrong.

7.3. It is important to not touch the heartbeat LED of the sensor. This part is sensitive and can damage the sensor. If any damage was caused to the sensor notify the supervisor immediately.

7.4. The supervisor will take further corrective actions which may include repairing or replacing the sensors.

8.0. CHANGES FROM PREVIOUS VERSION

8.2. Reviewed, revised, and approved by supervisor on 09 October 2018.
1.0. PURPOSE
This SOP explains the protocol for preparing the soybeans sample for a grain respiration test in a S-GRMS.

2.0. SCOPE
This SOP describes how to clean and re-wet soybeans for a grain respiration test.

3.0. RESPONSIBILITY
The supervisor will be responsible for training the personnel on proper use of S-GRMS and its components, preparing samples, and implementing this protocol/procedure.

4.0. MATERIALS AND EQUIPMENT
4.1. Refrigerated soybeans sample (about 3000 g for 4 desiccators).
4.2. Aluminum grain sieve (Grainman 10/64” x 3/4”, Miami, FL, USA) to remove impurities and split or damaged beans.
4.3. Portable moisture meter (Model No. SW16060, John Deere, Moline, IL, USA).
4.4. Roller mixers (qty = 2; Model No. MX-T6-S, Scilogex, Rocky Hill, CT, USA).
4.5. Digital precision scale range of 0 to 3100 g and 0.01 g resolution (Model iBalance i3100, MyWeigh, Phoenix, AZ, USA).
4.6. 2 L capacity plastic bottles (qty = 2; Model No. 2202-0005, U.S. Plastics, Lima Ohio, OH, USA) wrapped with 3 rubber bands to increase friction.
4.7. 100 mL capacity glass beaker (qty = 2).
4.8. 32 oz polyethylene funnel 6-3/8” Dia. x 7-1/8”H, (Model No. 832WN, U.S. Plastics, Lima Ohio, OH, USA).
4.9. Aluminum tray (92 x 62 x 3 cm).
4.10. Digital compact timer (Product No. 5806, Taylor, Oak Brooks, IL).
4.11. Deionized water.

5.0. PROCEDURES
5.1. Before starting to prepare the soybean sample, print one of the respiration test datasheets found in Section A.3. Static Respiration Test Datasheets. The datasheet type will depend on the respiration test, with or without split beans. If the respiration test will have split beans, follow the Document No. S-GRMS-003 before continuing this protocol.
5.2. Remove the soybean sample from the refrigerated storage.
5.3. Weigh the sample and write down the initial weight and the acclimation start time on the datasheet.
5.4. Clean the beans to remove foreign materials, splits, broken, and damaged seeds using the sieve.
5.5. Let the beans acclimate at room temperature for 20-30 min in the aluminum tray.
5.6. Check the moisture content of the cleaned sample with a moisture meter to see if the sample will need to be re-wetted. Follow the next steps if the moisture content is lower than desired or continue this protocol from Step 5.18.
5.7. Weigh two separate aliquots of 1,250 g, write down the measurement value \( m_{soy,0} \) for the assigned bottle (A or B) on the datasheet.
5.8. In triplicates, estimate the soybean moisture content per bottle using the portable digital moisture meter and write down on the datasheet the average measurement \( \bar{w}_{soy,0} \).
5.9. Calculate the amount of deionized water per bottle \( m_{H_2O} \) needed to achieve the desired moisture content \( \bar{w}_{soy,1} \) using the following equation:

\[
m_{H_2O} = m_{soy,0} \left( \frac{\bar{w}_{soy,1} - \bar{w}_{soy,0}}{100 - \bar{w}_{soy,1}} \right)
\]
5.10. Each bottle will have its \( m_{H_2O} \) calculated according to its individual soybeans sample weight and moisture content.
5.11. Write down the necessary \( m_{H_2O} \) for bottle A and B in the datasheet.
5.12. Place bottles on their sides on the roller mixer set it to 60 rpm and set the timer to 60 min.
5.13. Every 10 min add other aliquots of \( m_{H_2O} \) until the needed \( m_{H_2O} \) for each bottle has been added, replacing the bottle back on the roller mixer each time. The soybean sample with water should stay mixing in the roller for 60 min. Therefore, it is important to finish adding \( m_{H_2O} \) aliquots before this total time interval.
5.14. Spread a thin layer of soybeans on aluminum trays and let stand at room temperature to air-dry until \( \bar{w}_{soy,1} \) is reached.
5.15. Use the portable moisture meter to check \( \bar{w}_{soy,1} \). It is recommended when re-wetting the beans to check \( \bar{w}_{soy,1} \) every 10 min. When \( \bar{w}_{soy,1} \) is reached, from mixed triplicates samples, write down its moisture content using a moisture meter and save the subsamples for gravimetrically measured moisture content.
5.16. The sample will be ready to be used in the respiration tests; from 3000 g of sample 4 replications can be conducted in a S-GRMS (500 g per desiccator).

6.0. **PROCEDURE: FINAL STEPS AND REPORTING**

6.1. Do not forget to write down all the details listed above on the datasheet.
6.2. From the re-wetted sample, weigh 500 g of sample for further chemical analyses, place it in a labeled resealable plastic freezer bag and store it in the freezer.
6.3. Discard all foreign materials, split, broken, damaged and excess beans.
6.4. Clean all equipment after using it for this protocol.
6.5. Write down any observations during the execution of this protocol, including mold in the refrigerated sample. If there are any issues, see corrective action (Section 7.0).

7.0. CORRECTIVE ACTION

7.1. Pay attention to the digital scale, remember to always tare the scale before any measurement. Check the calibration using a known weight before using the digital scale. Be sure to use the same digital scale for all measurements throughout a grain respiration test or gravimetric moisture content measurement (not just individual tests or experiments). If any issues are noted with the digital scale notify the supervisor immediately.

7.2. When mold is detected from the refrigerated soybean tote, notify the supervisor immediately. Do not use any soybean with visible mold or off-odors in respiration tests.

7.3. The supervisor will take further corrective actions which may include discarding soybeans samples and requesting new one from Crop Sciences Research and Education Farm of the University of Illinois at Urbana-Champaign.

8.0. CHANGES FROM PREVIOUS VERSION

8.1. SOP drafted on 01 March 2017.
8.2. Reviewed, revised, and approved by supervisor on 09 October 2018.
1.0. PURPOSE

This SOP explains the protocol for preparing a split soybeans sample for a S-GRMS test.

2.0. SCOPE

This SOP describes how to split soybeans for a grain respiration test.

3.0. RESPONSIBILITY

The supervisor will be responsible for training the personnel on proper use of S-GRMS and its components, preparing samples, and implementing this protocol/procedure.

4.0. MATERIALS AND EQUIPMENT

4.1. Refrigerated soybeans sample, the quantity will depend on how many replications will be needed and the split beans content (% w/w).

4.2. Aluminum grain sieves (Grainman 10/64” x 3/4”, Miami, FL, USA, and USA Std. Sieve No. 8 Opening 0.0937”, Dual Manufcaturing Co., Fraklin Park, IL, USA) to remove impurities and damaged beans and keep splits beans.

4.3. Digital precision scale range of 0 to 3100 g and 0.01 g resolution (Model iBalance i3100, MyWeigh, Phoenix, AZ, USA).

4.4. Custom-fabricated degerminator located at the room 159 in the Agricultural Engineering Science Building (AESB).

4.5. 500 mL capacity polypropylene bottle (Model No. Nalgene™ Wide Mouth Economy Bottle, U.S. Plastics, Lima Ohio, OH, USA) wrapped with 2 rubber bands to increase friction.

4.6. 243 mL polypropylene funnel 104 mm Dia. & 21 mm Stem Dia. (Model No. Nalgene™ Powder Funnel, U.S. Plastics, Lima Ohio, OH, USA).


4.8. Resealable plastic bags.

5.0. PROCEDURES

5.1. Calculate the mass of splits beans needed for the proposed experimental design, according to the split beans content and a total of 500 g sample per desiccator. Add an extra amount of 500 g to clean the degerminator.

5.2. Follow the Steps 5.2 to 5.5 from Document No. S-GRMS-002 to clean the refrigerated beans. It is important to remove every split bean from the cleaned sample and use whole beans to split.

5.3. Bring the cleaned beans sample on a plastic tray and an extra tray to the room 159 at AESB and process about 500 g of the sample to clean remaining corn left on it. It is
important to add small aliquots until completing the total amount. Use the extra tray to collect the split beans.

5.4. Discard the split beans and then process and save the rest of the sample.

5.5. Back to the laboratory, clean the split beans first with USA Std. Sieve No. 8 Opening 0.0937” to remove small impurities and later to remove remaining whole beans or broken seeds use the Grainman 10/64” x 3/4” sieve.

5.6. Separate the clean split beans in aliquots for each respiration test and place them in labeled plastic bags using the funnel. Use the digital scale to weigh the aliquots. Remember that only 4 replications can be conducted at the same time.

6.0. PROCEDURE: FINAL STEPS AND REPORTING

6.1. Refrigerate the split beans until the day of the respiration test. It is important to always have “fresh” split beans, do not use split beans that were processed for more than 30 d.

6.2. Follow Steps 5.7 to 5.18 from Document No. S-GRMS-002 to re-wet the split beans. Re-wet the soybeans separately from the whole beans using a third bottle (500 mL).

6.3. Reserve a 500 g whole beans sample for further chemical analyses. Place it in a labeled resealable plastic bag and store it in the freezer.

6.4. Also, from the whole beans obtain the gravimetric moisture content (Document No. S-GRMS-004).

6.5. When split and whole beans have achieved the desired moisture content, then weigh out individually the mass of splits and whole beans to obtain 500 g samples per desiccator.

6.6. The samples will be ready for use in a grain respiration chamber.

6.7. Clean all equipment after using it for this protocol.

6.8. Write down any observations during the execution of this protocol, including mold on the refrigerated sample. If there are any issues, see corrective action (Section 7.0).

7.0. CORRECTIVE ACTION

7.1. Pay attention to the digital scale, remember to always tare the scale before any measurement. Check the calibration using a known weight before using the digital scale. Be sure to use the same digital scale for all measurements throughout a grain respiration test or gravimetric moisture content measurement (not just individual tests or experiments). Any issues with the digital scale notify the supervisor immediately.

7.2. When mold is detected from the refrigerated soybean tote, notify the supervisor immediately. Do not use any soybean with visible molds or off-odors in a respiration test.

7.3. The supervisor will take further corrective actions which may include discarding soybeans samples and obtaining more from Crop Sciences Research and Education Farm of the University of Illinois at Urbana-Champaign.

8.0. CHANGES FROM PREVIOUS VERSION


8.2. Reviewed, revised, and approved by supervisor on 10 October 2018.
1.0. PURPOSE

This SOP explains the protocol for determining the moisture content of soybeans according to ASAE Standard S352 (R2017).

2.0. SCOPE

This SOP describes how to determine gravimetric moisture content of soybeans before and after a grain respiration test.

3.0. RESPONSIBILITY

The supervisor will be responsible for training the personnel on proper use of S-GRMS and its components, preparing samples, and implementing this protocol/procedure.

4.0. MATERIALS AND EQUIPMENT

4.1. Soybeans subsamples before and after respiration test.
4.2. Convection oven (Model No. 160DM Thelco® Laboratory oven, Precision Scientific Inc., Chicago, IL, USA) set at 103°C or similar equipment.
4.3. Digital precision scale range of 0 to 3100 g and 0.01 g resolution (Model iBalance i3100, MyWeigh, Phoenix, AZ, USA).
4.4. Custom-fabricated desiccator cabinet with desiccant (Catalog No. 23025, WA Hammond Drierite Co., Ltd., Xenia, OH, USA).
4.5. Aluminum weighing or moisture dishes – 4 oz utility cup full curl (Product No. 42330, Pactiv, Lake Forest, IL, USA).
4.6. Perforated Aluminum Microbiological Basket/Carriers (16”x16”).

5.0. PROCEDURES

5.1. Label with a unique tag three aluminum moisture dishes. For example, “Test 1A-1a, Test 1A-2a, and Test 1A-3a” labels are for three subsamples for Test 1. The uppercase letters denote whether samples were taken (A) before and (B) after a grain respiration test. Lowercase letters denote whether samples were filled with (a) wet or (b) oven-dried soybeans.
5.2. Tare the empty digital scale. Place one dish at a time on the scale and write down its mass on the first column (dish mass) of the first gravimetric measurement of moisture content table in the printed datasheet (e.g., \(m_{1A}, m_{2A}, m_{3A}\)).
5.3. Repeat Step 5.2 for each dish.
5.4. Without touching or moving the dish, gently pour one of the soybean subsamples saved at Step 5.17 of Document No. S-GRMS-002 until reaching approximately 30 g. Record the total mass of the dish with wet soybeans at the second column (dish + wet
sample) of the same table (e.g., $m_{1A-1a}, m_{1A-2a}, m_{1A-3a}$). Carefully remove dish with wet soybeans from the scale.

5.5. Repeat Step 5.4 for the two remaining soybean subsamples saved.

5.6. Place the three filled dishes on a perforated aluminum basket and place it inside the convection oven set at 103°C for 72 h.

5.7. Remove the basket from the oven and let it cool down inside the desiccator cabinet for 20 min.

5.8. Carefully remove the basket from the cabinet and quickly weigh three times each dish’s dry weight e.g., $m_{1A-1b}, m_{1A-2b}, m_{1A-3b}$). Write down on the third column of the datasheet table (dish + dry sample) the measured values.

5.9. Per subsample, calculate the following:

- mass of wet soybeans: $m_{soy,1a} = m_{1A-1a} - m_{1A}$
- mass of dry matter: $m_{DM,1} = m_{1A-1b} - m_{1A}$
- mass of moisture removed: $m_{H2O,1} = m_{soy,1a} - m_{DM,1}$
- moisture content of soybean sample (% w.b.): $w_{soy,1a} = \frac{m_{H2O,1}}{m_{soy,1a}} \times 100$

5.10. Repeat step 5.9 for the other two soybeans subsamples and write down the moisture content values found on the last column of the datasheet table (calculated moisture content).

5.11. Calculate the average moisture content of soybeans taken before a grain respiration test:

$$\bar{w}_{soy,1} = \frac{w_{soy,1a} + w_{soy,2a} + w_{soy,3a}}{3}$$

5.12. To determine average moisture content of samples taken after a grain respiration test, repeat Steps 5.1 to 5.11 taking care to label the subsamples appropriately. Do not forget to write down all the measured weights on the second gravimetric measurement of moisture content table in the printed datasheet.

5.13. Compute the standard deviation of the average moisture content subsamples for record-keeping and quality control. If this number for one sample is larger than most (typically subsamples are within 0.5% moisture content) there may be a problem.

6.0. PROCEDURE: FINAL STEPS AND REPORTING

6.1. Record all weight measurements and calculations from the printed datasheet in the electronic datasheet template (A.4.1. Supplemental file: Static Respiration Test Electronic Datasheet) designated for the specific grain respiration test.

6.2. Record any issues with determining gravimetrically moisture content of soybeans. If there are any issues, see corrective action (Section 7.0).

7.0. CORRECTIVE ACTION

7.1. Pay attention to the digital scale, remember to always tare the scale before any measurement. Check the calibration using a known weight before using the digital scale. Be sure to use the same digital scale for all measurements throughout a gravimetric moisture content measurement (not just individual tests or experiments). Any issues with the digital scale notify the supervisor immediately.
7.2. The supervisor will take further corrective actions, which may include re-calibrating or replacing the digital scale.

8.0. CHANGES FROM PREVIOUS VERSION

8.1. SOP drafted on 01 July 2016 by L. R. Trevisan.
8.2. Reviewed, revised, and approved by supervisor on 01 October 2017.
8.3. Second review, revision, and approval by supervisor on 10 October 2018.
1.0. PURPOSE

This SOP explains the protocol for running a grain respiration test in a S-GRMS.

2.0. SCOPE

This SOP describes how to set-up S-GRMS prior to starting a test, collect the data from the sensor package built-in data logger, calculate dry matter loss over time, end the test, and clean up the system.

3.0. RESPONSIBILITY

The supervisor will be responsible for training the personnel on proper use of S-GRMS and its components, preparing samples, and implementing this protocol/procedure.

4.0. MATERIALS AND EQUIPMENT

4.1. Clean, re-wetted soybeans sample (approximately 500 g per desiccator) prepared according to Document No. S-GRMS-002.
4.2. Sensor package with built-in data logger (qty = 4; Model K33-BLG, CO2Meter, Inc., Ormond Beach, FL, USA) calibrated according to Document No. S-GRMS-001.
4.3. 10 L glass desiccators (qty = 4).
4.4. Custom-fabricated metal baskets and their lids to place soybeans sample and the sensor on top (qty = 4 each).
4.6. Digital thermometer (qty = 1, Model No. 1235D30, Traceable® Products, Houston, TX, USA).
4.7. Rubbing alcohol isopropyl 70%.
4.8. Battery charger (Model Digi charger D4, Nitecore®, Guangzhou, Guangdong, China).
4.9. Resealable plastic bags (qty = 5).
4.10. Vacuum grease (Model High vacuum grease, Dow Corning®, Dow Chemical, Midland, MI, USA).

5.0. PROCEDURES

5.1. Turn on and set up the temperature-controlled incubator to the desired test temperature one day before starting your test and place the digital thermometer inside it.
5.2. On the next day, first, clean the desiccators with rubbing alcohol and a paper towel and place them inside the incubator to preheat to the desired temperature.
5.3. Before starting to prepare the soybean sample, print one of the respiration test datasheets at A.3. Static Respiration Test Datasheets depending on whether your sample will have whole or split beans.

5.4. Write down on the printed datasheet the test number, starting date and time, the cold storage soybeans moisture content and temperature, and the temperature the incubator was set to.

5.5. Check the last calibration date of the sensors and write down on the datasheet. If the calibration has not been done in more than 6 months, the sensors will need a new calibration. To calibrate the sensors, follow Document No. S-GRMS-001, this procedure can take about 3 h. After double-checking the calibration, set the sensors internal log interval to 600s (10 min), sync the sensor date and time and clean the internal log memory. The instructions about how to set the log interval, sync date and time, and clean the internal memory are provided in Document No. S-GRMS-001.

5.6. Check also if the batteries for the sensors are fully charged, placing them in the charger.

5.7. Follow the instructions in the Document No. S-GRMS-002 to prepare the cleaned and re-wetted soybeans sample.

5.8. Weigh 500 g of sample per desiccator, measuring exactly the weight on the digital scale, recording the value on the datasheet. Pour each sample into the specific labeled basket.

5.9. From the remaining sample, take three subsamples for gravimetric measurement of moisture content following the Document No. S-GRMS-004 and write down the information on the gravimetric measurement of moisture content table placed on the datasheet. Take also 500 g of remaining sample for further chemical analyses, place it in a labeled resealable plastic bag and store it in the freezer.

5.10. Bring the baskets with the samples, their lids and the sensors packages to the incubator room.

5.11. Take one desiccator at a time from the incubator and place the assigned soybean basket covered with the lid. Place on top of the lid the sensor package.

5.12. Set the jumper of the sensor package and check the heartbeat LED to confirm that the data collection started. Seal the desiccator with vacuum grease, close it and place back to the incubator.

5.13. Repeat Steps 5.10 and 5.11 for each desiccator and write down in the datasheet the double-checked temperature of the incubator and the time that all the desiccators were placed inside the incubator. The sealed desiccators with their respective samples and sensors will look like figure A.2.10 inside the incubator.

5.14. After 10 days of measurements, it is time to finish the respiration test. At least every other day it is recommended to open the incubator without opening the internal glass door to check that the sensors are working properly and if the mold growth is excessive.
6.0. PROCEDURE: FINAL STEPS AND RECORDING

6.1. After a grain respiration test, write down on the datasheet the stopping criteria and any additional observations.
6.2. Turn off the incubator, open each desiccator, remove the sensor jumper to stop the data collection, and write down the date and time the test ended.
6.3. Bring the soybean basket to the digital scale and record the soybean sample weight on the datasheet.
6.4. Repeat Step 5.9 for each desiccator to gravimetrically measure moisture content, but instead of 500 g of sample, freeze only 400 g because 90 g will be needed for the moisture content test. Write down the information to measure the gravimetric measurement of moisture content.
6.5. To collect the data for each sensor, follow the Steps 6.11 to 6.13 from the Document No. S-GRMS-001. Save the data as “StaticData_SensorName_RepNumber_TestDate.csv”.
6.6. To calculate respired CO₂ and DML use the electronic datasheet template (A.4.1. Supplemental file: Static Respiration Test Electronic Datasheet) and copy the saved data from Step 6.5. into the row 22 and columns B to D. Also copy extra information from the datasheet on the blank cells (B7, B13, D5, D6, D7, D8, D9, I7, J7), including the two gravimetric measurement of moisture content tables.
6.7. After filling the electronic datasheet, respired CO₂ and DML will be automatically generated. Check section A.4.2. Supplemental File Example: Static Respiration Test Electronic Datasheet to see an example of calculated data.
6.8. Clean all equipment after using it for this protocol.
7.0. CORRECTIVE ACTION

7.1. Pay attention to the digital scale, remember to always tare the scale before any measurement. Check the calibration using a known weight before using the digital scale. Be sure to use the same digital scale for all measurements throughout a grain respiration test (not just individual tests or experiments). Any issues with the digital scale notify the supervisor immediately.

7.2. Pay attention to the sensor package data logger data. If any of them did not save data or showed a different behavior while collecting data notify supervisor immediately.

7.3. The supervisor will take further corrective actions which may include repairing or replacing the digital scale or system components.

8.0. CHANGES FROM PREVIOUS VERSION


8.2. Reviewed, revised, and approved by supervisor on 10 October 2018.
A.4. Static respiration test datasheets

A.4.1. Static respiration test datasheet for 4 replications

Test Nº.: _______ Soybeans moisture content: _______ %
Start date and time: _______/______/______ h: _______ min Soybeans storage temperature: _______ °C

Follow Document No. S-GRMS-005 to conduct a static grain respiration test.

SET UP INCUBATOR
Turn on and set the temperature one day before the respiration test begins.
Temperature set: _______ °C

SET UP RESPIRATION CHAMBERS
Clean desiccators: _______
Allocate desiccators inside the incubator before soybeans sample preparation: _______

SET UP K33-BLG SENSORS
1. Follow Document No. S-GRMS-001: _______
2. Calibration Date: _______/______/______
3. Set log interval to 600s: _______
4. Synchronize sensor time: _______
5. Clean sensor log memory: _______

SOYBEAN PREPARATION
☐ Hand-shelled ☐ Mechanically harvested ☐ Harvested in 2017 ☐ Harvested in ______
Additional notes: ___________________________________________
1. Follow Document No. S-GRMS-002: _______
2. Initial weight of beans taken out of refrigerated storage: _______ g
3. Acclimate the sample to room temperature (about 30 min):
   Start time: _______ h _______ min End time: _______ h _______ min
4. Clean beans: _______
5. Moisture content: _______ %
6. Necessary to re-wet soybeans: _______

REWETTING SOYBEANS
1. Follow Document No. S-GRMS-002 to re-wet beans: _______
2. Weight of soybeans to be re-wetting: Bottle A: _______ g Bottle B: _______ g
3. Amount of water needed: Bottle A: _______ g Bottle B: _______ g
4. Place bottles in roller mixer for 60 min: _______
   Start time: _______ h _______ min End time: _______ h _______ min

7. Follow Document No. S-GRMS-004 to gravimetrically determine the moisture content of the sample before the respiration test: _______
8. Moisture content check before the start of the respiration test:

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Estimated moisture content (% w.b.)</th>
<th>Sample No.</th>
<th>Dish mass (g)</th>
<th>Dish + Wet Sample (g)</th>
<th>Dish + Dry Sample (g)</th>
<th>Calculated moisture content (% w.b.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
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</tr>
</tbody>
</table>

Date and time put in oven: ___ / ___ h: ___ min
Date and time taken out of oven: ___ / ___ h: ___ min

*Max.: 3 days.

BACK TO RESPIRATION CHAMBER

1. Collect a 400 g sample from the re-wetted soybeans for further analyses: ☐ Bag Labeled: ☐ Fridge Stored: ☐
2. Weight of soybeans to be tested: Desiccator 1: _____ g Sensor: ______ Desiccator 2: _____ g Sensor: ______ Desiccator 3: _____ g Sensor: ______ Desiccator 4: _____ g Sensor: ______
3. Grain: Temperature: ______ ºC Moisture content: ______ % Time: _____ h _____ min
4. Pour soybeans inside the basket and place it inside each assigned desiccator chamber: ☐
5. Power sensors: ☐
6. Set sensors jumper: ☐
7. Check sensors heartbeat LED: ☐
8. Close desiccators sealing them with vacuum grease: ☐

STARTING THE RESPIRATION TEST

1. Check temperature of the incubator: ☐ Temperature: ______ ºC
2. Allocate desiccators inside the incubator: ☐
3. Time respiration test started: _____ h _____ min

TERMINATION OF RESPIRATION TEST:

1. Stopped criteria:
   a. Visible excessive mold growth: ☐
   b. End of the stipulated ____ days: ☐
   c. Other criteria: ☐
2. Turn off Incubator: ☐
3. Open desiccators: ☐
4. Remove sensors’ jumpers: ☐
5. Collect soybeans samples baskets: ☐
6. Date and time respiration test ended: ___ / ___ h: ___ min

END OF RESPIRATION TEST

CLEANING SYSTEM

1. Weight of tested soybeans: Desiccator 1: _____ g Desiccator 2: _____ g Desiccator 3: _____ g Desiccator 4: _____ g
2. Pour beans onto a tray to be observed: ☐
   Observations: ___________________________________________________________
3. Collect 400 g samples from each desiccator for further analyses: ☐
   Bags Labeled: ☐
   Fridge Stored: ☐
4. Follow Document No. S-GRMS-004 to gravimetrically determine the moisture content of each desiccator sample after the respiration test: ☐
5. Moisture content check after the respiration test:

<table>
<thead>
<tr>
<th>Moisture Meter</th>
<th>Gravimetric measurement of moisture content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample No.</td>
<td>Estimated moisture content (% w.b.)</td>
</tr>
<tr>
<td>1</td>
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<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

Desiccator 1 Sample
Desiccator 2 Sample
Desiccator 3 Sample
Desiccator 4 Sample

Date and time put in oven: / / h: min  Date and time taken out of oven: / / h: min

*Max.: 3 days.

6. Clean desiccators: ☐

SAVING DATA
1. Save data as StaticData_SensorName_TestNumber_TestDate (".csv") from each K33-BLG sensors using DAS Software and following steps from Document No. S-GRMS-001: ☐
A.4.2. Static respiration test datasheet for split beans content

Test N°: _______ Soybeans moisture content: _______ %
Start date and time: ___/___/___ h: ___ min Soybeans storage temperature: ______ °C

Follow Document No. S-GRMS-005 to conduct a static grain respiration test.

SET UP INCUBATOR
Turn on and set the temperature one day before the respiration test begins.
Temperature set: ______ °C

SET UP RESPIRATION CHAMBERS
Clean desiccators: ☐
Allocate desiccators inside the incubator before soybeans sample preparation: ☐

SET UP K33-BLG SENSORS
1. Follow Document No. S-GRMS-001: ☐
2. Calibration Date: ___/___/____.
3. Set log interval to 600s: ☐
4. Synchronize sensor time: ☐
5. Clean sensor log memory: ☐

SOYBEAN PREPARATION
☐ Hand-shelled ☐ Mechanically harvested ☐ Harvested in 2017 ☐ Harvested in ______
Additional notes: ____________________________________________________________
1. Follow Document No. S-GRMS-002 and S-GRMS-003: ☐
2. Prepare split beans samples: ☐
3. Initial weight of beans taken out of refrigerated storage: ________g
4. Acclimate the samples to room temperature (about 30 min):
   Start time: ________ h _______ min   End time: ________ h _______ min
5. Clean beans: ☐
6. Moisture content: _______%
7. Necessary to re-wet soybeans: ☐

REWETTING SOYBEANS
1. Follow Document No. S-GRMS-002 to re-wet beans: ☐
2. Weight of soybeans to be re-wetting: Bottle A: ________g   Bottle B: ________g
   Bottle Splits: ________g
   Amount of water needed: Bottle A: ________g   Bottle B: ________g
   Bottle Splits: ________g
3. Place bottles in roller mixer for 60 min: ☐
   Start time: ________ h _______ min   End time: ________ h _______ min
4. Follow Document No. S-GRMS-004 to gravimetrically determine the moisture content of the sample before the respiration test: ☐
5. Moisture content check before the start of the respiration test following Document No. S-GRMS-004:

<table>
<thead>
<tr>
<th>Moisture Meter</th>
<th>Gravimetric measurement of moisture content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample No.</td>
<td>Estimated moisture content (% w.b.)</td>
</tr>
<tr>
<td>1</td>
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<td>2</td>
<td></td>
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<tr>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>

Date and time put in oven: _______ / _______ h: _____ min  Date and time taken out of oven: _______ / _______ h: _____ min

*Max.: 3 days.

BACK TO RESPIRATION CHAMBER
1. Collect 400 g from the re-wetted whole soybeans sample for further analyses: ☐
   - Bag Labeled: ☐  Fridge Stored: ☐
2. Weight of soybeans to be tested:
   - Desiccator 1: _______ g  Split beans content: _______ %  Sensor: _______
   - Desiccator 2: _______ g  Split beans content: _______ %  Sensor: _______
   - Desiccator 3: _______ g  Split beans content: _______ %  Sensor: _______
   - Desiccator 4: _______ g  Split beans content: _______ %  Sensor: _______
3. Grain: Temperature: _______ °C  Moisture content: _______ %  Time: _______ h  _______ min
4. Pour whole and split beans inside the basket and place it inside each assigned desiccator chamber: ☐
5. Power sensors: ☐
6. Set sensors jumper: ☐
7. Check sensors heartbeat LED: ☐
8. Close desiccators sealing them with vacuum grease: ☐

STARTING THE RESPIRATION TEST
1. Check temperature of the incubator: ☐  Temperature: _______ °C
2. Allocate desiccators inside the incubator: ☐
3. Time respiration test started: _______ h  _______ min  \( t_{\text{initial}} \)

TERMINATION OF RESPIRATION TEST:
1. Stopped criteria:
   a. Visible excessive mold growth: ☐
   b. End of the stipulated _______ days: ☐
   c. Other criteria: ☐
2. Turn off Incubator: ☐
3. Open desiccators: ☐
4. Remove sensors’ jumpers: ☐
5. Collect soybeans samples baskets: ☐
6. Date and time respiration test ended: _______ / _______ h: _______ min  \( t_{\text{final}} \)

END OF RESPIRATION TEST
CLEANING SYSTEM
1. Weight of tested soybeans:
   - Desiccator 1: _______ g
   - Desiccator 2: _______ g
   - Desiccator 3: _______ g
   - Desiccator 4: _______ g

2. Pour beans onto a tray to be observed: □ Observations: ____________________________

3. Collect 400g samples from each desiccator for further analyses: □ Bags Labeled: □ Fridge Stored: □

4. Follow Document No. S-GRMS-004 to gravimetrically determine the moisture content of each desiccator sample after the respiration test: □

5. Moisture content check after the respiration test:

<table>
<thead>
<tr>
<th>Moisture Meter</th>
<th>Gravimetric measurement of moisture content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample No.</td>
<td>Estimated moisture content (% w.b.)</td>
</tr>
<tr>
<td>Desiccator 1 Sample</td>
<td></td>
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<tr>
<td>1</td>
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<td>Desiccator 2 Sample</td>
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<tr>
<td>Desiccator 3 Sample</td>
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<td>Desiccator 4 Sample</td>
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</table>

Date and time put in oven: _______/_____/______ h: _______ min Date and time taken out of oven: _______/_____/______ h: _______ min

*Max.: 3 days.

6. Clean desiccators: □
SAVING DATA

1. Save data as StaticData_SensorName_SplitContent_TestNumber_TestDate (".csv") from each K33-BLG sensors using DAS Software and following steps from Document No. S-GRMS-001: ☐

A.5. Supplemental files: Static respiration test electronic datasheet

The supplemental electronic file “APPENDIX C Supplemental file _da Silva_ Thesis” contains Excel spreadsheets tab named as “APPENDIX A.4.1” and “APPENDIX 4.2” with important formulas and example as an additional tool to follow the SOPs for S-GRMS. Appendix A.4.1. Supplemental File: Static Respiration Test Electronic Datasheet has instructions on how to use the spreadsheet with the purpose to have the data from the sensor and the information about respiration test copied to the electronic file. The formulas contained in this spreadsheet automatically calculate accumulated and specific mass of respired CO₂ and DML. It is important to also create a daily plot to see the accumulate DML (%) over time (d) and check if the respiration test is having the expected results. Appendix A.4.2. Supplemental File Example: Static Respiration Test Electronic Datasheet is an example of a respiration test that demonstrates how Appendix A.4.1 needs to be filled.
APPENDIX B. OVERVIEW OF DESIGN AND OPERATION: DYNAMIC GRAIN RESPIRATION MEASUREMENT SYSTEMS (D-GRMSs)

B.1. D-GRMS improvements and instrumentation

B.1.1. D-GRMS improvements

The D-GRMS designed by Sood (2015) and improved by Trevisan (2017) was used as a basis to design a new (second) replicate of D-GRMS and to improve the first replicate. The two D-GRMS (Figure B.3) had some important improvements from the design used by Trevisan (2017). First, a gas guard (Model No. 3050, Forma Scientific, Inc., Marjetta, OH, USA) was used to continuously control the source of compressed air, allowing automated switching between two gas cylinders. A backflow-prevention valve with push-to-connect tube fitting (Model No. 3208K18, McMaster-Carr®, Chicago, IL, USA) was used to maintain the air flow in only one direction, and then, a wye push-to-connect tube fitting for air (Model No. 5779K46, McMaster-Carr®, Chicago, IL, USA) was used to divide the flow for the two dynamic systems. Before each mass flow controller, an on/off valve with push-to-connect fitting (Model No. 4503K25, McMaster-Carr®, Chicago, IL, USA) to control the flow for each system, enabling a single system to run. A mass flow controller for the second system was calibrated for CO\textsubscript{2}, therefore the adjustment of volumetric flow rate was done by a separate microcontroller different from the first system developed by Trevisan (2017). Details about this instrumentation are in section Appendix B.1.2.

Each respiration chamber had its own water bath to control the water-jacket temperature; however, the bottles of water-glycerol solutions from both systems were maintained in the same water bath to control the air relative humidity at desired test temperature. The tubing and respiration chambers’ insulation was reinforced to have double insulation as shown in Figure B.1. The double insulation helps to prevent the condensation that was occurring on previous tests, especially at higher grain temperature in cooler lab conditions. The set of temperature, relative humidity and CO\textsubscript{2} concentration ($T$, $\phi$, and $C_{CO_2}$) sensors before each respiration chamber were placed together with their respective rotameters in an insulated and sealed electrical enclosure (Model No. NBE-10563, Bud Industries, Inc., Willoughby, OH, USA). To prevent condensation in the enclosure space, heat tape was assembled around the enclosure and then covered by an insulated metalized mylar double Foil bubble wrap, leaving only the cover clear to allow visibly of rotameter (Figure B.2a and b). The outlets from both systems after passing through individual rotameters were placed inside a glass jar to monitor $T$, $\phi$, and $C_{CO_2}$ double checking if there was any leak of CO\textsubscript{2} or humidified air (Figure B.2c). The outlet from the jar had a backflow-prevention valve with barbed fittings (Model No. 47245K27, McMaster-Carr®, Chicago, IL, USA) to maintain the air flow in only one direction.
The last improvement was in how $T$, $\phi$, and $C_{CO_2}$ were monitored. Measurements from each sensor were logged every minute onto a desktop computer and saved in a SD Card located on the shield of the microcontroller. The data were also displayed in an LCD digital display. More information about the monitoring is provided in section Appendix B.1.3.

Figure B.1. Respiration chamber (a) and tubing (b) double insulation.

Figure B.2. Location of rotameter, CO$_2$, temperature and relative humidity monitoring sensors (a) before respiration chamber inside an electrical enclosure and (b) after respired CO$_2$ scrubbers inside a glass jar.
Figure B.3. Schematic of two improved dynamic grain respiration measurement system (D-GRMS). Heat was applied to the flow tubes in Section A and B using a 19.6 W m\(^{-1}\) heating tape.
B.1.2. Adjustment of volumetric air flow rate

Because the mass flow controller (MFC) used on the second system was calibrated for CO$_2$ instead of air, a new program was coded to use a factor to adjust the desired flow rate based on the coefficient of specific heat of each gas (CO$_2$ and air). The factor is 0.7837 to convert the MFC reading (calibrated to CO$_2$) to an airflow reading, $Q_{air} = Q_{CO_2}/k$ (Operating Manual GFC Mass Flow Controller, Aalborg®, Orangeburg, NY, USA). Like Trevisan (2017), adjustment of the mass flow controller input voltage was controlled by a microcontroller (ATmega2560, Arduino, Ivrea, Italy) using a digital potentiometer (Model No. MCP4131-103, Microchip®, Chandler, AZ, USA) and the actual voltage and the air flow rate were displayed using an LCD digital display (Figure B.4). The digital potentiometer takes as input a digital value between 0 and 128 and changes resistance to produce discrete voltages; with a reference voltage of 4.9671 Vdc, the 128 voltage steps range from 0.0245 to 4.9663 Vdc. These discrete voltages are used to set the desired airflow rate in the MFC.

![Circuit diagram for the second system fine adjustment of volumetric flow rate. All components share a common ground.](image)

The mass flow control program code for the second system (listed below) was written and uploaded with the open-source software by Arduino (IDE Version 1.8.5r2, Arduino, Ivrea, Italy) and three supporting libraries: SPI, LiquidCrystal_I2C, Wire. The SPI library is a synchronous serial data protocol used to communicate quickly over short distances with peripheral devices. This library allows the Arduino Mega2560 to have a quicker communication with the digital potentiometer through the pins 50, 51, 52 and 53, respectively master in slave
out, master out slave in, serial clock and slave select). The LiquidCrystal_I2C allows controlling I2C displays, such as the LCD used. The Wire library allows the microcontroller to communicate with the I2C device used. The voltage and the flow rate for CO₂ were measured for 0 to 128 digital potentiometer steps. The data was used to convert the CO₂ flow rate to air flow rate by a factor equal to 0.7837 for every command for the momentary pushbutton switches.

“DigitalRead” command is used to collect the information from the momentary switches connected to the pins 33 and 36 respectively, which were used to change the internal resistance of the digital potentiometer counted as steps. The resulting step is correlated to the voltage change across the potentiometer. The voltage is then supplied to the remote-control circuitry of the MFC, which corresponds to the desired flow rate level. The flow is kept constant with constant input voltage as long as power and air are supplied. The LCD is connected to the SCL and SDA pins to display flow rate and voltage in the MFC.

Mass flow controller program code for the second system:

```c
/* Mass Flow Controller Code: LCD, Buttons, & digital potentiometer
 * 16 character 2 line I2C Display
 * Using digital potentiometer MCP4131
 */

/* Libraries*/
#include <SPI.h>
#include <Wire.h>
#include <LiquidCrystal.h>
#include <LiquidCrystal_I2C.h>

/*Declare Constants*/
LiquidCrystal_I2C lcd(0x3f, 2, 1, 0, 4, 5, 6, 7, 3, POSITIVE);  // Set the LCD I2C address
const int upButtonPin = 36; //Pin connected to the First Button
const int downButtonPin = 33; //Pin connected to the Second Button
const int CSPin = 53; //Pin connected to chip select (pin 1 of MCP4131)
const float k = 0.7837; //K MFC factor for CO2 and Air
const byte address = 0x00;

/* In addition to the pin descriptions above, we have the following Arduino - MCP4131 connections
 *  Pin 51 -> SDI/SDO (pin 3 on MCP4131)
 *  Pin 52 -> SCLK (pin 2 on MCP4131)
 *  Ground -> VSS & P0B (pins 4 & 7 on MCP4131)
 *  5V -> P0A & VCC (pins 5 & 8 on MCP4131)
 *  LED/load -> P0W (pin 6 on MCP4131)
 */

const float measuredVoltage[129] = {0.0245, 0.0628, 0.101, 0.1392, 0.1781, 0.2161, 0.2541, 0.2921, 0.3305, 0.3684, 0.4062, 0.444, 0.4833, 0.521, 0.5587, 0.5964, 0.6358, 0.6734, 0.711, 0.7485, 0.7881, 0.8255, 0.863, 0.9004, 0.9401, 0.9774, 1.0147, 1.052, 1.0911, 1.1283, 1.1657, 1.2029, 1.241, 1.2782, 1.3154, 1.3526, 1.3912, 1.4283, 1.4655, 1.5027, 1.543, 1.5802, 1.6174, 1.6545, 1.6918, 1.7289, 1.7662, 1.8033, 1.8413, 1.8784, 1.9157, 1.9528, 1.9908, 2.028, 2.0653, 2.1025, 2.1414, 2.1786, 2.216, 2.2532, 2.2942, 2.3315, 2.3689, 2.4062, 2.4439, 2.4813, 2.5188, 2.5562, 2.5956, 2.6332, 2.6709, 2.7084,
```
```

const float measuredFlow[129] = {0, 0, 0, 0.06, 0.08, 0.1, 0.11, 0.12, 0.14, 0.15, 0.16, 0.19, 0.2, 0.22, 0.23, 0.25, 0.26, 0.28, 0.29, 0.31, 0.32, 0.34, 0.35, 0.37, 0.39, 0.4, 0.41, 0.43, 0.45, 0.46, 0.48, 0.49, 0.51, 0.52, 0.53, 0.55, 0.57, 0.58, 0.6, 0.61, 0.63, 0.64, 0.66, 0.67, 0.69, 0.7, 0.72, 0.73, 0.75, 0.76, 0.78, 0.79, 0.81, 0.82, 0.84, 0.85, 0.87, 0.88, 0.9, 0.91, 0.93, 0.94, 0.96, 0.97, 0.99, 1, 1.02, 1.03, 1.05, 1.06, 1.08, 1.09, 1.11, 1.12, 1.14, 1.15, 1.17, 1.18, 1.2, 1.21, 1.23, 1.24, 1.26, 1.27, 1.29, 1.3, 1.32, 1.33, 1.35, 1.37, 1.38, 1.4, 1.41, 1.43, 1.44, 1.46, 1.48, 1.49, 1.51, 1.52, 1.54, 1.56, 1.57, 1.59, 1.6, 1.62, 1.63, 1.65, 1.67, 1.68, 1.7, 1.71, 1.73, 1.75, 1.76, 1.78, 1.79, 1.81, 1.83, 1.84, 1.86, 1.88, 1.89, 1.91, 1.93, 1.94, 1.96, 1.98, 1.99};

const float measuredFlow[129] = {0, 0, 0, 0.06, 0.08, 0.1, 0.11, 0.12, 0.14, 0.15, 0.16, 0.19, 0.2, 0.22, 0.23, 0.25, 0.26, 0.28, 0.29, 0.31, 0.32, 0.34, 0.35, 0.37, 0.39, 0.4, 0.41, 0.43, 0.45, 0.46, 0.48, 0.49, 0.51, 0.52, 0.53, 0.55, 0.57, 0.58, 0.6, 0.61, 0.63, 0.64, 0.66, 0.67, 0.69, 0.7, 0.72, 0.73, 0.75, 0.76, 0.78, 0.79, 0.81, 0.82, 0.84, 0.85, 0.87, 0.88, 0.9, 0.91, 0.93, 0.94, 0.96, 0.97, 0.99, 1, 1.02, 1.03, 1.05, 1.06, 1.08, 1.09, 1.11, 1.12, 1.14, 1.15, 1.17, 1.18, 1.2, 1.21, 1.23, 1.24, 1.26, 1.27, 1.29, 1.3, 1.32, 1.33, 1.35, 1.37, 1.38, 1.4, 1.41, 1.43, 1.44, 1.46, 1.48, 1.49, 1.51, 1.52, 1.54, 1.56, 1.57, 1.59, 1.6, 1.62, 1.63, 1.65, 1.67, 1.68, 1.7, 1.71, 1.73, 1.75, 1.76, 1.78, 1.79, 1.81, 1.83, 1.84, 1.86, 1.88, 1.89, 1.91, 1.93, 1.94, 1.96, 1.98, 1.99};

/*Declare variables*/
int upButtonState = 0;
int downButtonState = 0;
float flow = 0.00; //Flow Range variable
float vout = 0.00; // Voltage variable
int resistorStep = 0; // Digital Potentiometer Wiper Resistance Variable
boolean buttonPressed = false;

/*Setup: Runs once*/
void setup()  {
  Serial.begin(9600);  // Used to type in characters
  Serial.println("Start");
  Serial.print("resistorStep: ");
  Serial.println(resistorStep);

  SPI.begin(); // Initializes the communication protocol for the digital potentiometer
  lcd.begin(16,2);   // Initialize the lcd for 16 chars 2 lines, turn on backlight

  pinMode(upButtonPin, INPUT);
  pinMode(downButtonPin, INPUT);
  pinMode(CSPin, OUTPUT);

  for(int i = 0; i< 2; i++)//Quick 2 blinks of backlight
  {
    lcd.backlight();
    delay(250);
    lcd.noBacklight();
    delay(250);
  }
  lcd.backlight(); // finish with backlight on

  lcd.setCursor(1,0); //Start at character 1 on line 0
  lcd.print("MFC Program 2");
delay(1000);
lcd.setCursor(4,1); //Start at character 4 on line 1
cdc.print("By: Ana");
delay(4000);

digitalPotWrite(resistorStep);
updateLCD();
}

/*Loop repeats indefinitely*/
void loop()
{
upButtonState = digitalRead(upButtonPin);
downButtonState = digitalRead(downButtonPin);

if(upButtonState == HIGH) {
    buttonPressed = true;
    Serial.println("UpButton");
    if(resistorStep < 128) {
        resistorStep++;
    }
} else if(downButtonState == HIGH) {
    buttonPressed = true;
    Serial.println("DownButton");
    if(resistorStep > 0) {
        resistorStep--;
    }
}

if(buttonPressed == true) {
    digitalPotWrite(resistorStep);
    Serial.print("resistorStep: ");
    Serial.println(resistorStep);
    Serial.print("flow = ");
    Serial.println(flow);
    Serial.print("vout = ");
    Serial.println(vout);
    buttonPressed = false;
    delay(250);
}

updateLCD();
}

int digitalPotWrite(int value) {
digitalWrite(CSPin, LOW);
SPI.transfer(address);
SPI.transfer(value);
digitalWrite(CSPin, HIGH);
}
void updateLCD(){
    vout = measuredVoltage[resistorStep];
    flow = (measuredFlow[resistorStep])/k;

    lcd.setCursor(0,0); //Start at character 0 on line 0
    lcd.print("Voltage: ");
    lcd.print(vout);
    lcd.print("VDC");
    lcd.setCursor(0,1); //Start at character 0 on line 1
    lcd.print("Q air: ");
    lcd.print(flow);
    lcd.print("L/min");
}

B.1.3. Temperature, relative humidity and CO₂ monitoring

$T$, $\phi$, and $C_{CO_2}$ of the air flow were monitored before and after the $RC$ from both systems. After sensibly heating and humidifying air in the water-glycerol solution, the air was monitored inside an insulated electrical enclosure for each system. The exhaust air flow from both systems was monitored together inside a glass jar. The purpose of monitoring these variables was to ensure that the humidified airstream entering the $RC$ was at $T$ equal to $30 \pm 2^\circ C$, $\phi$ equivalent to the corresponding $\phi_e \pm 5$ %RH, and $C_{CO_2} \leq 20$ ppm, where the equilibrium $\phi$ were 77, 88, and 96% for soybeans at 14, 18, and 22% $w$, respectively. The monitoring of the exhaust airflow was critical to ensure $\phi$ equal to 0 %RH and $C_{CO_2} \leq 20$ ppm, showing that all moisture and CO₂ were absorbed in the $RC$ dehumidifiers and $RC$ CO₂ scrubbers, respectively. $T$, $\phi$, and $C_{CO_2}$ measurements were logged every minute onto a desktop computer and SD card using an ATmega microcontroller with simultaneously display of it in an LCD digital display (Figure B.5).

The monitoring and data acquisition program (listed below Figure B.5) utilized the open source software for Arduino and 4 supporting libraries: Wire, LiquidCrystal_I2C, DHT, and SD. As mentioned before, the Wire and LiquidCrystal_I2C libraries allow and control the communication between microcontroller and I2C device (LCD). The DHT library allows the communication between microcontroller and relative humidity and temperature sensors. And the SD library allows for reading from and writing to SD cards. The constants, variables, and DHT sensors are declared, then the sensors, LCD and SD card are initiated, and the LCD display is updated. Inside the void loop, the DHT and CO₂ sensors are read, then in an interval of 1 minute the read information is saved on the SD card and displayed on the LCD. In case there is an error message is printed. Pins 26, 36 and 46 on the microcontroller correspond to the digital data output collected from DHT sensors. Pins A8, A12, and A15 correspond to the analog voltage outputs collected from CO₂ sensors. Pins 26 and A8 correspond to the DHT and CO₂ sensors located before the $RC$ in the first system (S1), and pins 36 and A8 correspond to the sensors located before the $RC$ in second system (S2). Pins 46 and A15 corresponds to the sensors located at the end of both systems exhaust. Relative humidity (%RH), temperature ($^\circ C$) and CO₂ concentration in part per million (ppm) are recorded. The program printed the readings from all sensors on a serial monitor screen and LCD screen as well as saving to the SD card.

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Figure B.5. Circuit diagram of the power supply, sensors, and data acquisition system for monitoring temperature, relative humidity, and CO\textsubscript{2} concentration from both D-GRMS.

**Monitoring and data acquisition program code:**

```c
/* Sensor Monitoring: LCD, 3 DHT, and 3 VAISALA, saving in a SD card
 * 16 character 2 line I2C Display
 * 3 DHT - Temperature and Humidity Sensor
 * 3 VAISALA CO2 Sensor
 */

/* Libraries*/
#include<SPI.h>
#include <Wire.h>
#include <LCD.h>//Download the LiquidCrystal library I2C communication
#include <LiquidCrystal.h>
#include <LiquidCrystal_I2C.h>
#include <DHT.h> //Download DHT Library
#include <SD.h>

/*Declare Constants*/
LiquidCrystal_I2C lcd(0x3f, 2, 1, 0, 4, 5, 6, 7, 3, POSITIVE); // Set the LCD I2C address
const byte address = 0x00;
const int DHTTYPE = DHT11; //Defining DHT sensor type
const int DHT1Pin = 26; //Digital Pin connected to the First DHT sensor
const int DHT2Pin = 36; //Digital Pin connected to the Second DHT sensor
```
const int DHT3Pin = 46; //Digital Pin connected to the Third DHT sensor
const int VAI1Pin = 8; //Analog Pin connected to the First Vaisala sensor
const int VAI2Pin = 12; //Analog Pin connected to the Second Vaisala sensor
const int VAI3Pin = 15; //Analog Pin connected to the Third Vaisala sensor
const int CSPin = 4; //Chip Selected Pin of the Ethernet shield
const long saveInterval = 60000; // interval at which to run saveData (milliseconds)

/*Declare variables*/
int h1 = 0; //Relative humidity variable of the First DHT sensor
int h2 = 0; //Relative humidity variable of the Second DHT sensor
int h3 = 0; //Relative humidity variable of the Third DHT sensor
int t1 = 0; //Temperature variable of the First DHT sensor
int t2 = 0; //Temperature variable of the Second DHT sensor
int t3 = 0; //Temperature variable of the Third DHT sensor
int VAI1 = 0; //Voltage variable of the First Vaisala sensor
int VAI2 = 0; //Voltage variable of the Second Vaisala sensor
int VAI3 = 0; //Voltage variable of the Third Vaisala sensor
unsigned long previousMillis = 0; // It will store last time saveData was run
unsigned long currentMillis = 0; // It will store how long the program has been running (milliseconds), overflow at ~50 days
unsigned long currentInterval = 0; // It will store currentMillis - previousMillis so we can call abs() on it to avoid overflow restrictions

File myfile; //File that the data will be saved

/* DHT sensors*/
DHT dht1(DHT1Pin, DHTTYPE); //Setting the DHT sensor 1
DHT dht2(DHT2Pin, DHTTYPE); //Setting the DHT sensor 2
DHT dht3(DHT3Pin, DHTTYPE); //Setting the DHT sensor 3

/*Setup: Runs once*/
void setup() {  
  Serial.begin(9600); // Used to type in characters
  Serial.println("Sensor Monitoring: Start");
  pinMode(CSPin, OUTPUT); //Setting Up SD card

  lcd.begin(16,2); // Initialize the lcd for 16 chars 2 lines, turn on backlight
  dht1.begin(); //Initialize the first DHT sensor
  dht2.begin(); //Initialize the second DHT sensor
  dht3.begin(); //Initialize the third DHT sensor

  //Quick 2 blinks of backlight
  for(int i = 0; i< 2; i++) {  
    lcd.backlight();
    delay(25);
    lcd.noBacklight();
    delay(25);
  }

  lcd.backlight(); // Finish with backlight on

  lcd.setCursor(0,0); //Start at character 1 on line 0
  lcd.print("Sensor Monitor");
delay(500);
lcd.setCursor(4,1); //Start at character 4 on line 1
lcd.print("By: Ana");
delay(2000);

//SD card initialization
if (!SD.begin(4)) {
    Serial.println("SD card initialization failed.");
    return;
}
Serial.println("SD card is ready to use.");

myFile = SD.open("data.txt", FILE_WRITE);
if (myFile) {
    myFile.println("Arduino started");
    myFile.println("Humidity(%), Temperature(*C), CO2(ppm)\n");
    Serial.println("data.txt is ready to use.");
} else {
    Serial.println("Error opening file on SD card.");
}

updateLCD();

/*Loop repeats indefinitely*/
void loop() {
    // Reading the relative humidity and temperature of the three sensors
    // Relative humidity in Percentage
    // Default Temperature in Celsius
    h1 = dht1.readHumidity();
    t1 = dht1.readTemperature();
    h2 = dht2.readHumidity();
    t2 = dht2.readTemperature();
    h3 = dht3.readHumidity();
    t3 = dht3.readTemperature();

    //Checking if any reads failed
    if (isnan(h1)|| isnan(t1)) {
        Serial.println("Failed to read from DHT 1 sensor!");
        return;
    }

    if (isnan(h2)|| isnan(t2)) {
        Serial.println("Failed to read from DHT 2 sensor!");
        return;
    }

    if (isnan(h3)|| isnan(t3)) {
        Serial.println("Failed to read from DHT 3 sensor!");
        return;
    }

    //Reading the CO2 (ppm)
    VAI1 = map(analogRead(VAI1Pin),0,1023,0,5000);
    VAI2 = map(analogRead(VAI2Pin),0,1023,0,5000);
VAI3 = map(analogRead(VAI3Pin), 0, 1023, 0, 2500); // Error of the double value when used 5k so we used 2.5k

currentMillis = millis();
currentInterval = currentMillis - previousMillis;

// check to see if it's time to save the data; that is if the difference // between the current time and last time you saved is bigger than // the interval at which you want to save.
if (abs(currentInterval) >= saveInterval) {
    previousMillis = currentMillis;
    saveData();
}

void updateLCD();
{
    lcd.setCursor(1,0); //Start at character 0 on line 0
    lcd.print("System 1 Inlet");
    lcd.setCursor(0,1); //Start at character 0 on line 1
    lcd.print(h1);
    lcd.print("%");
    lcd.setCursor(4,1); //Start at character 4 on line 1
    lcd.print(t1);
    lcd.print("C");
    lcd.setCursor(8,1); //Start at character 4 on line 1
    lcd.print(VAI1);
    lcd.print("ppm");
    delay(2000);
}

cleanLCD();

lcd.setCursor(1,0); //Start at character 0 on line 0
lcd.print("System 2 Inlet");
lcd.setCursor(0,1); //Start at character 0 on line 1
lcd.print(h2);
lcd.print("%");
lcd.setCursor(4,1); //Start at character 4 on line 1
lcd.print(t2);
lcd.print("C");
lcd.setCursor(8,1); //Start at character 4 on line 1
lcd.print(VAI2);
lcd.print("ppm");

delay(2000);


cleanLCD();

lcd.setCursor(2,0); //Start at character 0 on line 0
lcd.print("S1+S2 Outlet");
lcd.setCursor(0,1); //Start at character 0 on line 1
lcd.print(h3);
lcd.print("%");
lcd.setCursor(4,1); //Start at character 4 on line 1
lcd.print(t3);
lcd.print("C");
lcd.setCursor(8,1); //Start at character 4 on line 1
lcd.print(VAI3);
lcd.print("ppm");

delay(2000);
cleanLCD();
}

void cleanLCD() {
lcd.setCursor (0, 0);
for (int i = 0; i < 16; ++i) {
    lcd.write(' ');
}
lcd.setCursor (0, 1);
for (int i = 0; i < 16; ++i) {
    lcd.write(' ');
}
}

void saveData() {
//Saving data on SD card
myFile = SD.open("data.txt", FILE_WRITE);

if (myFile) {
    Serial.println("Saving data on SD card...");
    myFile.print("S1_in: ");
    myFile.print(h1);
    myFile.print(",");
    myFile.print(t1);
    myFile.print(",");
    myFile.println(VAI1);
    myFile.print("S2_in: ");
    myFile.print(h2);
    myFile.print(",");
    myFile.print(t2);
    myFile.print(",");
    myFile.println(VAI2);
    myFile.print("S1+S2_out: ");
    myFile.print(h3);
    myFile.print(",");
    myFile.print(t3);
    myFile.print(",");
    myFile.println(VAI3);

    myFile.close(); //To close the file
} else {
    Serial.println("Error opening file on SD card.");
}

/*Send data to serial monitor*/
Serial.println("Humidity(%) , Temperature(*C), CO2(ppm)");
Serial.println("S1_in: ");
Serial.print(h1);
Serial.print("",");
Serial.print(t1);
Serial.print("{},");
Serial.println(VAI1);
Serial.print("S2_in: ");
Serial.print(h2);
Serial.print("{},");
Serial.print(t2);
Serial.print("{},");
Serial.println(VAI2);
Serial.print("S1+S2_out: ");
Serial.print(h3);
Serial.print("{},");
Serial.print(t3);
Serial.print("{},");
Serial.println(VAI3);
}
}
B.2. Dynamic respiration tests at 18% moisture content and 35°C

Three replicated respiration tests were conducted in each D-GRMS experimental unit. The system conditioned 18% w soybean samples in a controlled \( T \) of 35°C. During the first weeks of the replication 1, it was observed condensation inside the enclosed box where the sensors are placed and on the outlet of the \( RC \). To end the condensation a small heater was placed in front of the enclosed box and the room temperature was set to 30°C, which is the equivalent dewpoint for the respect relative humidity. The second and third replications were conducted at the same time. However, the level of respiration from these two last replications depleted and at the end of the test the moisture content was checked, and it had decreased about 4%.

The achieved \( DML \) was 0.60, 0.15, and 0.16% for replication 1, 2, and 3, respectively, of soybeans stored in the dynamic system for 20 days (Figure B.6). Replication 1 achieved \( t_{0.05} \) in 6.7 d, almost at the same time as replication 2 (6.8 d) and 3 (7.7 d). It can be observed that \( t_{0.05} \) was not different between replications and that the moisture content was not depleted. However, after 7 d, \( DML \) from the replications 2 and 3 started differing from replication 1, where probably the moisture content started depleted. And \( v_{DML} \) estimates from replication 1, 2, and 3 were 0.0374, 0.0085, and 0.0097 % d\(^{-1} \) (Table B.1.).

![Graph showing dry matter loss estimates (DML, %) over time (t, d) of soybeans at 35°C in dynamic grain respiration measurement systems (D-GRMS).](image)

Figure B.6. Dry matter loss estimates (\( DML, \% \)) over time (\( t, \) d) of soybeans at 35°C in dynamic grain respiration measurement systems (D-GRMS).
Table B.1. Dry matter loss rates of soybeans stored at 35°C in dynamic grain respiration measurement systems.

<table>
<thead>
<tr>
<th>Initial moisture ($w_1$, % w.b.)</th>
<th>Final moisture ($w_2$, % w.b.)</th>
<th>Replication ($r$)</th>
<th>Dry matter loss rate ($v_{DML}$, % d$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>17.78</td>
<td>18.91</td>
<td>1</td>
<td>0.0374</td>
</tr>
<tr>
<td>17.88</td>
<td>13.40</td>
<td>2</td>
<td>0.0085</td>
</tr>
<tr>
<td>18.09</td>
<td>13.90</td>
<td>3</td>
<td>0.0097</td>
</tr>
</tbody>
</table>
1.0. PURPOSE

This SOP explains the protocol for setting up the air supply CO₂ scrubber for the two dynamic grain respiration measurement system (D-GRMS) located in room 392 at National Soybean Research Center.

2.0. SCOPE

This SOP describes how to prepare the air supply CO₂ scrubber with Sodasorb®, clean and store the unit after each use.

3.0. RESPONSIBILITY

The supervisor will be responsible for training the personnel on proper use of D-GRMS and its components and implementing this protocol/procedure.

4.0. MATERIALS AND EQUIPMENT

4.1. Laboratory gas drying unit (qty = 1 per system, Product No. 26800, W.A. Hammond Drierite Co., Ltd., Xenia, OH, USA) – the unit includes 1 molded polycarbonate column, 1 polycarbonate cap fitting (screw-top) with an o-ring gasket, 2 desiccant supports or perforated metal disks one flat and another shaped, 1 coil spring, and 1 wrench.

4.2. Vincon flexible PVC tubing – 6.35 mm (0.25 in) ID (Part No. ABH02017, Saint-Gobain, Akron, OH, USA) or similar material with equivalent resistance.

4.3. Connectors – in-line hose barbs, non-spill coupling insert (Product Nos. 60719 and 60724, U.S. Plastics, Lima, OH, USA) or similar quick-disconnect coupler.

4.4. CO₂ absorbent – Sodasorb® (Product No. SODA-SORB-HP, Amron International, Vista, CA, USA) or similar material with equivalent absorbing capacity and particle size (e.g., granular).

4.5. Cotton rounds (Product No. 175480, Assured™, Chesapeake, VA, Canada).

4.6. 243 mL polypropylene funnel 104 mm Dia. & 21 mm Stem Dia. (Model No. Nalgene™ Powder Funnel, U.S. Plastics, Lima Ohio, OH, USA).

5.0. PROCEDURES

5.1. Print the respiration test datasheet at B.3. Dynamic Respiration Test Datasheet.

5.2. Start with a clean and dry laboratory gas drying unit.

5.3. Check if the tubing attached to the unit is secure and, on each end, a quick-disconnect coupler is securely inserted and attached.

5.4. Place the first perforated metal disk (flat) in the bottom of the column and after that place a cotton round on top of it to create a plenum.
5.5. Weigh 300 g of Sodasorb®.
5.6. Carefully pour the Sodasorb® into the unit using the funnel.
5.7. Place a cotton round and in sequence, the second perforated metal disk (shaped) on top of the Sodasorb®, followed by the coil spring.
5.8. Cover the filled column using the screw-top cap. Tighten cap using the supplied wrench.
5.9. Connect the filled drying unit in between the mass flow controller and the first glycerol-water reservoir of a D-GRMS. Connect tubing using quick-disconnects couple.
5.10. Check mark on the datasheet that the air supply CO₂ scrubber was filled.
5.11. When this air supply CO₂ scrubber has approximately 75% of its Sodasorb® color changed from white to purple, remove the scrubber from the system.
5.12. Open the unit and dispose of the Sodasorb® following chemical disposal guidelines by the UIUC Division of Research Safety.
5.13. Repeat Steps 5.5 to 5.19 to replace the Sodasorb® from the unit.
5.14. To assemble an air supply CO₂ scrubber for the second system, repeat Steps 5.1 to 5.13.

6.0. PROCEDURE: CLEANING AND REPORTING

6.1. After a grain respiration test, dispose the used Sodasorb® following chemical disposal guidelines by the UIUC Division of Research Safety.
6.2. Wash the column, metal disks, coil spring, and cap with warm soapy water and let dry at room temperature.
6.3. Check for scratches, cracks, and other defects in all components that would cause gas to leak in/out of the column. The Sodasorb® reaction is exothermic, and the acrylic cylinders can fail with repeated use.
6.4. Store clean and dry units in a cabinet at room temperature.
6.5. Record any issues with preparing, cleaning, and inspecting the units. If there are any issues, see corrective action (Section 7.0).

7.0. CORRECTIVE ACTION

7.1. When cracks or defects are found in the units and Sodasorb®, notify the supervisor immediately. Do not use damaged units or Sodasorb® in future grain respiration tests.
7.2. The supervisor will take further corrective actions which may include repairing or replacing damaged materials.

8.0. CHANGES FROM PREVIOUS VERSION

8.1. SOP drafted on 01 July 2016 by L. R. Trevisan.
8.2. Reviewed, revised, and approved by supervisor on 01 October 2017.
8.3. Second review, revision, and approval by supervisor on 10 October 2018.
1.0. PURPOSE

This SOP explains the protocol for preparing glycerol-water solutions for the first or second dynamic grain respiration measurement system (D-GRMS) located in room 392 at National Soybean Research Center.

2.0. SCOPE

This SOP describes how to prepare glycerol-water solutions used to control the humidity of the airstream during a grain respiration test, following guidelines by Forney and Brandl (1992).

3.0. RESPONSIBILITY

The supervisor will be responsible for training the personnel on proper use of D-GRMS and its components and implementing this protocol/procedure.

4.0. MATERIALS AND EQUIPMENT

4.1. Plastic vacuum bottles – heavy duty HDPE bottles with 83 mm cap, 2 L capacity (Product No. D1069702 Saint Gobain Performance, Akron, OH, USA) or similar vacuum bottle with the same volume capacity.
4.2. Digital precision scale range of 0 to 3100 g and 0.01 g resolution (Model iBalance i3100, MyWeigh, Phoenix, AZ, USA) or similar device with the same range and resolution.
4.3. Stirring hot plate – temperature range 30 to 540ºC and magnetic stirrer speed 60 to 1200 rpm (Model No. G33500, Fisher Scientific, Hampton, NH, USA) or similar device that could heat solution to 50ºC and stir at 100-200 rpm.
4.4. Magnetic stir bar and remover tool – octagonal with flat surfaces, 12.7 mm (0.5 in) long (Product No. S717737, Fisher Scientific, Hampton, NH, USA) or similar device.
4.5. Parafilm – 10.2 cm (4 in) wide (Product No. S37440, Fisher Scientific, Hampton, NH, USA) or similar material.
4.6. Glass beakers – 3000 mL beaker (qty = 2).
4.8. Glycerol – Certified ACS grade (Fisher Scientific, Hampton, NH, USA).
4.9. Deionized water.

5.0. PROCEDURES

5.1. Use the table and equations in Document No. D-GRMS-002a to calculate individual masses of glycerol and water needed in a 2 L mixture to deliver desired relative humidity at the temperature of the grain respiration test.
5.2. Weigh the amount of glycerol and water, according to results from Step 5.1, into separate 3000 mL beakers.
5.3. Carefully pour the glycerol into the 4.4 L glass bottle.
5.4. Rinse the glycerol beaker with a portion of the deionized water weighed out in Step 5.2 and transfer the rinse solution to the 4.4 L glass bottle. Repeat 2-3 more times to transfer all of the glycerol into the glass bottle.
5.5. Pour any remaining water from Step 5.2 into the 4.4 L glass bottle.
5.6. Gently drop the magnetic stir bar into the mixture.
5.7. Seal the bottle with parafilm to prevent evaporation.
5.8. Place the 4.4 L mixture on the stirring hot plate. Carefully set the temperature to 50°C and stir speed to 100-200 rpm.
5.9. Let the solution mix and warm up for 30 min. Use the timer to set the time.
5.10. Remove mixture from hot plate and let it cool to room temperature.
5.11. Pour mixture in equal parts into two vacuum bottles.
5.12. Connect the bottles in series, submerge them in the water bath placed after the mass flow controller, and let them reach the desired temperature prior to starting a grain respiration test. The same water bath can hold all four bottles from the two systems (2 of each).
5.13. Using the datasheet, check the humidification system steps. Record the temperature of the water bath, the preparation of the glycerol-water solutions with its respective equilibrium relative humidity value, and the weight of water and glycerol used.
5.14. Open the gas regulator and set the mass flow controller to 0.5 L min\(^{-1}\), close the enclosure box in which the sensors are located to measure temperature, relative humidity, and CO\(_2\) after the relative humidity.
5.15. Check the resulting relative humidity and adjust by adding glycerol in small increments (100-1000 µl at a time) to decrease humidity or by adding water in small increments (100-1000 µl at a time) to increase humidity. The tested relative humidity and added portions of water should be recorded in the datasheet to have a control about the water solution.
5.16. To prepare water-glycerol solutions for the second system repeat Steps 5.1 to 5.15.

6.0. PROCEDURE: FINAL STEPS AND REPORTING

6.1. Record the date when a fresh glycerol-water solution was made.
6.2. Glycerol-water solutions may only be re-used once. Store solutions for re-use at 4°C in a 4.4 L glass bottle labeling it with the equilibrium relative humidity, moisture content, date and time.
6.3. Prior to re-use, check for mold or off-odors and test the resulting relative humidity.
6.4. A solution that has been used for two grain respiration tests should be discarded by pouring it down the drain with copious amounts of water.
6.5. Vacuum bottles and beakers should be washed with warm soapy water and let dry at room temperature.
6.6. Check for scratches, cracks and other defects in vacuum bottles that could cause air to leak in/out of the bottle prior to each use.
6.7. Record any issues with preparing and re-using solutions. If there are any issues, see corrective action (Section 7.0).
7.0. CORRECTIVE ACTION

7.1. If the resulting relative humidity is below the desired humidity, calculate the amount of water needed to dilute the solution using information in D-GRMS-002a. Adjust accordingly by adding ½ the amount of water needed to each vacuum bottle.

7.2. Discard any glycerol-water solution that shows signs of molding or emits off-odors.

7.3. When cracks or defects are found in the bottles, notify the supervisor immediately.
   Do not use damaged bottles in future respiration tests.

7.4. The supervisor will take further corrective actions which may include repairing or replacing damaged materials.

8.0. CHANGES FROM PREVIOUS VERSION

8.1. SOP drafted on 01 July 2016 by L. R. Trevisan.

8.2. Reviewed, revised, and approved by supervisor on 01 October 2017.

8.3. Second review, revision, and approval by supervisor on 10 October 2018.
1.0. PURPOSE

This SOP provides a table and equations used to calculate the relative proportions of glycerol and water to make the mixture used for humidification in the two dynamic grain respiration measurement system (D-GRMS) located in room 392 at National Soybean Research Center.

2.0. SCOPE

This SOP provides glycerol-water concentrations for soybean respiration tests involving 12 to 22% moisture content soybeans to be stored at 25 to 35°C. Methodology followed guidelines of Forney and Brandl (1992).

3.0. RESPONSIBILITY

The supervisor will be responsible for training the personnel on proper use of D-GRMS and its components and implementing this protocol/procedure.

4.0. MATERIALS AND EQUIPMENT

4.1. Calculator or spreadsheet.
4.2. Laboratory notebook and respiration test datasheet to record calculations.

5.0. PROCEDURES

5.1. Important constants are the water density ($\rho_{H_2O}$) equal to 1 g mL$^{-1}$ and the glycerol density ($\rho_{C_3H_8O_3}$) equal to 1.262 g mL$^{-1}$.

5.2. The following equations should be used to calculate the specific gravity ($SG$) and concentration ($C_{C_3H_8O_3}$) of glycerol-water solutions:

$$SG = (-0.189\phi_{set} + 19.9)^{0.0806}$$

$$C_{C_3H_8O_3} = 383(SG - 1) \text{ (mass glycerol concentration)}$$

where $\phi_{set}$ is the setpoint (desired) relative humidity the D-GRMS will need to reach, which is equal to $\phi_e$ of the soybeans at desired moisture content.

5.3. The total mass ($M_{soln}$) and volume ($V_{soln}$) of the solution are dependent on the mass and/or volume of the glycerol ($V_{C_3H_8O_3}$) and water ($V_{H_2O}$) to be mixed:

$$M_{soln} = \frac{V_{H_2O} (100 \rho_{H_2O})}{(100 - C_{C_3H_8O_3})}$$

5.4. For example, for a 4000 g $M_{soln}$ and soybeans at 14% w and 30°C:

$$V_{H_2O} = \frac{(100 - C_{C_3H_8O_3}) M_{soln}}{(100 \rho_{H_2O})} = 1783.44 \text{ mL}$$

$$V_{C_3H_8O_3} = \frac{(C_{C_3H_8O_3}) M_{soln}}{(100 \rho_{C_3H_8O_3})} = 1766.25 \text{ mL}$$
5.5. The table B.2 has been developed to ease calculations.

**Table B.2. Calculated variables for preparing water-glycerol solutions for soybeans with 12, 14, 18, and 22% w and 25, 30, and 35°C.**

<table>
<thead>
<tr>
<th>w (% w.b.)</th>
<th>T (°C)</th>
<th>$\phi_e$ (%)</th>
<th>SG</th>
<th>$C_{\text{C}_3\text{H}_6\text{O}_3}$ (%)</th>
<th>$M_{\text{soln}}$ (g)</th>
<th>$V_{\text{H}_2\text{O}}$ (mL)</th>
<th>$V_{\text{C}_3\text{H}_6\text{O}_3}$ (mL)</th>
<th>$V_{\text{soln}}$ (mL)</th>
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</thead>
<tbody>
<tr>
<td>12</td>
<td>25</td>
<td>65</td>
<td>1.18</td>
<td>68.09</td>
<td>4000</td>
<td>1276.48</td>
<td>2158.10</td>
<td>3434.58</td>
</tr>
<tr>
<td>30</td>
<td>66</td>
<td>1.18</td>
<td>67.18</td>
<td>4000</td>
<td>1312.99</td>
<td>2141.13</td>
<td>3451.12</td>
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</tr>
<tr>
<td>35</td>
<td>68</td>
<td>1.17</td>
<td>66.24</td>
<td>4000</td>
<td>1350.37</td>
<td>2099.55</td>
<td>3449.92</td>
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<tr>
<td>14</td>
<td>25</td>
<td>76</td>
<td>1.15</td>
<td>56.64</td>
<td>4000</td>
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<td>3435.22</td>
<td>447.53</td>
<td>3882.75</td>
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</tr>
</tbody>
</table>

5.6. To prepare water-glycerol solutions for the second system repeat Steps 5.1 to 5.4.

6.0. **PROCEDURES: FINAL STEPS AND REPORTING**

6.1. Use calculations from Step 5.0 when preparing water-glycerol solutions when following Document No. D-GRMS-002.

6.2. Record any issues with preparing solutions. If there are any issues, see corrective action (Section 7.0).

7.0. **CORRECTIVE ACTION**

7.1. Discard any glycerol that shows signs of molding or emits off-odors.

7.2. The supervisor will take further corrective actions which may include buying new glycerol.

8.0. **CHANGES FROM PREVIOUS VERSION**

8.1. SOP drafted on 01 July 2016 by L. R. Trevisan.

8.2. Reviewed, revised, and approved by supervisor on 01 October 2017.

8.3. Second review, revision, and approval by supervisor on 10 October 2018.
1.0. PURPOSE

This SOP explains the protocol for setting up the respiration chamber ($RC$) dehumidifiers for the two dynamic grain respiration measurement system (D-GRMS) located in room 392 at National Soybean Research Center.

2.0. SCOPE

This SOP describes how to prepare two $RC$ dehumidifiers per system, clean, and store the unit after each use.

3.0. RESPONSIBILITY

The supervisor will be responsible for training the personnel on proper use of D-GRMS and its components and implementing the protocol/procedure.

4.0. MATERIALS AND EQUIPMENT

4.1. Laboratory gas drying units (qty = 2 per system, Product No. 26800, W.A. Hammond Drierite Co., Ltd., Xenia, OH, USA). Each unit includes 1 molded polycarbonate column, 1 polycarbonate cap fitting (screw-top) with an o-ring gasket, 2 desiccant supports or perforated metal disks one flat and another shaped, 1 coil spring, and 1 wrench.

4.2. Vincon flexible PVC tubing – 6.35 mm (0.25 in) ID (Part No. ABH02017, Saint-Gobain, Akron, OH, USA) or similar material with equivalent resistance.

4.3. Connectors – in-line hose barbs, non-spill coupling body and insert (Product Nos. 60719 and 60724, U.S. Plastics, Lima, OH, USA) or similar quick-disconnect couplers.


4.5. Desiccant – Drierite®, 8 mesh, with indicator (Product No. 23025, W.A. Hammond Drierite Co., Ltd., Xenia, OH, USA) or similar material.

4.6. Cotton rounds (Product No. 175480, Assured™, Chesapeake, VA, Canada).

4.7. 243 mL polypropylene funnel 104 mm Dia. & 21 mm Stem Dia. (Model No. Nalgene™ Powder Funnel, U.S. Plastics, Lima Ohio, OH, USA).

4.8. Convection oven (Model No. VWR-U50, VWR™, Radnor, PA, USA) set at 210°C, or similar equipment.

5.0. PROCEDURES

5.1. Start with one clean and dry laboratory gas drying unit.

5.2. Check that tubing attachments are secure, and on each end, a quick-disconnect coupler is securely attached.
5.3. Place the first perforated metal disk (flat) in the bottom of the column and after that place a cotton round on top of it to create a plenum.
5.4. Weigh 500 g of desiccant.
5.5. Carefully pour the desiccant into the unit using the funnel.
5.6. Place a cotton round and in sequence, the second perforated metal disk (shaped) on top of desiccant, followed by the coil spring.
5.7. Cover the filled column using the screw-top cap. Tighten cap using the supplied wrench.
5.8. Repeat Steps 5.1 to 5.7 to make a second RC scrubber dehumidifier unit.
5.9. Connect the two RC dehumidifiers in parallel in one D-GRMS immediately following the RC using two valves and set up the valves so that airstream passes through the first RC dehumidifier (designated as “A”; the second scrubber is designated as “B”).
5.10. Check the box the datasheet that two RC dehumidifiers were filled.
5.11. When the RC dehumidifiers have approximately 75% of their desiccant color changed from blue to purple, remove one dehumidifier by time from the system to change the desiccant switching the side of the air flow. For example, when changing dehumidifier “A”, switching the valve to side “B”.
5.12. Repeat Steps 5.1 to 5.11 to replace the desiccant in the dehumidifier.
5.13. Repeat Steps 5.11 and 5.12 to replace the desiccant in the other dehumidifier, but this time switch the valve to the opposite direction.
5.14. To set up RC dehumidifier for the second system repeat Steps 5.1 to 5.13.

6.0. PROCEDURE: CLEANING AND REPORTING

6.1. Each time that the desiccant has been changed or at the end of a respiration test, regenerate spent desiccant at 210°C for 1 h using the oven.
6.2. Wash the units, metal disks, coil springs, caps, and separators with warm soapy water. Be sure to scrub off any traces of mold. Let dry at room temperature.
6.3. Check for scratches, cracks, and other defects in all components that would cause gas to leak in/out of the column.
6.4. Store clean and dry units in a cabinet at room temperature.
6.5. Record any issues with preparing, cleaning, and inspecting the units. If there are any issues, see corrective action (Section 7.0).

7.0. CORRECTIVE ACTION

7.1. When cracks or defects are found in the dry gas units and desiccant, notify the supervisor immediately. Do not use damaged units or desiccant for future grain respiration tests.
7.2. The supervisor will take further corrective actions which may include repairing or replacing damaged materials.

8.0. CHANGES FROM PREVIOUS VERSION

8.1. SOP drafted on 01 July 2016 by L. R. Trevisan.
8.2. Reviewed, revised, and approved by supervisor on 01 October 2017.
8.3. Second review, revision, and approval by supervisor on 10 October 2018.
1.0. PURPOSE

This SOP explains the protocol for setting up the RC CO₂ scrubbers for the two dynamic grain respiration measurement system (D-GRMS) located in room 392 at National Soybean Research Center.

2.0. SCOPE

This SOP describes how to prepare RC CO₂ scrubbers per system, clean, and store the units after each use.

3.0. RESPONSIBILITY

The supervisor will be responsible for training the personnel on proper use of D-GRMS and its components and implementing the protocol/procedure.

4.0. MATERIALS AND EQUIPMENT

4.1. Laboratory gas drying units (qty = 2 per system, Product No. 26800, W.A. Hammond Drierite Co., Ltd., Xenia, OH, USA). Each unit includes 1 molded polycarbonate column, 1 polycarbonate cap fitting (screw-top) with an o-ring gasket, 2 desiccant supports or perforated metal disks one flat and another shaped, 1 coil spring, and 1 wrench.

4.2. Separators – each separator is custom-fabricated of a plastic cylinder (2.5 cm ID x 1.5 mm height) with a plastic perforated disk (40% open, 0.3 cm dia. holes) on each end. Two separators per unit is needed for a total of four separators.

4.3. Convection oven (Model No. VWR-U50, VWR™, Radnor, PA, USA) set at 210°C, or similar equipment.

4.4. Vincon Flexible PVC tubing – 6.35 mm (0.25 in) ID (Part No. ABH02017, Saint-Gobain, Akron, OH, USA) or similar material with equivalent resistance.

4.5. Connectors – in-line hose barbs, non-spill coupling body and insert (Product Nos. 60719 and 60724, U.S. Plastics, Lima, OH, USA) or similar quick-disconnect couplers.


4.7. CO₂ absorbent – Sodasorb® (Product No. SODA-SORB-HP, Amron International, Vista, CA, USA) or similar material with equivalent absorbing capacity and particle size (e.g., granular).

4.8. Desiccant – Drierite®, 8 mesh, with indicator (Product No. 23025, W.A. Hammond Drierite Co., Ltd., Xenia, OH, USA) or similar material.

4.9. Cotton rounds (Product No. 175480, Assured™, Chesapeake, VA, Canada).

4.10. 243 mL polypropylene funnel 104 mm Dia. & 21 mm Stem Dia. (Model No. Nalgene™ Powder Funnel, U.S. Plastics, Lima Ohio, OH, USA).
5.0. PROCEDURES

5.1. Start with one clean and dry laboratory gas drying unit.
5.2. Check if the tubing attached to the unit are secure and, on each end, a quick-
disconnect coupler is securely attached.
5.3. Place the first perforated metal disk (flat) in the bottom of the column and after that
place a cotton round on top of it to create a plenum.
5.4. Weigh 150 g of Sodasorb® and write down the measurement on datasheet
5.5. Carefully pour the Sodasorb® into the unit using the funnel.
5.6. Place one separator on top of the Sodasorb® and a cotton round on top of the
separator.
5.7. Weigh 290 g of desiccant and write down the measurement on datasheet.
5.8. Carefully pour the desiccant into the unit using the funnel.
5.9. Place a cotton round and in sequence, the second perforated metal disk (shaped) on
top of desiccant, followed by the coil spring.
5.10. Cover the filled column using the screw-top cap. Tighten cap using the supplied
wrench.
5.11. Weigh the filled column three times, rotating the column 120° in between
measurements. Record each weight and their average on the datasheet.
5.12. Repeat Steps 5.1 to 5.11 to make a second RC scrubber column.
5.13. Connect the two RC CO₂ scrubbers in parallel in the D-GRMS using two valves and
set up the valves so that airstream passes through the first RC scrubber (designated as
“A”; the second scrubber is designated as “B”).
5.14. Check the box on the datasheet that two RC CO₂ scrubbers were filled.
5.15. When the RC CO₂ scrubbers have approximately 75% of their desiccant color
changed from blue to purple or their Sodasorb® color changed from white to purple,
remove one scrubber by time from the system to change the desiccant or the
Sodasorb® switching the side of the air flow. For example, when changing
dehumidifier “A”, switching the valve to side “B”.
5.16. Repeat Steps 5.1 to 5.11 to replace the desiccant or Sodasorb® from the scrubber
and place it back on the system.
5.17. Repeat Steps 5.15 and 5.16 to replace the desiccant or Sodasorb® from the other
scrubber, but this time switch the valve for the opposite direction.
5.18. To set up RC CO₂ scrubbers for the second system repeat Steps 5.1 to 5.17.

6.0. PROCEDURE: CLEANING AND REPORTING

6.1. After a grain respiration test, repeat Step 5.10.
6.2. For every time that the desiccant has been changed or at the end of a respiration test,
regenerate spent desiccant at 210°C for 1 h using the oven.
6.3. Dispose used Sodasorb® following chemical disposal guidelines by the UIUC
Division of Research Safety.
6.4. Wash the gas units, metal disks, coil springs, caps, and separators with warm soapy
water. Be sure to scrub any traces of mold. Let dry at room temperature.
6.5. Check for scratches, cracks, and other defects in all components that would cause gas
to leak in/out of the column.
6.6. Store clean and dry units in a cabinet at room temperature.
6.7. Record any issues with preparing, cleaning, and inspecting the units. If there are any issues, see corrective action (Section 7.0).

7.0. **CORRECTIVE ACTION**

7.1. When cracks or defects are found in the gas drying units, Sodasorb®, and desiccant, notify the supervisor immediately. Do not use damaged columns, Sodasorb®, or desiccant future grain respiration tests.

7.2. The supervisor will take further corrective actions which may include repairing or replacing damaged materials.

8.0. **CHANGES FROM PREVIOUS VERSION**

8.1. SOP drafted on 01 July 2016 by L. R. Trevisan.
8.2. Reviewed, revised, and approved by supervisor on 01 October 2017.
8.3. Second review, revision, and approval by supervisor on 10 October 2018.
1.0. **PURPOSE**

This SOP explains the protocol for preparing the soybeans sample for a grain respiration test in two dynamic grain respiration measurement system (D-GRMS) located in room 392 at National Soybean Research Center.

2.0. **SCOPE**

This SOP describes how to clean and re-wet soybeans for a grain respiration test.

3.0. **RESPONSIBILITY**

The supervisor will be responsible for training the personnel on proper use of S-GRMS and its components, preparing samples, and implementing this protocol/procedure.

4.0. **MATERIALS AND EQUIPMENT**

   4.1. Refrigerated soybeans sample (qty = 3000 g per system).
   4.2. Aluminum grain sieve (Grainman 10/64” x 3/4”, Miami, FL, USA) to remove impurities and splits or damaged beans.
   4.3. Portable moisture meter (Model No. SW16060, John Deere, Moline, IL, USA).
   4.4. Roller mixers (qty = 2; Model No. MX-T6-S, Scilogex, Rocky Hill, CT, USA).
   4.5. Digital precision scale range of 0 to 3100 g and 0.01 g resolution (Model iBalance i3100, MyWeigh, Phoenix, AZ, USA).
   4.6. Temperature-controlled incubator (Model No. PT2445, Exo Terra®, Mansfield, MA, USA) set at the respiration test temperature.
   4.7. Custom-fabricated metal baskets (qty = 2 per system).
   4.8. 2 L capacity plastic bottles (qty = 2; Model No. 2202-0005, U.S. Plastics, Lima Ohio, OH, USA) wrapped with 3 rubber bands to increase friction.
   4.9. 100 mL capacity glass beaker (qty = 2).
   4.10. 32 oz polyethylene funnel 6-3/8” Dia. x 7-1/8”H, (Model No. 832WN, U.S. Plastics, Lima Ohio, OH, USA).
   4.11. Aluminum tray (92 x 62 x 3 cm).

5.0. **PROCEDURES**

   5.1. Soybeans need to be cleaned and acclimated inside the temperature-controlled incubator for 5 days at the desired test temperature before starting a respiration test.
   5.2. Print the respiration test datasheet at section B.3. Dynamic Respiration Test Datasheet.
5.3. Remove the 3000 g of soybean sample from the refrigerated storage.
5.4. Weigh the sample and write down the initial weight and the acclimation start time on the datasheet.
5.5. Clean the beans to remove foreign materials, splits, broken, and damaged seeds using the sieve.
5.6. Place the beans inside the two metal baskets.
5.7. Let the beans acclimate inside the temperature-controlled incubator at the desired respiration test temperature for 5 days.
5.8. After the period of 5 days acclimation, check the moisture content of the cleaned sample with a moisture meter to see if the sample will need to be re-wetted. Follow the next steps if the moisture content is lower than desired or continue this protocol from Step 5.18.
5.9. Weigh two separate aliquots of 1,250 g, write down the measurement value (m_{soy,0}) for the assigned bottle (A or B) on the datasheet.
5.10. In triplicates, estimate the soybean moisture content per bottle using the portable digital moisture meter and write down on the datasheet the average measurement (\bar{\hat{\omega}}_{soy,0}).
5.11. Calculate the amount of deionized water per bottle (m_{H_2O}) needed to achieve the desired moisture content (\bar{\hat{\omega}}_{soy,1}) using the equation:
\[ m_{H_2O} = m_{soy,0} \left( \frac{\bar{\hat{\omega}}_{soy,1} - \bar{\hat{\omega}}_{soy,0}}{100 - \bar{\hat{\omega}}_{soy,1}} \right) \]
5.12. Each bottle will have its m_{H_2O} calculated according to its individual soybeans sample weight and moisture content.
5.13. Record the necessary m_{H_2O} for bottle A and B in the datasheet.
5.14. Using a glass beaker for each bottle weigh out m_{H_2O} needed adding an extra amount of 10 g. Do not forget to tare the digital scale.
5.15. Carefully transfer the two aliquots soybeans samples into plastic its assigned bottle using a plastic funnel and add aliquots of m_{H_2O} from each bottle amount to the clean soybeans.
5.16. Place bottles on their sides on the roller mixer set it to 60 rpm and set the timer to 60 min.
5.17. Every 10 min remove the bottles, add another aliquot of m_{H_2O}, and place back on the roller mixer. Repeat until the needed m_{H_2O} for each bottle is achieved. The soybean sample and water should stay mixing in the roller for 60 min. Therefore, it is important to finish adding m_{H_2O} aliquots before this total time interval.
5.18. Spread a thin layer of soybeans on an aluminum tray and let stand at room temperature to air-dry until \bar{\hat{\omega}}_{soy,1} is reached.
5.19. Use the portable moisture meter to check \bar{\hat{\omega}}_{soy,1}. It is recommended when re-wetting the beans to check \bar{\hat{\omega}}_{soy,1} every 10 min. When \bar{\hat{\omega}}_{soy,1} is reached, from mixed triplicates samples, write down its moisture content using a moisture meter and save the subsamples for gravimetrically measure moisture content.
5.20. Follow the Document No. D-GRMS-006 to gravimetrically measure moisture content.
5.21. The sample is ready to be used in the respiration tests. One 3000 g sample provides one replication in a D-GRMS (1800 g per respiration chamber).

6.0. **PROCEDURE: FINAL STEPS AND REPORTING**

6.1. Do not forget to write down all the details listed above on the datasheet.
6.2. From the re-wetted sample, weigh 500 g of sample for further chemical analyses, place it in a labeled resealable plastic bag and store it at -18°C.
6.3. Discard all foreign materials, split, broken, damaged and excess bens.
6.4. Clean every equipment after using it for this protocol.
6.5. Write down any observations during the execution of this protocol, including mold on the refrigerated sample. If there are any issues, see corrective action (Section 7.0).

7.0. **CORRECTIVE ACTION**

7.1. Pay attention to the digital scale, remember to always tare the scale before any measurement. Check always for the calibration using a known weight. Be sure to use the same digital scale for all measurements throughout a grain respiration test or gravimetric moisture content measurement (not just individual tests or experiments). Any issues with the digital scale notify the supervisor immediately.
7.2. If mold is detected in the refrigerated soybean tote, notify the supervisor immediately. Do not use any soybean with visible molds or off-odors in a respiration test.
7.3. The supervisor will take further corrective actions which may include discarding soybeans samples and procuring new soybeans from Crop Sciences Research and Education Farm of the University of Illinois at Urbana-Champaign.

8.0. **CHANGES FROM PREVIOUS VERSION**

8.1. SOP drafted on 01 July 2016 by L. R. Trevisan.
8.2. Reviewed, revised, and approved by supervisor on 01 October 2017.
8.3. Second review, revision, and approval by supervisor on 10 October 2018.
1.0. PURPOSE

This SOP explains the protocol for determining the moisture content of soybeans according to ASAE Standard S352 (R2017).

2.0. SCOPE

This SOP describes how to determine gravimetrically moisture content of soybeans before and after a grain respiration test.

3.0. RESPONSIBILITY

The supervisor will be responsible for training the personnel on proper use of D-GRMS and its components, preparing samples, and implementing this protocol/procedure.

4.0. MATERIALS AND EQUIPMENT

4.1. Soybeans subsamples before and after respiration test.
4.2. Convection oven (Model No. 160DM Thelco® Laboratory oven, Precision Scientific Inc., Chicago, IL, USA) set at 103°C or similar equipment.
4.3. Digital precision scale range of 0 to 3100 g and 0.01 g resolution (Model iBalance i3100, MyWeigh, Phoenix, AZ, USA).
4.4. Custom-fabricated desiccator cabinet with desiccant (Catalog No. 23025, WA Hammond Drierite Co., Ltd., Xenia, OH, USA).
4.5. Aluminum weighing or moisture dishes – 4 oz utility cup full curl (Product No. 42330, Pactiv, Lake Forest, IL, USA).
4.6. Perforated Aluminum Microbiological Basket/Carriers (16”x16”).

5.0. PROCEDURES

5.1. Label with a unique tag three aluminum moisture dishes. For example, “Test 1A-1a, Test 1A-2a, and Test 1A-3a” labels are for three subsamples for Test 1. The uppercase letters denote whether samples were taken before (A) and after (B) a grain respiration test. Lowercase letters denote whether samples were filled with (a) wet or (b) oven-dried soybeans.
5.2. Tare the empty digital scale. Place one dish at a time on the scale and write down its mass on the first column (dish mass) of the first gravimetric measurement of moisture content table in the printed datasheet (e.g., $m_{1A}, m_{2A}, m_{3A}$).
5.3. Repeat Step 5.2 for each dish.
5.4. Without touching or moving the dish, gently pour one of the soybean subsamples saved at Step 5.19 of Document No. D-GRMS-005 until reaching approximately 30 g. Record the total mass of the dish with wet soybeans at the second column (dish +...
wet sample) of the same table (e.g., $m_{1A-1a}$, $m_{1A-2a}$, $m_{1A-3a}$). Carefully remove dish with wet soybeans from the scale.

5.5. Repeat Step 5.4 for the two remaining soybean subsamples.

5.6. Place the three filled dishes on a perforated aluminum basket and place it inside the convection oven set at 103°C for 72 h.

5.7. Remove the basket from the oven and let it cool down inside the desiccator cabinet for 20 min.

5.8. Carefully remove the basket from the cabinet and quickly weigh three times each dish’s dry weight e.g., $m_{1A-1b}$, $m_{1A-2b}$, $m_{1A-3b}$). Write down on the third column of the datasheet table (dish + dry sample) the measured values.

5.9. Per subsample, calculate the following:

- mass of wet soybeans: $m_{soy,1a} = m_{1A-1a} - m_{1A}$
- mass of dry matter: $m_{DM,1} = m_{1A-1b} - m_{1A}$
- mass of moisture removed: $m_{H_2O,1} = m_{soy,1a} - m_{DM,1}$
- moisture content of soybean sample (% w.b.): $w_{soy,1a} = \left( \frac{m_{H_2O,1}}{m_{soy,1a}} \right) \times 100$

5.10. Repeat step 5.9 for the other two soybeans subsamples and write down the moisture content values found on the last column of the datasheet table (calculated moisture content).

5.11. Calculate the average moisture content of soybeans taken before a grain respiration test:

$$\bar{w}_{soy,1} = \frac{w_{soy,1a} + w_{soy,2a} + w_{soy,3a}}{3}$$

5.12. To determine average moisture content of samples taken after a grain respiration test, repeat Steps 5.1 to 5.11 taking care to label the subsamples appropriately. Do not forget to write down all the measured weights on the second gravimetric measurement of moisture content table in the printed datasheet.

5.13. Compute the standard deviation and record it.

6.0. **PROCEDURE: FINAL STEPS AND REPORTING**


6.2. Record any issues with determining gravimetrically moisture content of soybeans. If there are any issues, see corrective action (Section 7.0).

7.0. **CORRECTIVE ACTION**

7.1. Pay attention to the digital scale, remind to always tare the scale before any measurement. Check always for the calibration using a known weight. Be sure to use the same digital scale for all measurements throughout a gravimetric moisture content measurement (not just individual tests or experiments). Any issues with the digital scale notify the supervisor immediately.

7.2. The supervisor will take further corrective actions which may include re-calibrating or replacing the digital scale.
8.0. **CHANGES FROM PREVIOUS VERSION**

8.1. SOP drafted on 01 July 2016 by L. R. Trevisan.

8.2. Reviewed, revised, and approved by supervisor on 01 October 2017.

8.3. Second review, revision, and approval by supervisor on 10 October 2018.
1.0. PURPOSE

This SOP explains the protocol for running a grain respiration test in a dynamic grain respiration measurement system (D-GRMS) located in room 392 at National Soybean Research Center.

2.0. SCOPE

This SOP describes how to set-up the D-GRMS prior to starting a test, collect and measure respired CO$_2$ by the soybeans, calculate dry matter loss over time, end the test, and clean up the system.

3.0. RESPONSIBILITY

The supervisor will be responsible for training the personnel on proper use of D-GRMS and its components, preparing samples, and implementing the protocol/procedure.

4.0. MATERIALS AND EQUIPMENT

4.1. D-GRMS with air conditioning and flow management, grain storage, moisture, and CO$_2$ absorption, and instrumentation sections see Figure 3.1 from Trevisan (2017).
4.2. Air supply CO$_2$ scrubber (qty = 1 per system) prepared according to Document No. D-GRMS-001.
4.3. Glycerol-water solution (qty = 4 L per system) prepared according to Document Nos. D-GRMS-002 and 002a.
4.4. RC dehumidifiers (qty = 2 per system) prepared according to Document No. D-GRMS-003.
4.5. RC CO$_2$ scrubbers (qty = 2 per system) prepared according to Document No. D-GRMS-004.
4.6. Clean, acclimated and re-wetted soybeans (qty = 1.8 kg per system) prepared according to Document No. D-GRMS-005.
4.7. Digital precision scale range of 0 to 3100 g and 0.01 g resolution (Model iBalance i3100, MyWeigh, Phoenix, AZ, USA).
4.8. Power supply – regulated power supply 24V (Model No. PSR-2/24, EMCO, Allen, TX, USA) for mass flow controller and Vaisala sensors.
4.9. Power supply (110 V ac) for water baths and heat tape.
4.12. Aluminum tray (92 x 62 x 3 cm).
4.13. Vacuum grease lubricant (Model High vacuum grease, Dow Corning®, Dow Chemical, Midland, MI, USA).
4.15. Glass bottle – 4.4 L capacity.
5.0. PROCEDURES

5.1. Soybean samples need to be clean and acclimated for 5 days before starting the respiration test, follow Steps 5.1 to 5.7 from Document No. D-GRMS-005 to clean and acclimate the soybeans.

5.2. Turn ON all components of the D-GRMS. Allow water baths to reach test temperatures.

5.3. Before starting to prepare the soybean sample, print the respiration test datasheet at B.3. Dynamic Respiration Test Datasheet.

5.4. Write down on the datasheet the test and system numbers, desired moisture content and temperature, and date and time that the preparation for the test started.

5.5. Prepare and connect the air supply CO$_2$ scrubber, glycerol-water solutions, $RC$ dehumidifiers, and $RC$ CO$_2$ scrubbers in the D-GRMS following their respective Document Nos. D-GRMS-001, 002, 003 and 004. The air supply scrubbers and dehumidifiers need to have their air valves flow all for the same side (A). Record all the information on the datasheet.

5.6. Turn ON the gas guard, open the two compressed gas cylinders, and adjust their regulators valves to supply 15 psi to the system. Close the enclosure where the $T$, $\phi$, and CO$_2$ sensors are placed, sealing it with vacuum grease. Leave the $RC$ open to monitor the desired storage conditions coming in to it.

5.7. Check the flow rate as indicated on the LCD display of the Arduino-based fine adjustment of the mass flow controller. If $Q \neq 0.50 \pm 0.02$ L min$^{-1}$, adjust by tuning the digital potentiometer accordingly by pressing the buttons on the top of the controller to increase or decrease the flow.

5.8. Check all components, tubing, and insulation of the assembled D-GRMS for any cracks, loose connections, or other defects that could allow gas to leak in/out of the system during a test.

5.9. For 10 min, let the compressed air flow through the system to flush out air, moisture, and CO$_2$ initially present in the tubing.

5.10. Turn ON the serial monitor of the Arduino software to start recording temperature, relative humidity, and CO$_2$ levels at the inlet of the respiration chamber ($RC$) and at the exhaust of the D-GRMS.

5.11. After 20 min, check the temperature, relative humidity, and CO$_2$ levels on the LCD display that monitoring these conditions. If temperature and relative humidity are outside their limits, adjust water bath thermostats and glycerol-water solution concentration, as needed. If CO$_2$ concentration is outside its limit, look for leaks by checking for any cracks, loose connections, or other defects. However, if the problem persists, abort the test by turning OFF all system components.

5.12. Take the clean acclimated soybean sample from the incubator and re-wet it following Steps 5.7 to 6.5 from Document No. D-GRMS-005. Write down the information about the soybean sample preparation, including the gravimetrically moisture content estimate.

5.13. Weigh 1.8 kg of clean, re-wetted soybeans using the 3000 L beaker. Record actual weight ($m_{soy,1}$) in the datasheet and from the remaining sample collect 500 g for further chemical analyses.
5.14. Using the moisture content of the soybeans \( w_{soy,1} \), estimate the mass of dry solids of the soybeans:

\[
m_{DM,1} = m_{soy,1} \left( 1 - \frac{w_{soy,1}}{100} \right)
\]

5.15. Carefully pour beans into the \( RC \). Cover the \( RC \), place the lid sealing it with vacuum grease, and connect the \( RC \) in the D-GRMS.

5.16. Check mark that all tubing, connectors, and flowmeters were verified. Record the time when the test conditions have been achieved.

5.17. Let the system run for 12-14 h undisturbed. During this period, the soybeans are acclimating to their new storage environment.

5.18. Quickly divert airflow from \( RC \) CO\(_2\) scrubber A to \( RC \) CO\(_2\) scrubber B, switching the four valves.

5.19. Detach the CO\(_2\) scrubber A from D-GRMS. Determine its average weight from three measurements, taking care to rotate the scrubber 120° in between measurements. Record all measurements in the respiration test table at the datasheet, along with the time of measurement (hh:mm).

5.20. Connect scrubber A back into D-GRMS.

5.21. After 2 h, divert airflow from scrubber B to scrubber A.

5.22. Repeat Steps 5.18 and 5.19 with scrubber B.

5.23. Conduct weight measurements every 2 h during the daytime, alternating between the two scrubbers each time. Check Document No. DGRMS-008 for extra information on daily measurements.

5.24. All weight measurements represent the accumulated respired CO\(_2\) in the scrubbers. Normalize each weight measurement to \( m_{DM,1} \).

5.25. The criteria to stop the respiration test will depend on a criterion decision to be developed with the supervisor. It can be for a period of time, for example, 20 d if the respiration rates are low, or until 14.7 g CO\(_2\) (kg dry beans\(^{-1}\)) equivalent to 1.0 % \( DML \) is reached. Once the criterion is achieved, terminate the grain respiration test.

5.26. During testing, if the air supply CO\(_2\) scrubber, \( RC \) dehumidifier or \( RC \) CO\(_2\) scrubbers have approximately 75% of their Sodasorb® color changed from white to purple or their desiccant color changed from blue to purple, they should be refreshed. Remove one scrubber at a time from the system to change the Sodasorb® or desiccant, for the scrubbers with sides A and B, switch the side of the air flow first before changing. For example, when changing dehumidifier “A”, switching the valve to side “B”. Follow their respective protocols, including recording initial weights.

6.0. **PROCEDURE: FINAL STEPS AND REPORTING**

6.1. Open the electronic datasheet template (Appendix B.4.1. Supplemental file: Dynamic Respiration Test Electronic Datasheet) and save the worksheet using a unique name that denotes the specific grain respiration test (e.g., 14%-35C-rep1-s1).

6.2. Copy all weight and time of measurements data on the rows 20 and columns A, E, F, G, K, L, and M from the respiration test table and the extra information on cells B8, B12, D6, D7, D8, D9, and G8. Do not forget to include the two gravimetric moisture content tables.
6.3. After filling the electronic datasheet, respired CO₂ (g CO₂ [kg dry beans]⁻¹) and DML (%) are automatically calculated when the data is inserted on the worksheet. Check section B.4.2. Supplemental File Example: Static Respiration Test Electronic Datasheet to see an example of calculated data.

6.4. Steps 6.1 and 6.2 can be done right after the beginning of a respiration test and over time the electronic datasheet can be filled with the weight and time measurements. This is helpful to see if something is wrong with the system, such as any leaks or excess mold growth causing increased respiration.

6.5. At the end of each respiration test, turn OFF all system components and remove the lid of RC. Record the date and time when the test terminated on the printed datasheet.

6.6. Place a 3000 L glass beaker on a digital scale. Tare the weight.

6.7. Gently transfer soybeans into the beaker to determine and record its weight after being tested, write down this value on the datasheet.

6.8. Spread the soybeans onto an aluminum tray. Mix manually by hand and retrieve three samples (25-30 g each).

6.9. Determine the moisture content of the soybeans gravimetrically following procedures outlined in Document No. D-GRMS-0006 writing down the moisture content information on the datasheet.

6.10. Set aside 500 g of soybeans for future chemical analyses. Discard remaining soybeans following disposal guidelines by the UIUC Division of Research Safety.

6.11. Save the water-glycerol solution in a 4.4 L glass bottle and refrigerate the solution if it was only used one time in a respiration test. If it was used twice, dispose of the solution following Document No. D-GRMS-002.

6.12. Record any issues encountered during a grain respiration test. If there are any issues, see corrective action (Section 7.0).

6.13. Both systems follow this same protocol, so all the steps should be repeated to start a second system.

7.0. CORRECTIVE ACTION

7.1. Pay attention to calibration, drift, bias, etc. issues with the digital scale. Be sure to use the same digital scale for all weight measurements throughout a grain respiration study (not just individual tests or experiments). When scale issues arise, notify the supervisor immediately.

7.2. Pay attention to all sensor readings. If temperature, relative humidity, and flow rate stray from their limits, adjust accordingly. Note adjustments in the electronic datasheets and notify supervisor immediately. If CO₂ levels go off limits, abort grain respiration test and notify supervisor immediately.

7.3. The supervisor will take further corrective actions which may include testing for leaks and repairing or replacing system components.

8.0. CHANGES FROM PREVIOUS VERSION

8.1. SOP drafted on 01 July 2016 by L. R. Trevisan.

8.2. Reviewed, revised, and approved by supervisor on 01 October 2017.

8.3. Second review, revision, and approval by supervisor on 10 October 2018.
1.0. PURPOSE

This SOP explains the protocol for the daily measurements of the $RC \ CO_2$ scrubber measurements while running a grain respiration test in a dynamic grain respiration measurement system (D-GRMS) located in room 392 at National Soybean Research Center.

2.0. SCOPE

This SOP describes how to check the respiration test daily and how to measure the accumulated CO$_2$ in D-GRMS.

3.0. RESPONSIBILITY

The supervisor will be responsible for training the personnel on proper use of D-GRMS and its components, preparing samples, and implementing the protocol/procedure.

4.0. MATERIALS AND EQUIPMENT

4.1. D-GRMS with air conditioning and flow management, grain storage, moisture, and CO$_2$ absorption, and instrumentation sections see Figure 3.1 from Trevisan (2017).

4.2. Air supply CO$_2$ scrubber (qty = 1 per system) prepared according to Document No. D-GRMS-001.

4.3. $RC$ dehumidifiers (qty = 2 per system) prepared according to Document No. D-GRMS-003.

4.4. $RC$ CO$_2$ scrubbers (qty = 2 per system) prepared according to Document No. D-GRMS-004.

4.5. Digital precision scale range of 0 to 3100 g and 0.01 g resolution (Model iBalance i3100, MyWeigh, Phoenix, AZ, USA).


5.0. PROCEDURES

5.1. Every day before the first measurement, check the gas guard to see if any of the gas tanks are low. If the pressure of one tank is low the instrument is going to beep and the red LED of the tank with the low pressure is going will be on. To turn off the beep, use the “Silence” switch (Figure B.7).

---

**Figure B.7. Gas guard silence switch button.**
5.2. If one of the tanks has low pressure double check it by confirming from the gas cylinder regulator (Figure B.8) primary pressure. The gauge will show zero for the tank with low pressure.

![Figure B.8. Tanks 1 and 2 gas cylinders regulators guard silence switch button.](image)

5.3. Close the valves of the regulator and the valve on top of the gas cylinder (Figure B.9).

![Figure B.9. Air tanks valves to be closed.](image)
5.4. Check all flowmeters to see if the flow is correct. The flowmeter inside the enclosure is supposed to be at 0.5 l/min (Figure B.10a) and the one at the end of the system at 1 scfh (Figure B.10b).

![Flowmeter inside enclosure (a) and at system exhaust (b).](image)

**Figure B.10. Flowmeter inside enclosure (a) and at system exhaust (b).**

5.5. If the air supply flow is correct, the \( RC \) CO\(_2\) scrubber can be measured.

5.6. Quickly divert airflow from \( RC \) CO\(_2\) scrubber A to \( RC \) CO\(_2\) scrubber B or vice-versa depending on the side of the last measurement. Switch all the four valves for the same side.

5.7. Detach the air supply CO\(_2\) scrubber A from D-GRMS (Figure B.11a). Determine its average weight from three measurements, taking care to rotate the scrubber 120\(^\circ\) in between measurements. Record all measurements in the respiration test table at the datasheet, along with the time of measurement (hh:mm). Do not disconnect the dehumidifier, which has a large amount of insulation around it (Figure B.11b).

![Air supply CO\(_2\) scrubber (a) and uncovered dehumidifier (b).](image)

**Figure B.11. Air supply CO\(_2\) scrubber (a) and uncovered dehumidifier (b).**

5.8. Connect scrubber A or B back into D-GRMS.
5.9. Check the flowmeters at the end (Figure B.12a). If they are not at 1 scfh, it may be that some of the valves were not switched. Double-check all of them. If all of them are switched correctly, then maybe some of the quick-disconnect (B.12b) are not connected correctly. Double check all the quick-disconnects.

![System exhaust flowmeter (a) and quick-disconnect coupler (b).](image)

5.10. After 2 h, divert airflow from scrubber B to scrubber A or vice-versa depending on the side that was switched last time.

5.11. Repeat Steps 5.17 and 5.18 with scrubber B or A.

5.12. Conduct weight measurements every 2 h during the daytime, alternating between the two scrubbers each time. A total of 5 measurements per day are commonly taken.

5.13. All weight measurements represent the accumulated respired CO$_2$ in the scrubbers. Normalize each weight measurement to $m_{DM,1}$.

5.14. When the air supply CO$_2$ scrubber, RC dehumidifier or RC CO$_2$ scrubbers have approximately 75% of their Sodasorb® color changed from white to purple or their desiccant color changed from blue to purple, remove one scrubber at a time from the system to change the Sodasorb® or desiccant. For the scrubbers with sides A and B, switch the side of the air flow first before changing. For example, when changing dehumidifier “A”, switch the valve to side “B”. Follow their respective protocols.

5.15. The gas guard and flow should be checked twice a day, before the first RC CO$_2$ scrubber’s measurement and after the last measurement.

6.0. PROCEDURE: FINAL STEPS AND REPORTING

6.1. The criteria to stop the respiration test will depend on a criterion decision to be developed with the supervisor. It can be for a period of time, for example, 20 d if the respiration rates are low, or until 14.7 g CO$_2$ (kg dry beans)$^{-1}$ equivalent to 1.0 % $DML$ is reached. Once the criterion is achieved, terminate the grain respiration test.

6.2. Follow Document No. D-GRMS-007 for more details about how to terminate a respiration test.

6.3. Record any issues encountered during a grain respiration test. If there are any issues, see corrective action (Section 7.0).
7.0. **CORRECTIVE ACTION**

7.1. Pay attention to calibration, drift, bias, etc. issues with the digital scale. Be sure to use the same digital scale for all weight measurements throughout a grain respiration study (not just individual tests or experiments). When scale issues arise, notify the supervisor immediately.

7.2. The supervisor will take further corrective actions which may include testing for leaks and repairing or replacing system components.

8.0. **CHANGES FROM PREVIOUS VERSION**

8.1. SOP drafted on 01 July 2016 by L. R. Trevisan.

8.2. Reviewed, revised, and approved by supervisor on 01 October 2017.

8.3. Second review, revision, and approval by supervisor on 10 October 2018.
**B.4. Dynamic respiration test datasheets**

Test No. ________  System No. ________  Moisture Content: ________%

Start date and time: / / ________ h: ________ min  Storage Temperature: ________ °C

Follow Document No. S-GRMS-007 to conduct a dynamic grain respiration test.

**SET UP COLUMNS**

1. Supply air CO₂ scrubber following Document No. D-GRMS-001: filled with Sodasorb®  
2. RC dehumidifiers A & B following Document No. D-GRMS-003: filled with desiccant  
3. RC CO₂ scrubbers A & B following Document No. D-GRMS-004: filled with proportion of Sodasorb® and desiccant  
4. Initial weight of RC CO₂ scrubbers A & B:

<table>
<thead>
<tr>
<th>RC CO₂ Scrubber</th>
<th>Scrubber A</th>
<th>Scrubber B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodasorb</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drierite</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Full Column</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (initial)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**SET UP RESPIRATION CHAMBER**

Water bath from respiration chamber temperature setting: ________ °C

**HUMIDIFICATION SYSTEM**

1. Set water bath from humidification system temperature: ________ °C
2. Prepare glycerol-water solution following Document No. D-GRMS-002 and 002a:  
2.a. Equilibrium relative humidity ($\phi_e$) from table: ________%RH  
2.b. Solution stored:  
2.c. Mass glycerol: ________g  Mass water: ________g  
2.d. Preparation date and time: / / ________.
3. Place solution into sealed vacuum bottles:  
4. Place bottles into water bath and attach tubing:  
5. Open air supply (15 psi):  
6. Test $\phi_e$ (actual): ________%RH  Period tested: ________  
7. Adjustment: Mass of water added: ________g  Mass of solution removed: ________g  
8. Test $\phi_e$ (actual): ________%RH  Period tested: ________

**SOYBEAN PREPARATION**

■ Hand-shelled  ■ Mechanically harvested  ■ Harvested in ________

Additional notes: ________________________________________________
1. Follow Document No. D-GRMS-004 to prepare and re-wet the beans.
2. Initial weight of beans taken out of storage: ___________ g
3. Beans cleaned: ☐
4. Acclimation to desired temperature (about 5 days) inside incubator:
   Start Date and Time: __/__/_____ h       End Date and Time: __/__/_____ h
5. Follow Document No. S-GRMS-004 to gravimetrically determine the moisture content of the sample before the respiration test: ☐
6. Moisture content checked after rewetting soybeans:

<table>
<thead>
<tr>
<th>Moisture Meter</th>
<th>Gravimetric measurement of moisture content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample No.</td>
<td>Estimated moisture content (% w.b.)</td>
</tr>
<tr>
<td>1</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>

Date and time put in oven: __/__/____ h:____ min  Date and time taken out of oven: __/__/____ h:____ min

*Max.: 3 days.

BACK TO RESPIRATION CHAMBER
1. Weight of soybeans to be tested: ___________ g
2. Beans mixed and 500g sample collected for further testing: ☐   Bag labeled: ☐
   Stored in freezer: ☐
3. Mass of dry solids from tested sample: ___________ g
4. Pour soybeans into respiration chamber: ☐   Time: _____ h _____ min
5. Grain temperature: ___________ °C   Time: _____ h _____ min
6. Place lid sealing it with vacuum grease: ☐

STARTING RESPIRATION TEST
1. Check if all tubing is connected: ☐
2. Check for leak: flowmeter 1 ☐  flowmeter 2 ☐  Exhausted air CO₂ sensor (zero) ☐
3. Allocate desiccators inside the incubator: ☐
4. Time respiration test started: ______ h ______ min
5. Take measurements periodically, write down the information of each measurement on the respiration test table and save on the electronic datasheet.

ENDING RESPIRATION TEST:
1. Stopped criteria:
   a. Vaisala more than 50 ppm: ☐   b. Leak detected: ☐ at: __/__/____.
   c. Stipulated stop criteria: ☐
Additional notes: ____________________________________________________________.

2. Turn OFF all the system components ☐
3. Date and time respiration test ended: ___/___/___ h: ___ min t= final

END OF RESPIRATION TEST

CLEANING SYSTEM
1. Weight of soybeans tested: __________ g
2. Poor Beans onto a tray: ☐
   Observations:________________________________________________________
3. Beans mixed and 500g sample collected for further testing: ☐ Bag Labeled: ☐
   Stored in freezer:☐
4. Follow Document No. S-GRMS-004 to gravimetrically determine the moisture content of the sample before the respiration test: ☐
5. Moisture content checked after rewetting soybeans:

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</tr>
<tr>
<td>2</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>

Date and time put in oven: ___/___/___ h: ___ min Date and time taken out of oven: ___/___/___ h: ___ min

*Max.: 3 days.

6. Save glycerol-water solution: ☐ Check $\phi_e$ _______%RH
   Label and store glass bottle in the refrigerator ☐
7. Discard used Sodasorb®: ☐
8. Regenerate Drierite®: ☐
9. Wash canisters and respiration chamber: RC CO$_2$ scrubbers ☐ RC dehumidifiers ☐
   air supply CO$_2$ Scrubber ☐

SAVING DATA
1. Place respiration test table on portfolio folder: ☐
2. Save the electronic datasheet on Box: ☐

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## Respiration Test Table

<table>
<thead>
<tr>
<th>Date</th>
<th>Real Time</th>
<th>Observations</th>
<th>$RC$ CO$_2$ scrubber A</th>
<th>$RC$ CO$_2$ scrubber B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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B.5. Supplemental files: Dynamic respiration test electronic datasheet

The supplemental electronic file “APPENDIX C Supplemental file _da Silva_ Thesis” contains Excel spreadsheets tab named as “APPENDIX B.4.1” and “APPENDIX B.2” with important formulas and example as an additional tool to follow the SOPs for D-GRMS. Appendix B.4.1. Supplemental File: Static Respiration Test Electronic Datasheet has instructions on how to use the spreadsheet with the purpose to have all $RC$ CO$_2$ scrubbers’ measurements and respiration test information copied to the electronic file. The formulas contained in this spreadsheet automatically calculate accumulated and specific mass of respired CO$_2$ and $DML$. It is important to also create a daily plot to see the accumulate $DML$ (%) over time ($d$) and check if the respiration test is having the expected results. Appendix B.4.2. Supplemental File Example: Static Respiration Test Electronic Datasheet is an example of a respiration test that demonstrates how Appendix B.4.1 needs to be filled.
APPENDIX C. SUPPLEMENTAL FILE: DA SILVA THESIS

The supplemental electronic file “APPENDIX C Supplemental file _da Silva_ Thesis” contains excel spreadsheets for all supplemental materials referenced throughout the thesis and the respiration tests data from Chapter 3, 4 and 5. Below is the title of each spreadsheet:

- **APPENDIX A.4. Supplemental Files: Static Respiration Test Electronic Datasheet.**
- **APPENDIX B.4: Supplemental Files: Dynamic respiration test electronic datasheet.**
- **APPENDIX C.1. Chapter 3 Supplemental File: Static respiration tests data: Soybeans at 18% m.c. and 35°C.**
  - Table C.1.1. Soybeans at 18% m.c. and 30°C - Replication No. 1.
  - Table C.1.2. Soybeans at 18% m.c. and 30°C - Replication No. 2.
  - Table C.1.3. Soybeans at 18% m.c. and 30°C - Replication No. 3.
  - Table C.1.4. Soybeans at 18% m.c. and 30°C - Replication No. 4.
- **APPENDIX C.2. Chapter 4 Supplemental File: Static respiration tests data: Soybeans at 18% m.c. and 35°C with 0, 4, 8, and 16% (w/w) split beans content.**
  - Table C.2.1. Soybeans at 18% m.c. and 35°C with 0% (w/w) splits- Replication No. 1.
  - Table C.2.2. Soybeans at 18% m.c. and 35°C with 0% (w/w) splits- Replication No. 2.
  - Table C.2.3. Soybeans at 18% m.c. and 35°C with 0% (w/w) splits- Replication No. 3.
  - Table C.2.4. Soybeans at 18% m.c. and 35°C with 0% (w/w) splits- Replication No. 4.
  - Table C.2.5. Soybeans at 18% m.c. and 35°C with 0% (w/w) splits- Replication No. 5.
  - Table C.2.6. Soybeans at 18% m.c. and 35°C with 4% (w/w) splits- Replication No. 1.
  - Table C.2.7. Soybeans at 18% m.c. and 35°C with 4% (w/w) splits- Replication No. 2.
  - Table C.2.8. Soybeans at 18% m.c. and 35°C with 4% (w/w) splits- Replication No. 3.
  - Table C.2.9. Soybeans at 18% m.c. and 35°C with 4% (w/w) splits- Replication No. 4.
  - Table C.2.10. Soybeans at 18% m.c. and 35°C with 4% (w/w) splits- Replication No. 5.
  - Table C.2.11. Soybeans at 18% m.c. and 35°C with 8% (w/w) splits- Replication No. 1.

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Table C.2.12. Soybeans at 18\% m.c. and 35°C with 8\% (w/w) splits- Replication No. 2.
- Table C.2.13. Soybeans at 18\% m.c. and 35°C with 8\% (w/w) splits- Replication No. 3.
- Table C.2.14. Soybeans at 18\% m.c. and 35°C with 8\% (w/w) splits- Replication No. 4.
- Table C.2.15. Soybeans at 18\% m.c. and 35°C with 8\% (w/w) splits- Replication No. 5.
- Table C.2.16. Soybeans at 18\% m.c. and 35°C with 16\% (w/w) splits- Replication No. 1.
- Table C.2.17. Soybeans at 18\% m.c. and 35°C with 16\% (w/w) splits- Replication No. 2.
- Table C.2.18. Soybeans at 18\% m.c. and 35°C with 16\% (w/w) splits- Replication No. 3.
- Table C.2.19. Soybeans at 18\% m.c. and 35°C with 16\% (w/w) splits- Replication No. 4.
- Table C.2.20. Soybeans at 18\% m.c. and 35°C with 16\% (w/w) splits- Replication No. 5.

- APPENDIX C.3. Chapter 4 Supplemental File: Dynamic respiration tests data: Soybeans at 14, 18, and 22 \% m.c. and 30°C.
  - Table C.3.1. Soybeans at 14\% m.c. and 30°C - Replication No. 1
  - Table C.3.2. Soybeans at 14\% m.c. and 30°C - Replication No. 2
  - Table C.3.3. Soybeans at 14\% m.c. and 30°C - Replication No. 3
  - Table C.3.4. Soybeans at 14\% m.c. and 30°C - Replication No. 4
  - Table C.3.5. Soybeans at 18\% m.c. and 30°C - Replication No. 1
  - Table C.3.6. Soybeans at 18\% m.c. and 30°C - Replication No. 2
  - Table C.3.7. Soybeans at 18\% m.c. and 30°C - Replication No. 3
  - Table C.3.8. Soybeans at 18\% m.c. and 30°C - Replication No. 4
  - Table C.3.9. Soybeans at 22\% m.c. and 30°C - Replication No. 1
  - Table C.3.10. Soybeans at 22\% m.c. and 30°C - Replication No. 2
  - Table C.3.11. Soybeans at 22\% m.c. and 30°C - Replication No. 3
  - Table C.3.12. Soybeans at 22\% m.c. and 30°C - Replication No. 4

- APPENDIX C.4. Supplemental File: Static respiration tests data: Soybeans at 18\% m.c. and 35°C.
  - Table C.4.1. Soybeans at 18\% m.c. and 35°C - Replication No. 1.
  - Table C.4.2. Soybeans at 18\% m.c. and 35°C - Replication No. 2.
  - Table C.4.3. Soybeans at 18\% m.c. and 35°C - Replication No. 3.
  - Table C.4.4. Soybeans at 18\% m.c. and 35°C - Replication No. 4.
  - Table C.4.5. Soybeans at 18\% m.c. and 35°C - Replication No. 5 (sensor on top).
  - Table C.4.6. Soybeans at 18\% m.c. and 35°C - Replication No. 5 (bottom sensor).