CARBON DIOXIDE EVOLUTION FROM FRESH AND PRESERVED SOYBEANS

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ABSTRACT. *Carbon dioxide evolution has proven to be a good indicator of deterioration in studies of stored cereal grains and oilseeds. Since little work has been done with stored soybeans, a study was conducted measuring carbon dioxide from stored soybeans using freshly harvested and preserved soybean samples. The objective of the study was to determine the effects of harvesting method, storage temperature, storage moisture content, and storage time on soybean deterioration. Following storage treatment, samples were held under aeration in a respirometer at 26°C and 21% moisture, and evolved carbon dioxide mass was measured until samples had lost 1.0% of original dry matter. At high harvest moistures, combine−harvested soybeans deteriorated faster, but at low harvest moistures, the deterioration rate of hand−harvested soybeans was greater. After 48 weeks of storage, the soybeans harvested at 22% moisture and preserved at −18°C deteriorated in a respirometer like freshly harvested soybeans, but soybeans harvested at 9% deteriorated in a respirometer significantly faster than those freshly harvested at 13% moisture.*

Keywords. *Carbon dioxide, Deterioration, Preservation, Soybeans, Storage fungi, Storage quality.*

At the time an oilseed reaches physiological maturity, it is considered to be at its prime state of quality. It then begins to deteriorate with time, slowly at low moisture contents and temperatures but very rapidly when temperature and moisture are high. Deterioration of most biological material is associated with decomposition of carbohydrates as a result of respiration. The selective respiratory utilization of carbohydrates in soybeans is similar to the oxidative combustion of carbohydrates such as glucose (Ramstad and Geddes, 1942).

Carbohydrate decomposition during deterioration of soybeans is discussed by Milner and Geddes (1946). They found that during this biological phase of respiratory behavior of seeds, the increased rate of respiration, a symptom of deterioration, was accompanied by a decrease in both reducing and nonreducing sugars but no change in the fat content. The protein concentration was found to be slightly increased, but was not speculated to have any role in the decomposition process. The increase was, in fact, attributed to the decrease in sample dry matter. A similar reduction in the sugar content of soybeans was observed by Howell et al. (1959) when they studied the respiration of ripening soybean seeds. Wilson (1995) reported similar changes in protein and carbohydrates in fungi−damaged soybeans, but either no change or an increase in oil concentration was observed.

The decomposition process that results in a loss of dry matter has been modeled as a breakdown of simple sugars:

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + 2{,}835 \text{ kJ/mole} \quad (1)$$

Following this reaction, the evolution of 14.7g of CO_2 per kg dry matter corresponds to a loss of 1.0% carbohydrate (dry matter).

Muir et al. (1985) treated the rising concentration of CO_2 in interseed air of stored wheat, rapeseed, barley, and corn as a measure of quality. The analysis of seed samples from locations that registered high CO_2 concentrations indicated that the kernels had undergone spoilage. Steele et al. (1969) demonstrated that decomposition of dry matter during deterioration of shelled corn can be determined by measuring the CO_2 produced. An equivalent dry matter loss was then calculated based on equation 1. In the case of commercial soybeans, the loss of dry matter may signify a loss of grade, as is evident in the case of stored shelled corn (Saul and Steele, 1966). Saul and Steele (1966) evaluated the length of time that shelled corn can be stored before 0.5% of its dry matter is lost. The 0.5% loss level was considered the threshold value before the grade is lowered because of an increase in damaged kernel total (DKT). An allowable storage timetable and its defining equations have been developed using information from Steele et al. (1969) and other sources (Bern et al., 2002). Table entries are days storage time at a specified temperature and moisture content before the corn loses 0.5% of its original dry matter.

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A model capable of predicting quality loss in stored soybeans has not been developed. The limitation of laboratory space needed to conduct such experiments requires that soybeans be preserved at harvest for later testing. Any changes in soybean characteristics during this preservation that influence CO_2 evolution during a later test, if not accounted for, can introduce error in the final analysis.

In the case of shelled corn, there was no significant difference in the CO_2 evolution for samples preserved at −10°C and 22% moisture (all reported moisture values are % wet basis) for a period of 100 days when compared with freshly harvested corn (Fernandez et al., 1985). Exponential and linear models for CO_2 versus time relationships were compared, and the model explaining more variability was chosen in each case. Reports on effects of temperature and moisture content in maintaining the quality of stored soybeans, in terms of CO_2 evolution during the deterioration process, were not found in the literature.

OBJECTIVES

This study was undertaken to define the impact of preservation in maintaining the initial condition of soybeans as measured by CO_2 evolution. In this study, "preservation" refers to the time period between harvest and laboratory storage testing.

The specific objectives of the study were:

- To determine effects of harvesting practices on the rate of CO_2 evolution from freshly harvested soybeans.
- To determine effects of preservation time, storage temperature during preservation, and soybean moisture during preservation on CO_2 evolution during a short−term respiration test.

MATERIALS AND METHODS

Experiments were conducted in which combine−harvested or hand−harvested soybeans were tested in a respirometer at 26°C and about 21% moisture. In the first experiment, respirometer tests were conducted on freshly harvested soybeans. In the second experiment, soybeans were subjected to a preservation treatment prior to respirometer testing.

SOYBEANS

Soybeans used in the study were Kruger 2555, grown at the Iowa State University Agronomy and Agricultural Engineering Research Center, 15 km west of Ames. The soybean lots were combine−harvested or hand−harvested at moisture contents of 22% to 20% (high) or 8% to 13% (low). The lots were cleaned using a Carter Day model XT3 dockage tester using 13 mm (0.5 in.) square hole, 8.5 mm (21/64 in.) round hole, and 7.9 × 19 mm (5/16 × 3/4 in.) slotted sieves. The slotted sieve removes splits. This dockage tester uses sloping screens that oscillate to facilitate flow of material.

Damage levels for the combine−harvested samples were determined using the hypochlorite test, as described by Van Utrecht et al. (2000). The high and low moisture content soybeans had 35% ±4% and 14% ±5% swollen beans, respectively, when soaked in 1% hypochlorite solution.

CARBON DIOXIDE RESPIROMETER SYSTEM

A CO_2 respirometer system similar to that described by Aljinovic et al. (1995) and Dugba et al. (1996) was used for short−term storage tests (fig. 1). During the tests, carbon dioxide produced by 1 kg soybean samples stored under constant aerated storage conditions was measured. Compressed air that had been cleaned by filtering, stripped of CO_2, and conditioned to 26°C and 93% ±2.7% relative humidity to maintain soybean moisture was forced at a rate of 0.45 m^3/min/Mg of soybeans through the 1 m long glass tubes containing the soybean samples. Tubes were autoclaved at 120°C for 20 min before use. The conditions of 26°C and 93% relative humidity were chosen to accelerate the process of soybean deterioration during this storage study and are not recommended for storage of soybeans.

CO_2 produced by the soybean samples while in storage was trapped by the CO_2 absorbing section of the system (fig. 1). The sulaimanite CO_2 absorbent agent (Al−Yahya, 1991) was packed in Plexiglas tubes, and its weight gain every 24 h allowed calculation of the CO_2 produced during the period. The weight of the CO_2 gained was corrected to account for the residual CO_2 present in the airstream (Rukunudin, 1997). Observations of visible fungi growth were made during and after the respirometer testing.

EXPERIMENT I: FRESH SOYBEANS

Soybeans used in experiment I were freshly harvested under two modes of harvesting and at two moisture contents. Harvesting was carried out manually (hand harvesting followed by hand shelling) and by combine. A total of four treatments were used (table 1).

Before the start of the experiment, the low−moisture soybean samples (both hand harvested and combine harvested) were raised to about 21% moisture content by direct addition of a calculated weight of distilled water. The approach used was quite similar to that described by Milner and Geddes (1945), although Ramstad and Geddes (1942) earlier found this to be unsatisfactory with soybeans, noting a problem in ensuring uniform distribution of moisture because some of the beans swelled very greatly and seed coats loosened. To ensure minimum swelling of the beans and uniform distribution of water, the addition of water to a particular bag of soybeans was accomplished in three or four stages by use of a spray bottle. After spraying, the bag was rotated by hand for 2 to 3 min to uniformly distribute the water. Each stage was separated by a 6 to 12 h storage period at 4°C to 5°C. This prevented a sudden swelling of beans. Samples were then kept at room temperature for about 12 h before being used in an experiment. Most of the soybeans soon presented a normal appearance as the water was taken up by the cotyledons. Samples were poured into randomly arranged glass storage columns.

EXPERIMENT II: PRESERVATION OF SOYBEAN SAMPLES

Preservation of soybean lots at −18°C and 10°C involved only combine−harvested soybean lots, harvested at 22% and 13% moisture. The cleaned soybean lot, combine harvested at 22% (high moisture), was packed in polyethylene bags, which each contained about 1200g, and stored at −18°C. High−moisture soybeans were not preserved at 10°C because excessive fungal deterioration occurs during the preservation period. The 13% (low moisture) soybean lot was ambient air dried to 9% and 10% moisture before being preserved at −18°C or 10°C in bags, which each contained about 1200g.

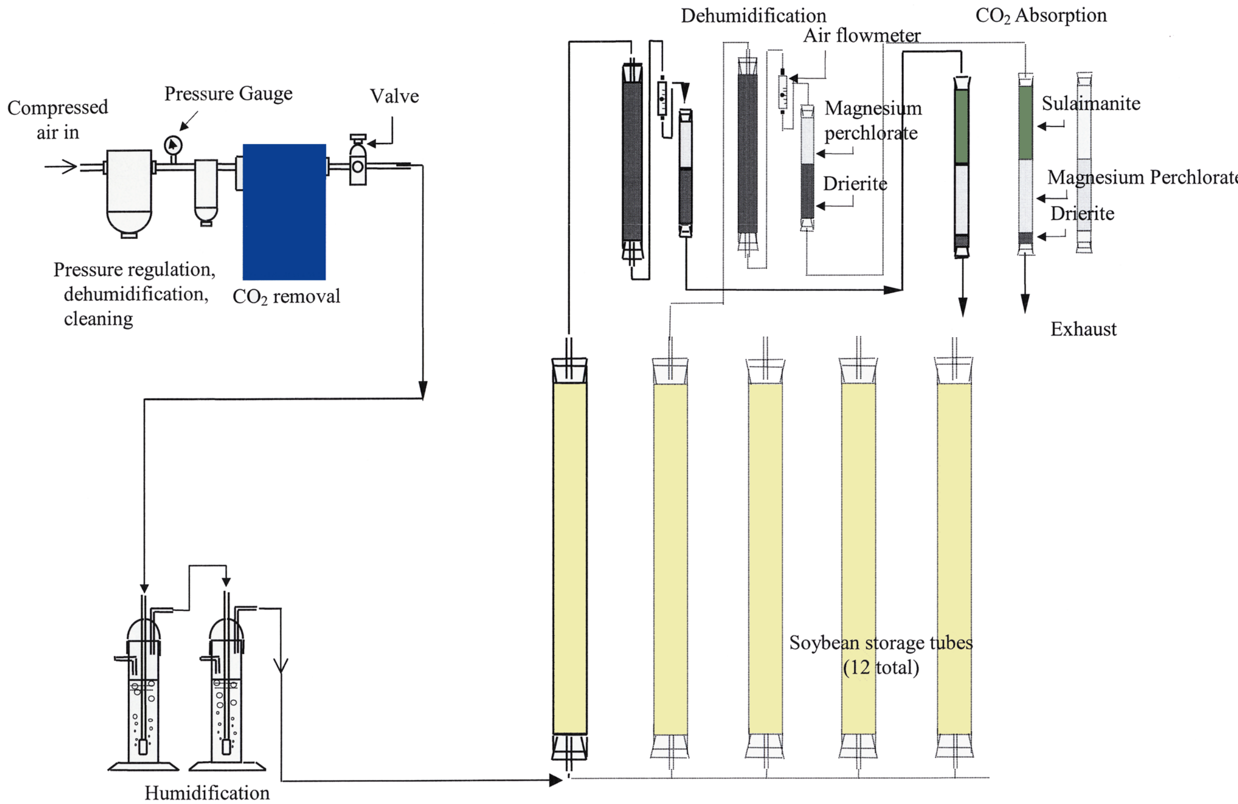

Figure 1. CO_2 respirometer system.

Table 1. Treatments for experiment I.

Treatment	Harvesting Condition
1	Combine harvested at 21% moisture
2	Combine harvested at 13% moisture
3	Hand harvested at 20% moisture
4	Hand harvested at 9% moisture

For each of the preservation temperatures, three different temperature−controlled chambers were used, representing three replications. A total of six chambers were used, and six bags of cleaned soybean samples were preserved in each of the chambers. The soybeans were preserved for 48 weeks, with sampling done at 0, 26, and 48 weeks after harvest.

This experiment investigated effects of moisture, preservation temperature, and period of preservation on CO_2 evolution during laboratory respirometer testing using three treatments:

- Combine harvested at 22% moisture and preserved at −18˚C.
- Combine harvested at 13% moisture, air dried to 9% to 10% moisture, and preserved at ?18˚C.
- Combine harvested at 13% moisture, air dried to 9% to 10% moisture, and preserved at 10˚C.

Respirometer testing was done using the same apparatus, relative humidity, and temperature as previously described. Fresh soybeans, combine harvested at 21% and 13% moisture content, represented two types of control. Before testing, the low−moisture soybeans were reconstituted with water to about 21% moisture, as described previously.

Statistical Analysis

Each treatment was replicated three times in a restricted randomization design. Complete randomization of the environmental chambers at each temperature could not be achieved. Statistical analysis of data was carried out using Statistical Analysis Software (SAS, 1990). Polynomial regression models to describe the CO_2 evolution (dependent variable) versus time of storage (independent variable) were established using the General Linear Model Procedure (Proc GLM) to the third order, with zero intercept. Coefficients of the terms were included in the model if they were significant ($p < 0.05$) as indicated by t−statistics. Comparisons of rates of deterioration between treatments were made by means of the analysis of variance (ANOVA), where measurement for samples preserved 26 and 48 weeks were considered repeated measures. Significance was established by calculating the least significant different (LSD) between the means (Steel et al., 1997). If selected regression models were significant, then the model was used to characterize for moisture content and dry matter loss. Unless otherwise stated, the significance was established at $p < 0.05$. Visible microbial growth during storage was also noted.

Results and Discussion

Effect of Harvesting Practices on CO_2 Evolution from Fresh Soybeans

Combine Harvested

Figure 2 shows curves describing CO_2 evolution from fresh soybeans combine−harvested at high (21%) and low (13%) moistures and held at 26˚C and 22% moisture. The curves were derived from third−order polynomial regressions on the data of three replications (Rukunudin, 1997). Coefficients of terms (table 2) were used only if their respective t−statistics were shown to be significant.

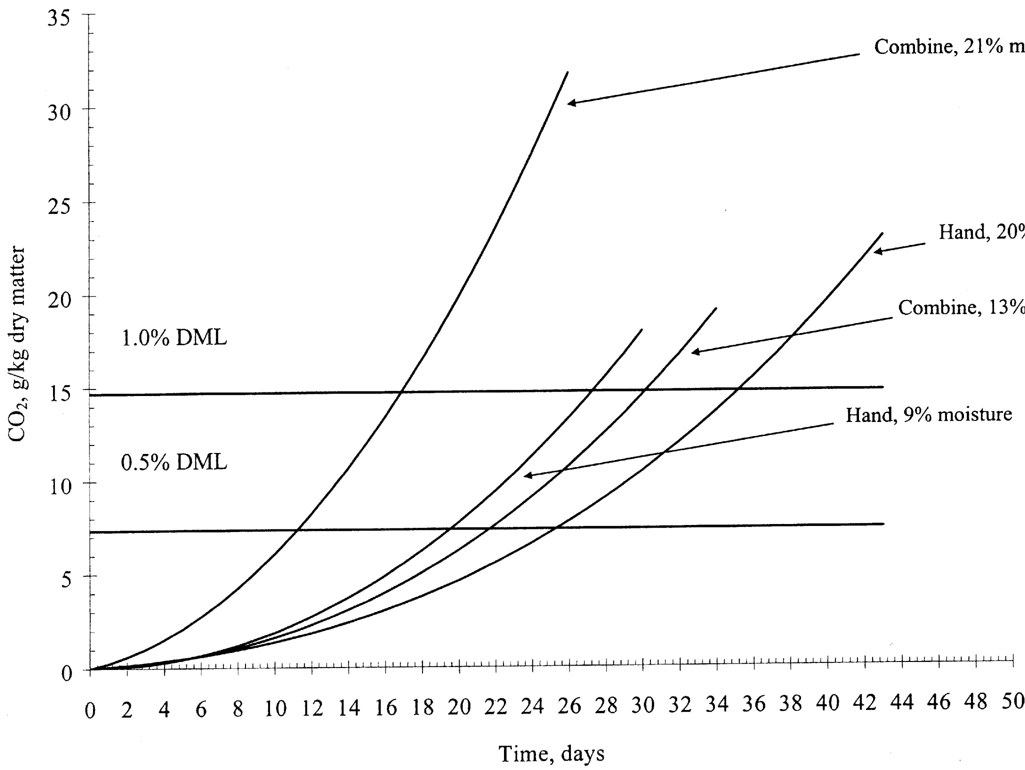

Figure 2. CO_2 evolution from soybeans held at 26°C and 22% moisture, for two modes of harvesting with two harvest moistures.

Table 2. Regression models for CO_2 evolution from fresh soybean samples held at 26°C and 22% moisture.

	General Model[a] Coefficients of Polynomials		
Treatment	c_1	c_2	c_3
Combine−harvested/21% moisture	0.195	ns[b]	0.038
Combine−harvested/13% moisture	ns	0.012	0.0001
Hand−harvested/20% moisture	0.034	0.006	0.0001
Hand−harvested/9% moisture	ns	0.018	ns

[a] Y (g CO_2/kg dm) = $c_1 t + c_2 t^2 + c_3 t^3$, where t = number of days.
[b] ns = not significant.

Table 3. Average aerated storage times of freshly harvested soybeans.

Moisture at Harvest	Number of Days to Reach 0.5% DML	Number of Days to Reach 1.0% DML
Combine−harvested soybeans		
21%	11.5	17.8
13%	22.5	31.2
Hand−harvested soybeans		
20%	26.2	37.1
9%	19.8	28.1

[a] Mean from three replicates: $LSD_{\alpha=0.05}$ at 0.5% DML = 2.13; $LSD_{\alpha=0.05}$ at 1.0% DML = 2.69.

Table 3 shows storage times, defined as the number of days of aerated storage before soybeans lost 0.5% and 1.0% of their dry matter. LSDs of the two moisture treatment means were established at the two dry matter loss (DML) levels. In every case, times for low−moisture soybeans were greater than those for high−moisture soybeans ($p \leq 0.05$). Soybeans combine−harvested at high moisture were found to lose 0.5% dry matter in about half the time required for soybeans harvested at low moisture content. Mechanical damage from combining high−moisture soybeans no doubt contributed to the faster rate of deterioration. The rate of deterioration of soybeans harvested at 13% moisture, which is within the range of 11% to 14% moisture associated with optimum toughness (Paulsen, 1977), should therefore demonstrate approximately the minimum rate of deterioration achievable when soybeans are combine−harvested.

Hand Harvested

The experiment also tracked deterioration of soybeans hand−harvested at high (20%) and low (9%) moistures. The plots of CO_2 evolution measured during laboratory storage for the two treatments are shown in figure 2, with the equations of the models in table 2. Soybeans hand−harvested at 20% moisture showed the slowest deterioration in both moisture treatments. Soybean quality is considered at its prime level at physiological maturity, which is usually at 50% to 60% moisture content (Howell et al., 1959; Rose, 1979). According to Howell et al. (1959) and Hurburgh and Benson (1995), full maturity, i.e., when the pods are brown in color and ready to harvest, is reached at about 18% to 20% moisture content. Thus, the deterioration curve of hand−harvested soybeans at 20% may describe soybeans near their highest quality.

Soybeans hand−harvested at low (9%) moisture exhibited a significantly faster rate of deterioration than the high−moisture treatment (fig. 2, table 2). These aerated storage times, however, were also found to be significantly less than for soybeans combine−harvested at 13%. Prolonging field drying after soybeans have reached harvest moisture content (13%) apparently causes damage. Any cracks due to overdrying that develop in the hulls of those lots render them more susceptible to microbial attack. According to Milner and Geddes (1946), damaged seeds present a more hospitable medium for mold mycelial penetration and growth of

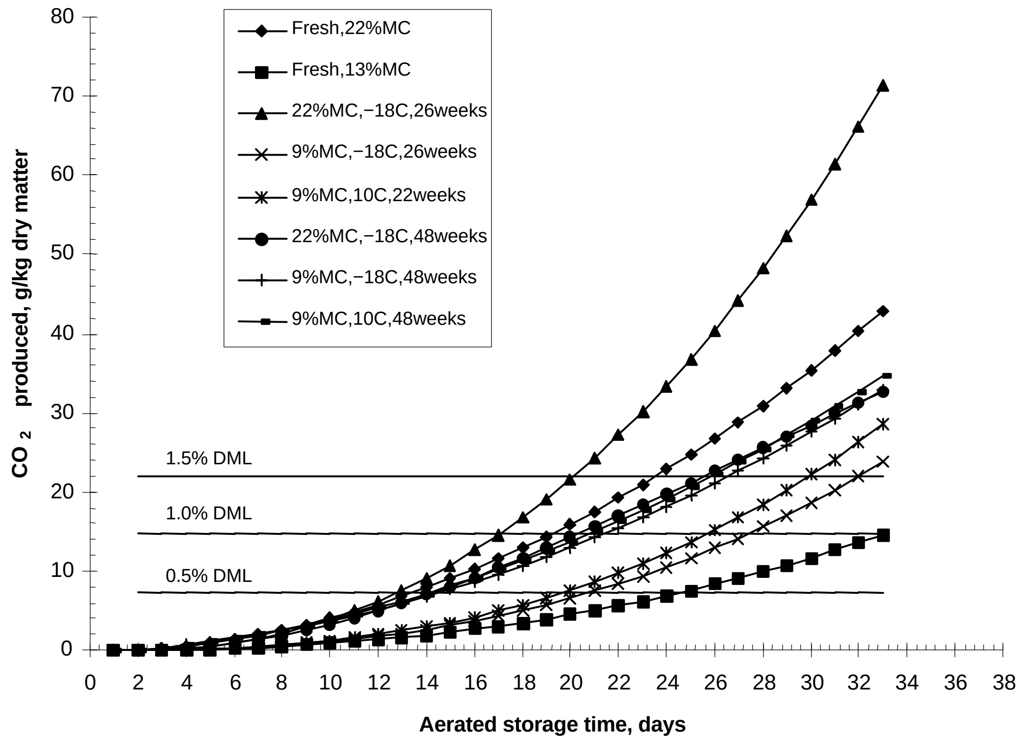

Figure 3. CO_2 evolution of soybeans held at 26°C and 22% moisture after different preservation conditions. (Symbols differentiate curves and are not data points.)

Table 4. Regression models for CO_2 evolution from preserved, combine−harvested soybean samples held 26°C and 22% moisture.

Treatment	General Model[a] Coefficients of the Polynomials c_1	c_2	c_3
22%[b], −18°C[c], 26 weeks[d]	ns[e]	0.056	0.001
9%, −18°C, 26 weeks	ns	0.014	0.0003
9%, 10°C, 26 weeks	ns	0.014	0.0005
22%, −18°C, 48 weeks	ns	0.057	−0.001
9%, −18°C, 48 weeks	0.244	0.026	ns
9%, 10°C, 48 weeks	0.258	0.028	ns

[a] Y (g CO_2/kg dm) = $c_1 t + c_2 t^2 + c_3 t^3$, where t = number of days.
[b] Harvest moisture.
[c] Preservation temperature.
[d] Preservation period.
[e] ns = not significant.

Table 5. Average aerated storage times of soybeans combine−harvested at 13% moisture, preserved at 9% moisture and −18°C or 10°C, and then held at 26°C and 22% moisture.

Preservation Period (weeks)	Number of Days to Reach 0.5% DML[a] −18°C[b]	10°C[b]	Number of Days to Reach 1.0% DML[a] −18°C[b]	10°C[b]
0	22.5	22.5	31.2	31.2
26	18.4	17.9	23.9	22.9
48	12.4	11.8	18.8	17.8

[a] Mean from three replicates: $LSD_{\alpha=0.05}$ at 0.5% DML = 1.34; $LSD_{\alpha=0.05}$ at 1.0% DML = 1.62.
[b] Preservation temperature.

microorganisms than undamaged beans. It is in these cracks and broken parts of the beans that mold first appears.

Carbon Dioxide Evolution Curves of Preserved Samples

Polynomial regression of carbon dioxide evolved per kg dry matter and storage time for the preserved samples yielded the curves shown in figure 3. Models defining the respective curves were derived from three replications and are listed in table 4.

Effect of −18°C and 10°C Preservation Temperatures on CO_2 Evolution

In an attempt to determine how preservation temperature affects CO_2 evolution, soybeans combine−harvested at 13% moisture were preserved in −18°C and 10°C chambers. Soybean samples were taken out of the chambers after 26 and 48 weeks of preservation and used in aerated storage tests along with freshly harvested samples. Based on corresponding fitted carbon dioxide evolution curves, average times for soybeans to lose 0.5% and 1.0% dry matter are summarized in table 5. Data for a preservation period of 0 weeks are the same as listed in table 3 for combine−harvested soybeans at 13% moisture.

Analysis of variance (table 6) shows that aerated storage times of samples, averaged over preservation period for the two preservation temperatures, show no significant difference between the two preservation temperatures ($p > F = 0.45$). There is, however, a high level of significance of the effect of preservation period on time to lose 0.5% and 1.0% dry matter, averaged over temperatures, ($p > F = 0.00$). No preservation period by temperature interaction effect was detected (table 6). The deterioration in 9% moisture samples is linear over the three time periods and is independent of storage temperature (tables 5 and 6). Although the deviation or the lack of fit to a linear component is significant ($p > F = 0.1$), the larger mean square (320.3) of the linear compared to the deviation (2.3) shows that a straight line is sufficient to explain the relationship.

Table 6. ANOVA table (relevant sources only) aerated storage times of 9% soybean samples harvested at 13% moisture.

Source	DF	MS	F	p > F
Temperature (T)	1	0.90	0.7	0.45
Chamber/T	4	1.32	−−	−−
Period (P)	2	161.30	320.0	0.00
Linear	(1)	(320.3)	616.0	0.00
Deviations	(1)	(2.3)	4.6	0.10
P × T	2	0.24	0.50	0.64
T × linear	(1)	(0.30)	0.60	0.50
T × deviations	(1)	(0.22)	0.44	0.50
P × C/T	8	0.50		

Effect of Moisture Content During Preservation in −18°C on CO_2 Evolution

The effect of moisture content during preservation in maintaining the freshness of soybeans was investigated by comparing soybean samples at 22% and 9% when preserved at−18°C. Based on the carbon dioxide evolution curves, the average deterioration times are summarized in table 7. Analysis of variance on data at 0.5% dry matter loss (table 8) shows there was a significant effect of soybean moisture content during preservation (p > F = 0.004) on the rate of deterioration, averaged over period, during the storage studies. There were also significant effects of preservation period (p > F = 0.00) and preservation period by moisture (p > F = 0.00) on the deterioration times for the samples. Analysis of the linear component averaged over all moisture contents indicated that the slope differs from zero (p > F = 0.0001), and the linear by moisture component (p > F = 0.0001) implies that slopes differ among moisture contents.

Observation of Microbial Growth

During the storage study, observations were made on the growth and development of visible fungi on the soybeans. Mycelial growth was visible after 4 to 13 days of storage. The growth was first spotted on soybeans at the bottom of the storage unit, at the end where the air enters. The range depends on the history of the sample. Samples that were combine−harvested and preserved at high moisture were found to show faster visible mold growth than samples that were hand−harvested at high moisture. The first and predominant mold to appear during storage was the grayish growth of *Penicillium* spp., common storage fungi that require relative humidity between 85% and 90% for minimum growth (Christensen and Saur, 1982). The dominance of the species is expected because the storage condition in the studies was maintained at 95% relative humidity. In the fresh samples, there were also visible spots of whitish cotton−like mold growing alongside the grayish mold, especially at the bottom section of the storage unit. This could either be *Fusarium* or *Phombosis* spp., both of which belong to a group of fungi collectively known as field fungi.

Table 7. Average aerated storage times of combine−harvested soybean preserved at 22% or 9% moisture in −18°C environments.

Storage Period (weeks)	Number of Days to Reach 0.5% DML[a]		Number of Days to Reach 1.0% DML[a]	
	22% MC[b]	9% MC[b]	22% MC[b]	9% MC[b]
0	11.5	22.5	17.8	31.2
26	10.1	18.4	14.4	23.9
48	11.6	12.4	18.0	18.3
Mean	11.1	17.8	16.7	24.5

[a] Mean from three replicates: $LSD_{\alpha=0.05}$ at 0.5% DML = 0.86; $LSD_{\alpha=0.05}$ at 1.0% DML = 1.18.
[b] Preservation moisture content.

Table 8. ANOVA table (relevant sources only) for aerated storage times to 0.5% DML, during storage of 22% and 9% moisture soybean samples preserved in −18°C environment.

Source	DF	MS	F	p > F
Moisture (M)	1	203.4	251	0.004
Period (P)	2	36.4	173.1	0.0001
lin	(1)	72.5	72.5	0.0001
Deviations	(1)	0.2	0.3	0.6000
P × M	2	42.2	200.9	0.0001
lin × M	(1)	78.5	78.5	0.0001
Deviations × M	(1)	5.8	8.5	0.04

Growth of the grayish mold intensified within the bottom 200 mm of the storage unit at the initial storage, but eventually spread over the entire storage unit. Depending on the history of the soybeans, it took between 12 to 24 days to cover the entire storage unit. It was during this phase that spots of orange mycelial growth of *Aspergilus* spp. also became visible. In tubes containing fresh soybeans, there were also spots of black mold growth resembling *Chaetomiun* spp., another field fungus.

Conclusions

Based on the results of this study, the following conclusions can be drawn:

- The moisture content of soybeans during harvest has the greatest impact on the rate of deterioration during storage. A moisture content of 13% at harvest was optimal for mitigating deterioration. Even soybeans that were hand−harvested at 8% exhibited a faster rate of deterioration than the 13% combine−harvested sample.
- Soybeans with a moisture content of 9%, when preserved at −18°C, had a slower rate of deterioration than soybeans preserved at 10°C. The difference in the rate, however, was not significant.
- Soybeans at 20% moisture content maintained the same rate of deterioration as fresh beans when preserved at −18°C.
- The rate of deterioration of 9% moisture soybeans preserved in −18°C and 10°C environments increased linearly with period of preservation. The rate was faster for soybeans preserved in a 10°C environment.

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