# Deterioration of soybeans during storage

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*Abstract*

Soybeans can deteriorate during storage when moisture, time or temperature levels are outside recommended ranges Laboratory studies were undertaken to determine effects of harvest method, variety, and splits percentage on deterioration. Deterioration was tracked by measurement of $CO_2$ emission and free fatty acid (FFA) formation during aerated storage at about 22 % moisture and 26℃. Hand harvested, high moisture soybeans deteriorated slowest, followed by low-moisture combine harvest, low-moisture hand harvest, and high-moisture combine harvest. Varieties exhibited different rates of $CO_2$ production, but FFA formation rates did not differ significantly among the three varieties. $CO_2$ production and FFA formation increased with splits content Within the 0 to 10% splits range, splits had a greater effect on FFA formation than on $CO_2$ production.

## Introduction

Major deterioration of stored soybeans is not a common occurrence in the Central United States because harvest usually takes place near the 13 to 14% moisture range which is suitable for storage for up to nine months with proper aeration When these moisture, time, or temperature levels are exceeded, however, deterioration can occur Deterioration due to fungi is associated with decomposition of carbohydrates as a result of respiration The selective respiratory utilization of carbohydrates in soybeans is assumed to be 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 (1946a). 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 non-reducing sugars There was no change in the fat content during this phase The protein content has been found to be slightly increased, but was not speculated to have any role during the decomposition process The increase was, in fact, attributed to the decrease in the 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 fungus-damaged soybeans, but either no change or an increase in the oil concentration was observed

The decomposition process that results in the loss of dry matter is usually modeled as a breakdown of simple sugar represented by the following equation:

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + 2.835kJ \quad (1)$$

Following this equation, the evolution of 14.7g of $CO_2$ per kg dry matter is equivalent to a loss of 1.0% dry matter.

### $CO_2$ production

Steele et al (1969) demonstrated that decomposition of dry matter during deterioration of shelled corn can be determined by measuring $CO_2$ 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). They evaluated the length of time that shelled corn can be stored before 0.5% of its dry matter is lost The 0.5% was considered the threshold value of dry matter loss in shelled corn before the grade is lowered because of an increase in damaged kernel total (DKT) No such threshold value has been proposed for soybeans An allowable storage time table for corn was developed using data from Steele et al 1969 (MWPS 1980). Table entries list days storage time at a specified temperature and moisture content before the corn's grade will be lowered Table entries were calculated by applying temperature and moisture multipliers to storage times at 'standard conditions' of 24% moisture and 16℃.

A model capable of predicting quality loss in stored soybeans has not been developed. 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.

### Free fatty acid

Free fatty acid (FFA) level in the oil in stored soybeans is an important quality indicator, since FFA level influences refining costs and refined oil quality. FFA forms during hydrolysis of soybean oil. The rate of the reaction increases

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with soybean temperature and moisture content. The rate of FFA increase goes up with the level of mechanical damage to the soybeans (Urbanski et al , 1980).

## Objectives

The specific objectives for three studies discussed here are:

- To determine the effect of harvesting practices on the rate of $CO_2$ evolution in freshly harvested soybeans held in aerated storage
- To compare $CO_2$ production and FFA development during aerated storage among three soybean varieties.
- To quantify the effects of splits on $CO_2$ production and FFA development in stored soybeans

## Materials and Methods

### Harvesting practices study

Soybeans used in the study were of a common variety adapted to Central Iowa. They were combine harvested or hand harvested and shelled in September 1995 at 21 to 22% (high) or 9 to 13% (low) moisture contents The lots were cleaned by use of a Carter Day Model XT3 dockage tester A total of four treatments were used (Table 1)

**Table 1.** Treatment for harvesting practice study.

| Treatment | History of sample |
|---|---|
| 1 | Combine harvested at 21% MC |
| 2 | Combine harvested at 13%MC |
| 3 | Hand harvested at 20% MC |
| 4 | Hand harvested at 9% MC |

MC - moisture content

Before the start of the experiment, the low moisture soybean samples were raised to about 21% moisture content by direct addition of a calculated weight of distilled water The approach used was quite similar to the method 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 minutes to uniformly distribute the water Each stage was separated by a 6-to 12-hrs storage period at 4 to 5℃. This prevented a sudden swelling of beans Samples were then kept at room temperature for about 12 hrs before being used in an experiment. Most of the soybeans soon presented a normal appearance as the water was taken up. Moisture was added to ensure that measurement of $CO_2$ was made from soybean samples with the same initial moisture content Samples were poured into the glass storage columns, which were arranged randomly in the $CO_2$ measuring system.

A $CO_2$ measuring system similar to that described by Dugba et al (1996) was used. During the study, carbon dioxide produced by 1-kg soybean samples stored under constant aerated storage conditions was measured Compressed air that had been filtered, stripped of $CO_2$ and conditioned to 93 ± 2.7% relative humidity and 26℃ was forced through the 1-m-long glass tubes containing soybeans at a rate of 0.45 $m^3$/min/mg. Tubes were autoclaved at 120℃ for 20 minutes before use $CO_2$ produced by the soybean samples while in storage was trapped by the $CO_2$ absorbing section of the system The sulaimanite $CO_2$ absorbent agent (Al-Yahya, 1991), was packed in plexiglass tubes. The weight gain recorded every 24 hrs was a measure of the amount of $CO_2$ produced during the period. The weight of the $CO_2$ gained was corrected to account for the residual $CO_2$ present in the air-stream (Rukunudin 1997)

### Statistical analysis

Each treatment was replicated three times Statistical analysis of data was carried out using the Statistical Analysis Software (SAS Institute, 1990). Polynomial regression models to describe the $CO_2$ evolution with time of storage 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 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 difference (LSD) between the means (Steel et al., 1997) at $P < 0.05$. Visible microbial growth and development during storage were also noted

### Varieties study

Soybeans used in the study were of three varieties adapted to Central Iowa and are designated as varieties A, B, and C They were grown 15 km west of Ames, Iowa

### Soybean shelling and cleaning

The soybean lots were hand harvested in pods on September 30, 1997 at a moisture content of about 8.5% Harvested pods were run through an Almaco soybean sheller, which employed the rubbing motion imposed of rubber belts moving at different speeds. Distance between belts was such that 90% of the soybeans were shelled on a

first run. Considering low moisture content of soybeans and small force needed to open up the pods, it could be assumed that the damage caused by shelling is about equal to hand shelling damage.

The first step of cleaning was provided by the sheller fan which, being set on a maximum speed, removed all the straw material and pods but failed to remove even small and immature seeds During cleaning with a Carter Day Dockage Tester (Model XT3), unshelled pods went over a 7.9-mm round hole sieve and small amount of foreign material and immature seeds went through the 4-mm round-hole sieve There were no splits in a pan underneath the 4-mm sieve It indicated that the damage of the soybeans was minimum The shelling and cleaning machines were not sterilized. Therefore, soybeans were assumed equally contaminated with fungi spores after passage through the machines.

### Raising soybean moisture content

Each variety of soybeans was split into two 2500-g bags Soybean moisture content was raised to 22% by direct addition of water into the bag. Water addition was accomplished in 5 steps, as 60 to 100 g of water was added at a time into each bag raising moisture content by 2 to 3 points. Soybeans were mixed in a bag for 30 seconds after each addition Soybeans and distilled water used were stored in a cooler at 3 to 5℃ for 3 to 17 hrs between each step. After the last batch of water was added soybeans were kept in a cooler until total time in a cooler reached 36 hrs.

### Filling the tubes

Soybeans were split into 800-g batches and poured into 90-cm by 50-mm diameter glass tubes, closed from each side with one-hole stoppers A layer of glass wool was placed from each side between the stopper and the beans to improve air distribution. Tubes were previously sterilized at 120℃ for at least 20 min.

### $CO_2$ collection system

A preservation system similar to that described by Dugba at al. (1996) was used. Air in the environmental chamber was maintained at 95 % RH, 26℃ throughout the experiment. An air pump pulled air from the environmental chamber through each tube containing soybean samples. The airflow rate was set at 0.45 $m^3$/min/Mg Air carrying out $CO_2$ produced by deteriorating soybeans was stripped of moisture and then of $CO_2$ and additional moisture. Tubes containing both Sulaimanite (for $CO_2$ removal) and Drierite (water removal) were weighed every two days. Increase in weight was a direct measure of $CO_2$ collected during the period. Tubes were changed during the trial as needed.

### Correction for atmospheric air $CO_2$

Five airlines not connected to the soybean tubes were operated to collect atmospheric $CO_2$ from the air pulled from the environ-mental chamber The average reading of five tubes was subtracted from the reading of each line collecting $CO_2$ from soybeans, thus giving the value of carbon dioxide produced by each soybean sample

### Soybean oil extraction and analysis

Samples of soybeans taken at the beginning of the trial, at first sampling (540 hrs), and at second sampling (997 hrs) were dried and ground in a Magic Mill III flour mill. The time of the second sampling was also the end of the experiment Oil was extracted from the samples as specified in AOCS Official Method Aa 4-38 (AOCS, 1989). FFA content in the oil samples was determined using a revised version of AOCS Official Method 5a-40 for the determination of free fatty acid in soybean oil (Rukundin et al., 1998).

### Experimental design & statistical analysis

The experimental design used was completely randomized. Six replications of the 3 treatments were randomized according to the location inside the chamber and tube location on a rack. Statistical analysis included tests for the effects of variety on $CO_2$ production and FFA development. The GLM procedure was used for analysis.

### Splits study

Splits are defined as soybeans with foreign material removed and more than 25% of the soybean missing. Soybeans are susceptible to splitting due to their seed structure that consists of an embryo enclosed by two hemispherical cotyledons, held together by a seed coat

Six treatments were used to investigate effects of splits on the rate of soybean deterioration The different treatments contained 0, 3, 7, 12, 22, and 40% splits by weight, respectively Three replications of each split treatment were tested in a totally randomized design

### Preparation of treatments

The soybeans used for the experiment were of the same variety used in the harvesting practice study They were combine harvested at 16% moisture content (wet basis) in September 1997 and stored in a freezer at −18℃ until the time of the experiment. Once removed from the freezer, they were spread out on trays and allowed to dry at room temperature. This was done to aid in splitting the soybeans, and to effectively halt deterioration.

Once dry, the soybeans were cleaned in a Kice model 6DT4 aspirator After aspiration, the soybeans were again spread on trays, one layer deep, to be sorted by hand. Once all of the naturally created splits had been removed, the soybeans were again cleaned in the aspirator The lots of soybeans and splits were weighed to determine the maximum size of each sample treatment. Next, more splits were

created by running soybeans through a Quaker City Mill model F, No. 4 burr mill.

The final step of preparation of the treatments was to raise the moisture content of the soybeans to approximately 22.5% (wet basis moisture content) by adding distilled water The hydrating process was carried out by adding water three times, with time to equilibrate in between. This was done in an effort to match what was predicted to be the equilibrium moisture content attained by the soybeans in the 26℃, 95% relative humidity environment where they would be placed to deteriorate during the experiment. This reduced any lag times in deterioration associated with hydrating soybeans.

Immediately after the final moisture content had been reached, the six treatments were separated into samples and placed in eighteen sterilized, 90-cm-long, by 50-mm-diameter glass tubes. The tubes were then placed in a constant environment of 26℃ and 95% relative humidity, and connected to an apparatus that aerated the soybeans and collected any $CO_2$ produced during deterioration

### Carbon dioxide collection

Quantification of the rate of deterioration was accomplished by capturing all of the $CO_2$ respired by the soybeans. The apparatus and the procedure were the same as used in the variety study.

### FFA measurement

Once before aeration was started, twice during aeration, and again at the completion of aeration, a 250-g sample was drawn from each tube for FFA testing. These samples were dried and ground in a Magic Mill III flour mill Oil was then extracted from this soybean meal with an apparatus as described in AOCS Official Method Aa 4-38 (AOCS, 1989). The amount of FFA present in each soybean oil sample was determined using a revised version of the AOCS Official Method 5a-40 for the determination of free fatty acid in refined and crude soybean oil (Rukunudin et al., 1998).

## Results and Discussion

### Harvesting practice study

Figure 1 shows curves describing the $CO_2$ evolution from fresh soybeans combine or hand-harvested and held at 26℃ and 22% moisture. The curves were derived from third order polynomial regressions on the data of three replications (Rukunudin, 1997). Coefficients of the terms (Table 2) were considered as part of the model only if their respective t-statistics were shown to be significant.

**Table 2.** Regression models for $CO_2$ evolution from harvesting practices study.

| Treatment | General model: Y, g $CO_2$/kg dm = $c_1 t + c_2 t_2 + c_3 t_3$ Coefficients of the polynomials | | |
|---|---|---|---|
| | $c_1$ | $c_2$ | $c_3$ |
| Combine-harvested/21% MC | 0.19 | ns | 0.038 |
| Combine-harvested/13% MC | ns | 0.012 | 0.0001 |
| Hand-harvested/20% MC | 0.034 | 0.006 | 0.0001 |
| Hand-harvested/9% MC | ns | 0.018 | ns |

MC - moisture content; t - number of days, ns - not significant

*Combine harvested*

Table 3 shows respective average 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 treatment means were established at the two dry matter loss levels. In each case, times for low-moisture soybeans were significantly greater than those for high-moisture soybeans. Soybeans combine-harvested at high moisture content were found to lose 0.5% dry matter at about twice the rate of dose at low moisture content Mechanical damage from combining high moisture content soybeans undoubted contributed to the faster rate of deterioration. The rate of deterioration of soybeans harvested at 13% moisture content, which is within the range of 11 to 14% moisture content associated with optimum toughness (Paulsen, 1977), should therefore demonstrate approximately the minimum rate of deterioration achievable when soybeans are combine harvested.

*Hand harvested*

Soybeans hand harvested at 20% moisture showed the slowest deterioration of all 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, that is when the pods are brown in color and ready to harvest, is reached about 18 to 20% moisture content. Thus the deterioration curve of hand harvested soybeans at 20% may describe soybeans near their highest quality

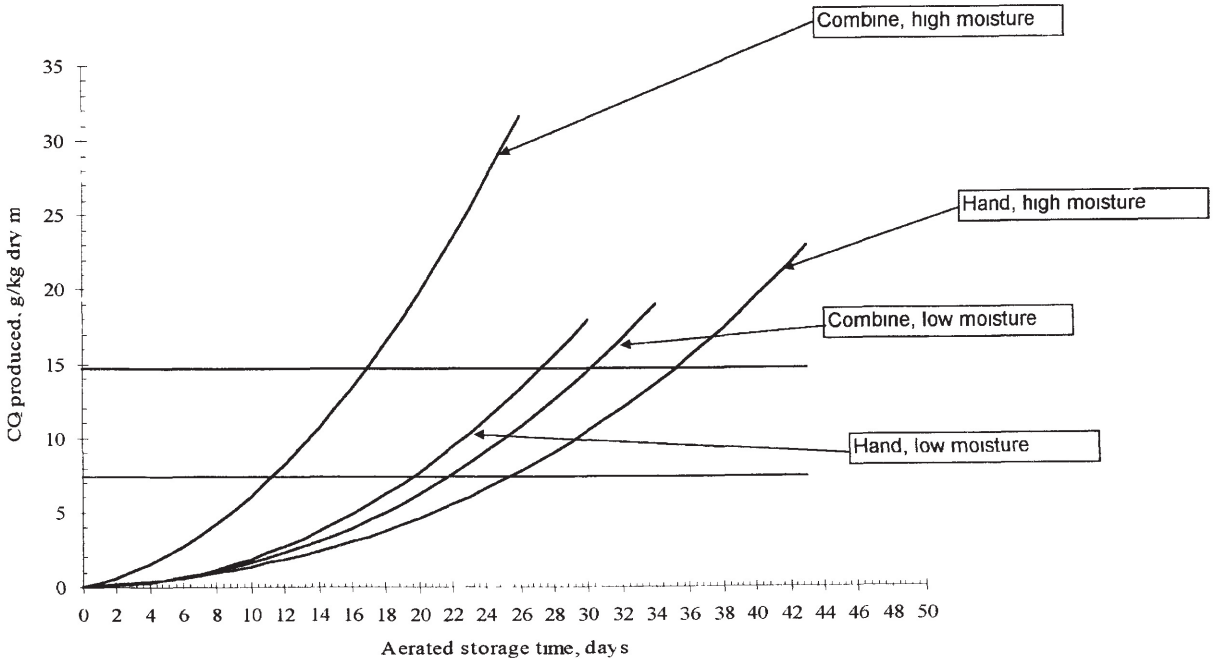


**Fig. 1.** $CO_2$ evolution from soybeans under two modes of harvesting with two harvest moisture contents.

Soybeans hand-harvested at low (9%) moisture content exhibited a significantly faster rate of deterioration than the high moisture content treatment (Figure 1, Table 3). 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 developed in the hulls of those lots render them more susceptible to microbial attack According to Milner and Geddes (1946 b), damaged seeds present a more hospitable medium for mold mycelial penetration and growth of microorganisms than undamaged beans It is in these cracks and broken parts of the beans that mold growth first appears

**Table 3.** Aerated storage times of freshly harvested soybeans stored at 26℃ and 22% moisture.

| Number of days[a] to reach respective DML level | | | | | |
|---|---|---|---|---|---|
| | Combine harvest | | | Hand harvest | |
| Moisture | Dry matter loss | | Moisture | Dry matter loss | |
| at harvest, % | 0.5% | 1.0% | at harvest, % | 0.5% | 1.0% |
| 21 | 11.5 | 17.8 | 20 | 26.2 | 37.1 |
| 13 | 22.5 | 31.2 | 9 | 19 8 | 28 1 |

[a] Mean from three replicates

$LSD_{\alpha=0\ 05}$@ 0 5% DML = 2 13, $LSD_{\alpha=0\ 05}$@ 1 0% DML = 2.69

## Varieties study

Average values of the total $CO_2$ production, g/kg of dry matter, for three varieties presented in Table 4. Statistical analysis showed no differences among the varieties in $CO_2$ production. The curves describing deterioration process are presented in Figure 2. $CO_2$ evolution from soybean samples was accelerating for the first 200 hrs of the experimental trial, reaching an average value of 0.25 g of $CO_2$ production per day This average rate was about constant for the next 797 hrs until the end of the experiment Even though the exponential stage is clearly observed at the beginning of the experiment, the linear curve sufficiently descries $CO_2$ production for each variety

**Table 4.** $CO_2$ production means and standard deviations at three sampling times, g/kg of dry matter

| | 0 h | 540 h | 997 h |
|---|---|---|---|
| Variety B | 0 | 8.23± .63 | 18.72±1.79 |
| Variety A | 0 | 7.1±0.55 | 15.02±1.56 |
| Variety C | 0 | 7.94±0.55 | 18±1.57 |

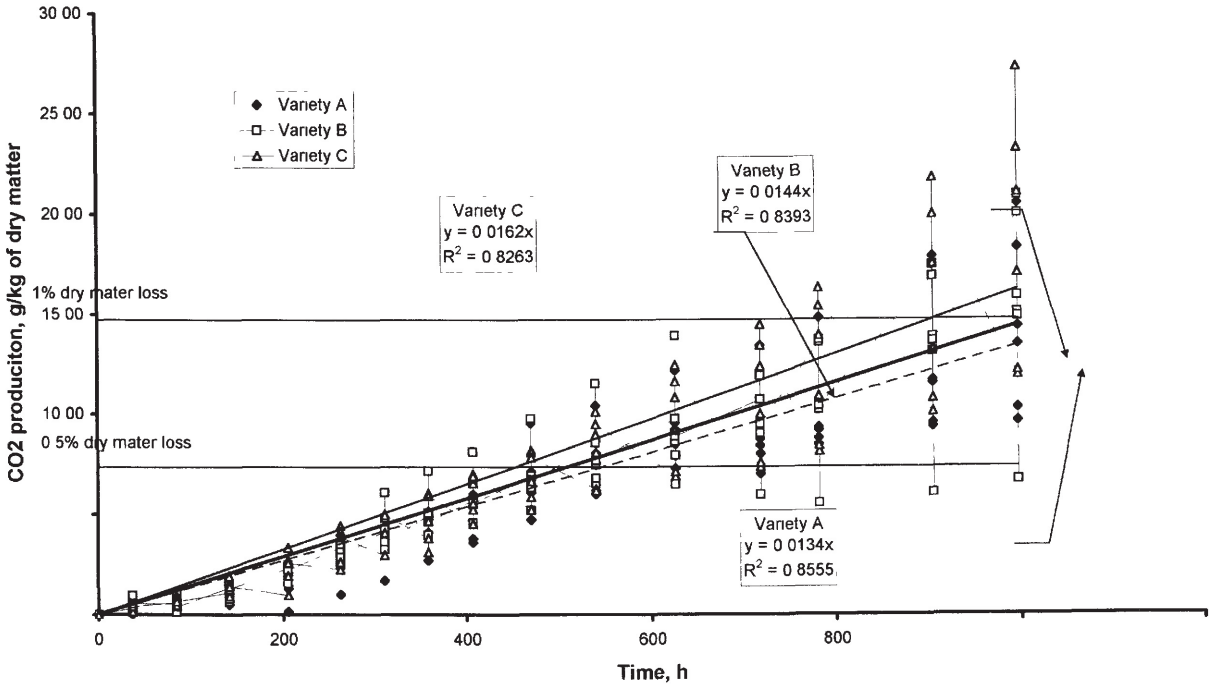


**Fig. 2.** Cumulative $CO_2$ production for 3 varieties.

*FFA development*

The averages of FFA oil content at the time of the beginning of the experimental trial, and at first and second sampling are presented in Table 5 Fitted curves are shown in Figure 3. At the beginning of the trial Variety C, visually defined as the most sound soybeans, showed the lowest FFA content of 0.16%. Variety B showed the second high value of 0.21% FFA and Variety A was the highest (0.29% FFA)

**Table 5.** FFA means and standard deviations at three sampling times, %

| | 0 h | 540 h | 997 h |
|---|---|---|---|
| Variety B | 0 21 | 0 38±0.033 | 0.68±0 12 |
| Variety A | 0.29 | 0 24±0.029 | 0.56±0 11 |
| Variety C | 0.16 | 0.41±0.029 | 1.2±0 11 |

$LSD_{\alpha=0\ 05}$@ 540 h 0 082 $LSD_{\alpha=0\ 05}$@ 997 h 0.30

At the time of first sampling Variety A, starting with the highest initial value of FFA, showed the lowest value of 0.24 % FFA. The other two varieties redistributed with Variety C having the highest level of FFA developed 0.4% and Variety B between the other two with 0.38% This order also was kept at the time of a second sampling when Varieties A, B, and C showed corresponding values of 0.56, 0.68 and 1.2%, respectively This high FFA increases for Variety C, which started lowest, suggests that there is a difference in FFA production that may be due to the effect of variety at 540 hrs, the differences between A and B, and A and C exceeded the LSD At 997 h, differences between B and C, and A and C exceeded the LSD.

Figure 4 shows correlation between $CO_2$ evolution and FFA produced for Variety A The linear model sufficiently fits the data with $R^2 = 0.89$ for Variety A. This correlation suggests that fungi may be involved in FFA development.

## Splits study

The cumulative mass of $CO_2$ produced per kilogram of soybean dry matter for each treatment is shown in Fig 5. During the first 75 hrs of deterioration, only few $CO_2$ was evolved by the soybeans Samples with the lowest percentages of splits were initially deteriorating faster than those with a greater split content. It has been theorized that this happens because soybean fungi spores are generally found on the seed hull, and, if the outer seed coat is missing (as generally occurs with splits), fewer spores will be present During the midpoint of the logarithmic growth phase, soybeans containing higher levels of splits began to deteriorate much faster than those with fewer splits. This is likely because the rate of fungi reproduction can quickly overcome any differences in initial levels of fungi, and fungi on splits have much greater access to starch.

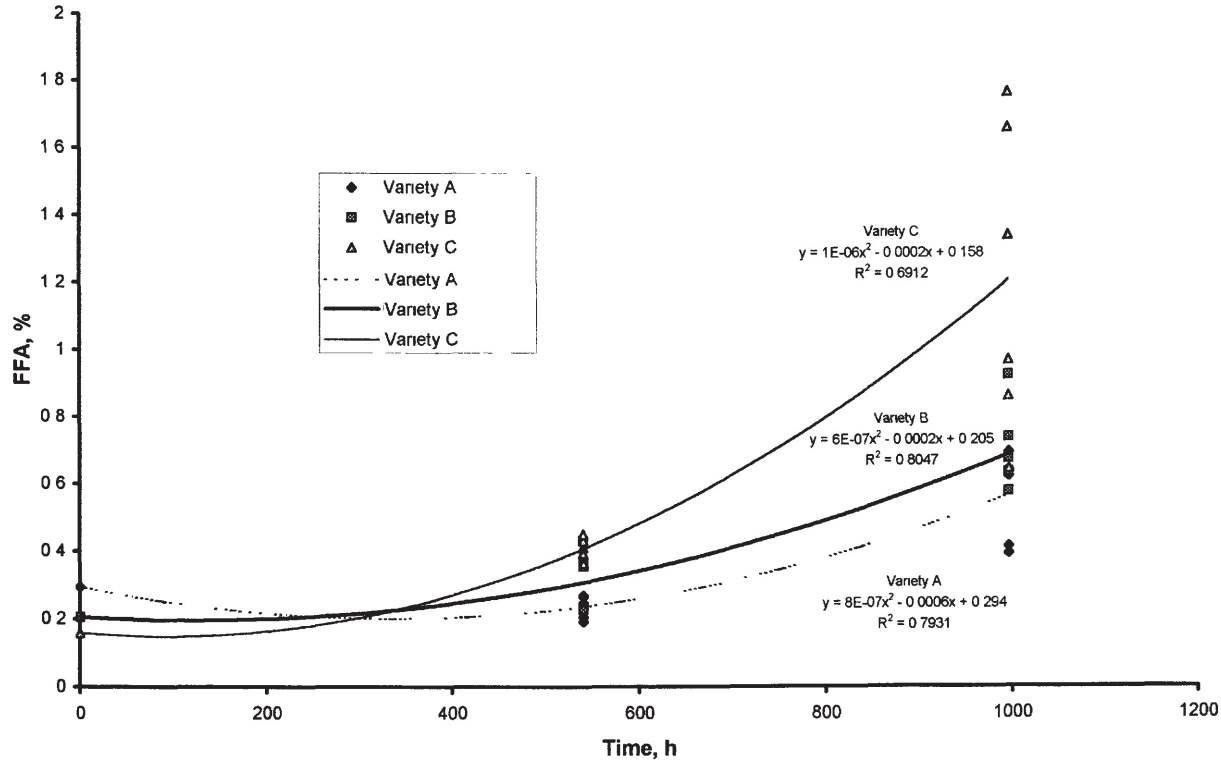


**Fig. 3.** FFA production for three varieties.

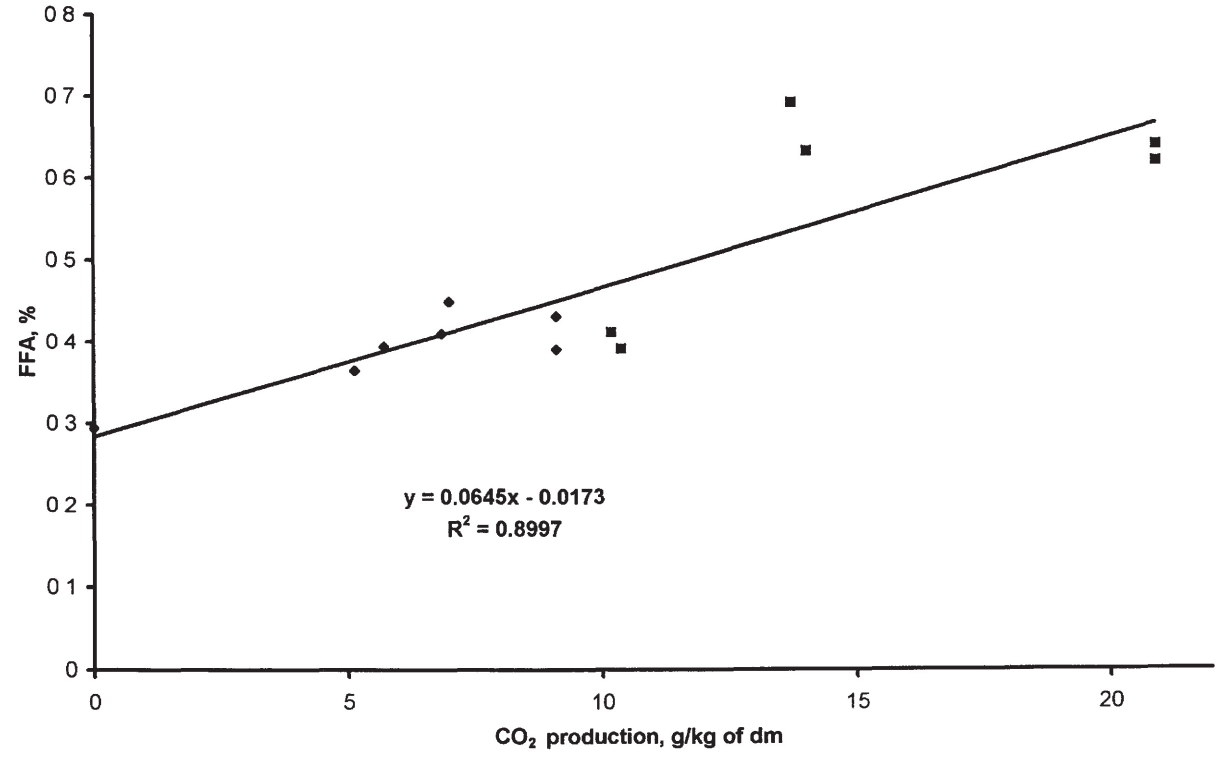


**Fig. 4.** Correlation of FFA production to $CO_2$ production for variety A.

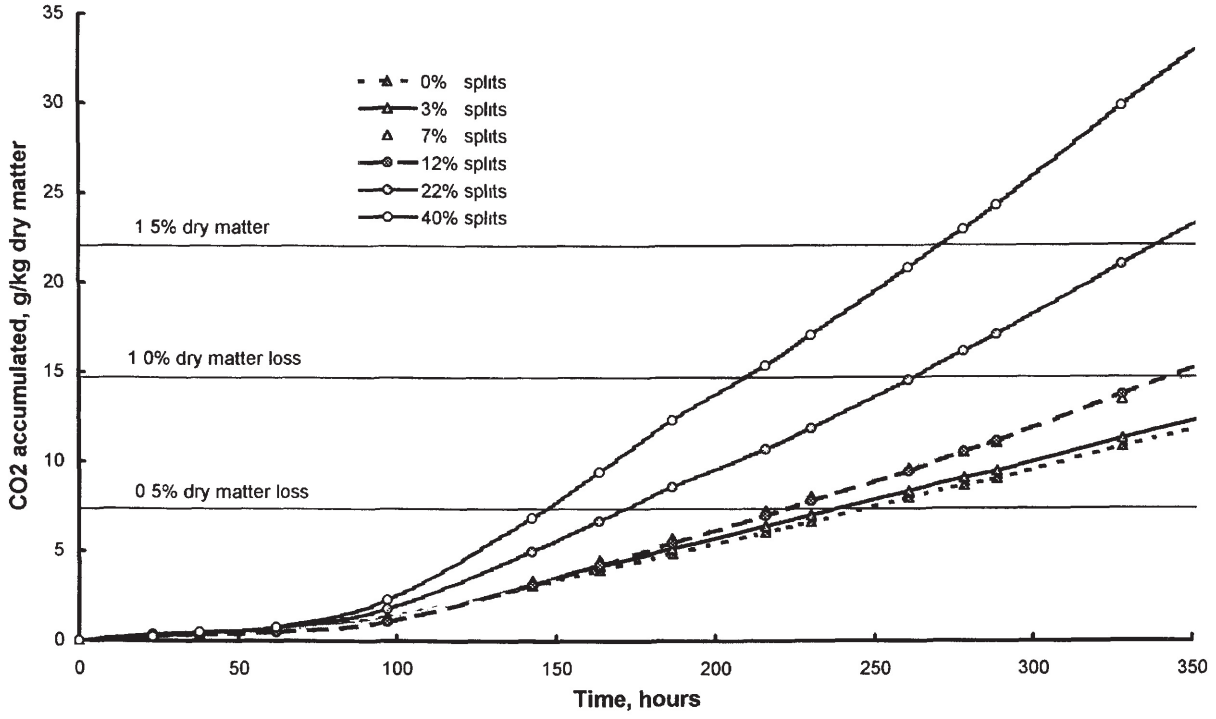


**Fig. 5.** Carbon dioxide accumulated per kg dry matter vs Time (average of three repetitions).

In the period between 75 and 200 hrs, storage fungi grew linearly. The 40% splits treatment was deteriorating fastest, the 22% treatment was deteriorating more slowly, while the 0, 3, 7, and 12% treatments were deteriorating the slowest at nearly identical rates

From 200 hrs until the end of the experiment (350 hrs), the same general trend was maintained. The only difference was that the 0 and 3% treatments were deteriorating at nearly the same rate, and the 7 and 12% treatments were also performing alike, but at a higher rate. The higher overall rate of deterioration of the treatments with greater percentages of splits is most likely due to the much greater availability of starch to the fungi.

*FFA Measurement*

FFA levels were measured for samples of each treatment taken before the start of monitored deterioration, at (about) 0.5% DML, 1.0% DML, and at the end of the experiment (Figure 6). This data corresponds very well with data shown on the $CO_2$ evolution graph The treatments with the lowest split percentages began with higher FFA levels, but were quickly surpassed by the treatments with 40% and 22% splits.

## Split multipliers

FFA data and $CO_2$ data were compiled to form split multiplier curves (Fig. 7) based on the time of each treatment to reach 0.75% FFA, and 0.5% DML versus a treatment of 0% splits. A soybean split multiplier could be used in conjunction with a soybean allowable storage time table or equation The predicted allowable storage time of a mass of soybeans would be multiplied by the value of the soybean split multiplier curve applied at the level of splits pertaining to the mass of soybeans in question. This product would reflect the decrease in allowable storage time due to the presence of splits For example:

Assume a lot of whole soybeans can be stored 300 days before an economically notable loss in quality is realized. Consider, however, a lot of soybeans containing 10% splits The $CO_2$ multiplier would be $(0.00001x^3) - (0.0006x^2) - (0.006x) + 1.0 = 0.89$, and the FFA multiplier would be $(-0.00001x^3) + (0.0009\ x^2) - (0.0261x) + 1.0 = 0.839$. Thus the lot of soybeans containing 10% splits could be stored $300 \times 0.89 \approx 267$ days for $CO_2$ and $300 \times 0.839 \approx 251$ days for FFA, before significant quality is lost.

A quick examination of the multiplier curves may yield some useful insights concerning the deterioration characteristics of soybeans as an effect of split content. It appears FFA content is affected most in the lower split contents (0 to 18%), while mainly holding constant in the ranges of high split content. The effects on respiration tend to exhibit the opposite relationship, with the rate of $CO_2$ production being affected more at the highest split level.

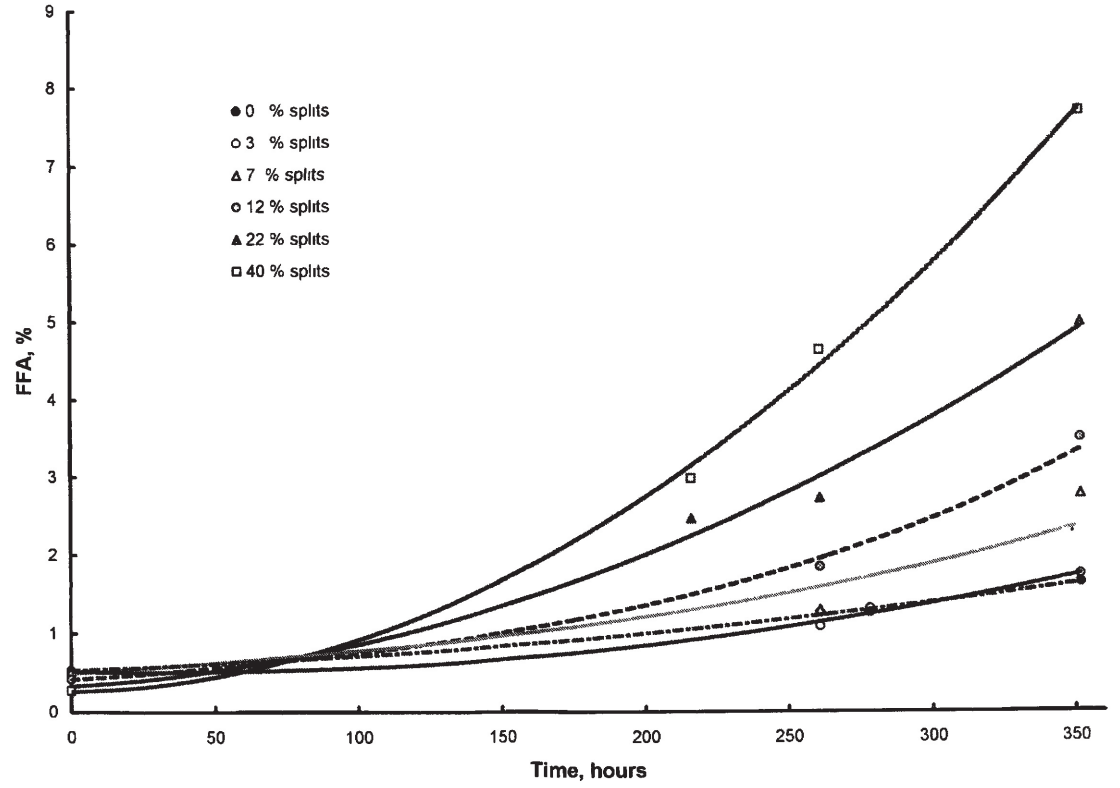


**Fig. 6.** Soybean oil FFA content vs Time (average of three repetitions).

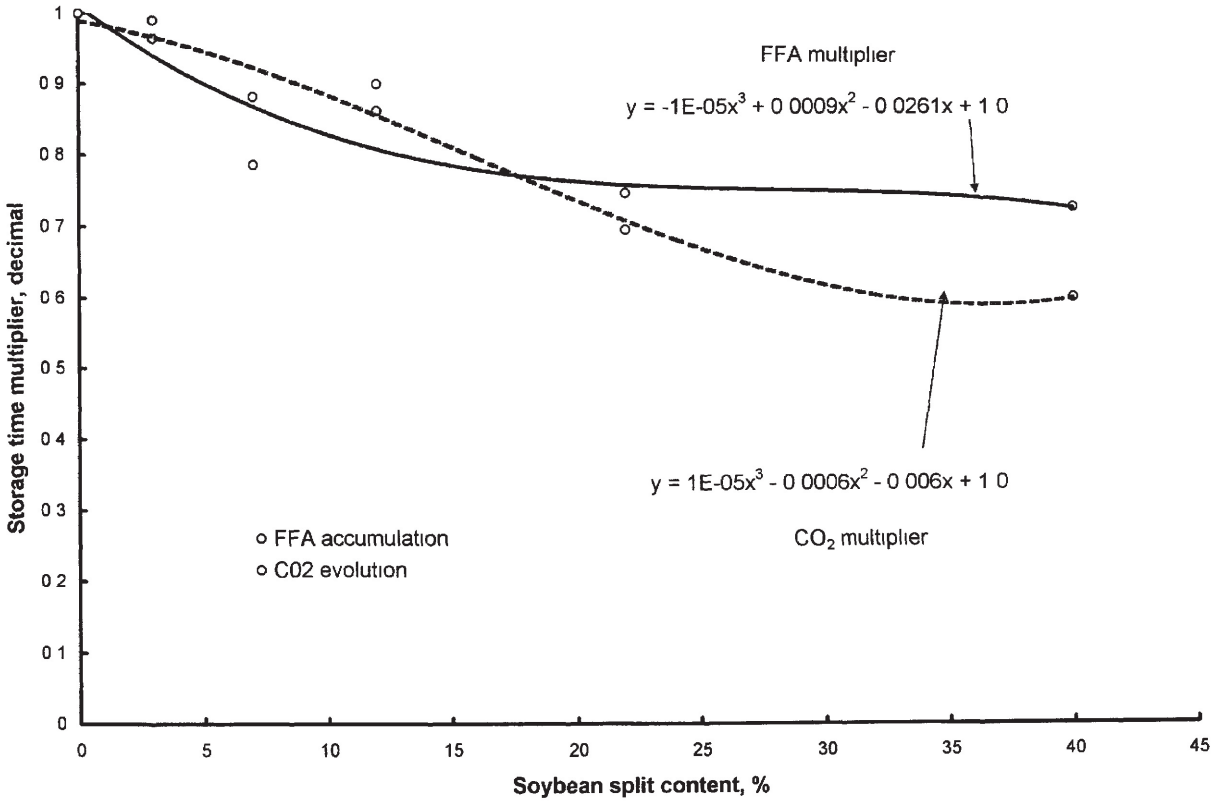


**Fig. 7.** Split multiplier curves.

## Conclusions

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

1 Moisture content of soybeans during harvest has the greatest impact on the rate of deterioration during storage. A moisture content of 13% can be considered best for harvest, in terms of deterioration Even soybeans manually harvested at 8% exhibited a higher rate of deterioration than the 13% machine-harvested sample.

2 Varieties A, B, and C produced $CO_2$ during storage at 22% moisture at statistically similar rates.

3 Varieties A, B, and C produced FFA during storage at 22% moisture at statistically different rates.

4. The fraction of whole soybean storage time to reach 0.5% DML ($y$) versus the percentage of splits ($x$) can be modeled by the following equation:

$$y = 0.00001x^3 - 0.0006x^2 - 0.006x + 1.0$$

5 The fraction of whole soybean storage time to reach 0.75% FFA content ($y$) versus the percentage of splits ($x$) can be modeled by the following equation:

$$y = -0.00001x^3 + 0\ 0009x^2 - 0.0261x + 0.9968$$

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