

Relating Electrolyte Leakage to Shelled Corn Storability¹

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ABSTRACT

Cereal Chem. 75(5):651–655

Corn from three growing seasons was harvested, dried, and stored in small bins. Samples taken at harvest and after various periods of bin storage (up to 77 months) were evaluated by two primary tests: carbon dioxide evolution during accelerated storage at $\approx 20.5\%$ moisture content and 26°C and electrolyte leakage from bulk (100 g) samples soaking in deionized water. The initial ($t < 72$ hr) slope of carbon dioxide evolution rate curves (SLOPE72) was used as a base measure of storability (i.e.,

potential for safe storage without significant mold invasion). In electrolyte leakage tests, both test temperature and initial sample moisture content influenced results. Additionally, water conductivity after 10 min was correlated ($r = 0.79$) with SLOPE72, implying that electrolyte leakage has the potential to provide rapid information regarding future storability.

One of the objectives of grain standards (as stated in the 1986 U.S. Grain Quality Improvement Act) is to provide information to aid in determining grain storability (Hill 1990). Current grain testing practices, however, fail to meet this objective. In a comprehensive study of U.S. grain quality, the U.S. Office of Technology Assessment (Office of Technology Assessment 1989) reported that no rapid test is available to determine the stage of deterioration or the remaining storage life of grain. Therefore, a measurement procedure is needed to evaluate differences in storability (i.e., the potential for safe future storage without economically significant mold invasion) among samples that appear to be equal on the basis of existing grading factors.

The need for a rapid storability test has been expressed by both the academic community and grain industry. Several researchers (Multon 1988, Hurburgh 1990, Sauer et al 1992) have noted a need for a test that can predict the storability of grain with unknown storage history. Also, Marks et al (1994) determined, via a mail survey, that 62% of the managers in the U.S. corn industry probably would pay for such a test if it were sufficiently fast and inexpensive (e.g., < 15 min and \$5 per sample).

Numerous researchers have used cumulative CO_2 evolution during accelerated storage tests as an index of mold-induced deterioration for freshly harvested corn (Saul and Steele 1966, Steele et al 1969, Seitz et al 1982, Friday et al 1989, Al-Yahya et al 1991, Wilcke et al 1993). These tests measure actual grain deterioration; however, samples have been limited to freshly harvested corn, and the tests are slow (i.e., typically requiring a test time of > 10 days). Based on comparisons with slower tests, Marks and Stroshine (1995) demonstrated that the initial slope of CO_2 evolution rate curves (as opposed to cumulative CO_2 evolution) is an acceptable indicator of storability. This new test method requires only 72 hr of test data, compared to the 200–500 hr required for cumulative CO_2 evolution. Even for corn previously stored in long-term bin storage (up to 77 months), the initial slope is a valid measure of storability.

Although this new analysis method reduced the required test time, 72 hr is still too slow for routine use by the grain industry. The survey conducted by Marks et al (1994) indicated that, even

without consideration of cost, $< 10\%$ of elevator managers who store corn would be likely to use a storability test that required 72 hr. Consequently, there is still a need to develop substantially faster (e.g., ≤ 15 min) test methods to provide information regarding storability. Electrolyte leakage testing may meet this need.

Measuring seed viability historically has been the primary motivation behind most electrolyte leakage studies. Fick and Hibbard (1925) and Hibbard and Miller (1928) reported a relationship between electrolyte leakage from soaked seeds and the percent viability of the seed lots. However, subsequent studies did not agree completely; based on differing criteria, some concluded that electrolyte leakage testing is effective (Brouwer and Mulder 1982, Keys 1982), and others concluded that it is ineffective (Hallowin 1975, Hepburn et al 1984, Herter and Burris 1989) as a test for seed viability.

In spite of the mixed results for viability testing, previous research indicates that electrolyte leakage can be affected by several other factors that also impact grain storability. For example, researchers do agree that electrolyte leakage increases with seed aging (Ching and Schoolcraft 1968, Gill and Delouche 1973, Ghosh et al 1981, Keys 1982, Pandey 1988, Ferguson et al 1990). Electrolyte leakage increases even before loss of viability in storage (Ferguson et al 1990) and continues to increase after seeds are no longer viable (Ching and Schoolcraft 1968, Ghosh et al 1981). Pandey (1988) found that electrolyte leakage from beans correlates better with seed aging ($r = 0.99$) than with seed viability ($r = 0.81$). Additionally, electrolyte leakage from seeds is affected by mechanical damage (Tao 1978), excessive heating or improper drying (Hallowin 1975, Keys 1982, Seyedin et al 1984, Herter and Burris 1989), and genotype (Gill and Delouche 1973, Odiemah 1989). All of these factors also affect future storability of corn (Friday et al 1990, Marks and Stroshine 1995). However, electrolyte leakage testing has not been evaluated previously as a rapid indicator of future storability.

On the basis of results reported by Marks and Stroshine (1995), CO_2 evolution in accelerated storage tests was assumed to be a suitable reference measure of storability for this study. Given this assumption, the specific objective was to assess the relationship between electrolyte leakage from soaking corn seed and corn storability, as measured by CO_2 evolution during accelerated storage tests.

MATERIALS AND METHODS

Harvest, Drying, and Bin Storage

Study samples included corn from three harvest seasons (1986, 1991, and 1992). Three hybrids (B73 \times Mo17, FR35 \times FR20, and Pioneer Brand hybrid P3377) were grown at the Purdue University Throckmorton Farm, near West Lafayette, IN, and harvested with an axial-flow combine during September or October of each year. Certain lots of corn were dried in a low-temperature (ambient air) drying system (Friday et al 1990), and others were dried

¹ Published with the approval of the director of the Arkansas Agricultural Experiment Station, University of Arkansas, Fayetteville, manuscript 97089, and the director of the Indiana Agricultural Experiment Station, Purdue University, West Lafayette, manuscript 15510. Mention of a commercial name does not imply endorsement by the University of Arkansas or Purdue University.

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in a high-temperature (95°C) cross-flow batch dryer (Martins 1988). Details of production, harvest, drying, and storage procedures have been described previously by Marks (1993).

After completion of drying, the corn lots were stored in small unsealed bins (0.56 m diameter, 3.66 m deep), without aeration, for up to 77 months in an unheated pole-construction building. A summary of ambient and grain temperatures during drying and storage has been reported by Marks (1993). During storage, grain samples were drawn periodically from each bin at various depths. The probed samples were temporarily stored at 3°C (<96 hr). For subsequent analyses, samples were divided randomly into subsamples using a Precision divider (Seedbuco, Chicago, IL).

Accelerated Storage and Carbon Dioxide Evolution Tests

Two types of samples were tested in accelerated storage tests: fresh samples from harvest (20–24% moisture content [mc]) and samples of dry corn removed from bin storage (12–17% mc). Sample preparation for testing the two types differed slightly; a complete description of the test procedures has been given by Marks and Strohshine (1995). In both cases, each sample was divided into three 500-g subsamples, and each subsample was tested in a separate test column at ≈20.5% mc and 26°C. All results are reported as mean values of three replicate samples.

The CO₂ measurement system included an air-humidifying section, an air manifold, flow sensors, corn storage columns, valves and switches to direct effluent air, a flow-through infrared gas analyzer, and a data acquisition system. Every 2 min, the system measured the flow rate and increase in CO₂ concentration of air flowing through the samples. The CO₂ evolution rate was calculated by multiplying the air-flow rate by the increase in CO₂ concentration. A detailed description of the system has been given by Marks (1993).

Electrolyte Leakage Tests

For each sample tested in the CO₂ evolution tests, a corresponding subsample was subjected to an electrolyte leakage test. Subsamples for this procedure were divided from the original bin samples with a Precision divider. The corn was passed over a 4.76-mm (¹²/₆₄-in.) round-hole sieve to remove fine material without removing broken pieces and damaged kernels (>4.76 mm). Each test was performed on three 100-g replicate samples of corn. All analyses are reported as mean values of triplicate measures.

Each replicate sample was soaked in 400 mL of deionized water (initial conductivity <100 × 10⁻⁶ S/m) in a 600-mL beaker. Beakers were held in a water bath at 25 ± 1°C, unless otherwise stated. The conductivity of the soak water was measured with a YSI (Yellow Springs, OH) model 35 conductivity meter and a YSI model 3417 dip-cell conductivity probe. Once beaker water reached the desired temperature, one 100-g replicate sample of corn was placed in the beaker, and conductivity was measured immediately and recorded manually. Five minutes after the start of the test, the corn was stirred by gently rotating a glass stirrer rod five times around the beaker, following the inside edge of the glass. The conductivity of the soak water was measured immediately without removing the sample. Before each measurement, the probe was rinsed in deionized water. This cycle was repeated every 5 min for 1 hr for each beaker.

To verify the effects of test temperature on electrolyte leakage, three subsamples of fresh B73 × Mo17 corn grown in 1992 were tested in soak water at 20.0, 24.8, and 34.5°C, respectively, with three replications at each temperature.

An additional series of these tests was conducted specifically to evaluate the effects of initial sample moisture content on electrolyte leakage. Samples of fresh FR35 × FR20, B73 × Mo17, and P3377 corn grown in 1992 were each divided into four subsamples. For each hybrid, subsamples were air-dried during a period of several

TABLE I
Sample Identification, History, Conditions, and Estimated Initial Slopes from Carbon Dioxide Evolution Tests

Corn Hybrid	Height (m) ^a	Bin Storage (months)	Storage mc ^b (% , wb)	Note ^c	DI ^d	SLOPE72 ^e (g CO ₂ /[kg DM]/hr/hr)
1986 harvest						
FRB73 × Mo17	1.83	56	13.4	...	na	142 (25.2)
Pioneer P3377	0.61	56	13.2	...	na	357 (47.9)
FRB73 × Mo17	1.83	77	13.1	...	16.1	199 (28.0)
FRB73 × Mo17	2.44	77	14.5	...	33.2	288 (38.5)
1991 harvest						
FR35 × FR20	1.83	4	13.0	...	19.5	289 (44.4)
Pioneer P3377	2.44	4	13.2	...	19.8	503 (53.9)
FR35 × FR20	2.29	18	15.1	...	18.2	328 (50.4)
Pioneer P3377	2.44	18	13.4	...	22.3	654 (96.4)
FR35 × FR20	1.83	18	12.7	...	21.8	196 (92.1)
FR35 × FR20	1.98	18	13.1	...	20.4	251 (37.2)
1992 harvest						
FR35 × FR20	ns ^f	ns	18.0	-9.47 (8.0)
Pioneer P3377	ns	ns	17.7	6.76 (4.5)
B73 × Mo17	ns	ns	21.9	-8.25 (5.8)
Pioneer P3377	0.61	3	14.9	...	21.4	220 (8.8)
Pioneer P3377	0.61	3	14.7	HTD	21.0	540 (14.5)
B73 × Mo17	1.83	7	16.9	...	23.4	24.0 (29.2)
B73 × Mo17	1.22	7	16.0	HTD	24.7	255 (21.4)
FR35 × FR20	1.83	7	16.3	...	19.9	-46.6 (23.3)
Pioneer P3377	1.83	7	16.6	...	21.6	66.8 (41.3)
Pioneer P3377	0.61	7	13.8	HTD	27.4	386 (48.6)
FR35 × FR20	0.61	7	15.7	...	19.8	-101 (10.8)
Pioneer P3377	0.61	7	15.7	...	19.4	27.2 (30.3)

^a Height above bin floor from which sample was withdrawn.

^b Moisture content.

^c HTD = high-temperature dried.

^d DI = damage index (Chowdhury and Buchele 1976). na = data not available.

^e SLOPE72 = initial slope of CO₂ evolution curve. Standard deviations in parentheses.

^f ns = not stored; corn was tested without previous storage.

hours in a laboratory dryer ($\approx 30^{\circ}\text{C}$) to four moisture contents, ranging from 12.0 to 24.8%. These samples were tested in triplicate for electrolyte leakage, and the relationships between moisture content and conductivity were evaluated via simple linear regression.

Laboratory Analyses

In addition to accelerated storage and electrolyte leakage, each corn lot was subjected to several laboratory analyses. Moisture content was determined by the whole-kernel, air-oven method (ASAE 1992), with triplicate samples, and is reported on a wet mass basis. Mechanical damage was evaluated by the procedure of Chowdhury and Buchele (1976) to determine a weighted damage index (DI). Additionally, percent germination was determined by placing 50 kernels on moist filter paper and counting any kernel that produced a root and shoot after seven days at room temperature.

Statistical Analyses

Data were analyzed using statistical analysis software (release 6.07.02, SAS Institute, Cary, NC). PROC CORR was used to analyze the correlation between electrolyte leakage data and CO_2 evolution data (SLOPE72 [initial ($t < 72$ hr) slope of carbon dioxide evolution rate curves]). PROC REG was used to conduct stepwise multiple linear regression, with the significance level for entry set at $\alpha = 0.15$.

RESULTS AND DISCUSSION

Samples representing 22 postharvest treatments (Table I) were analyzed by both accelerated storage and electrolyte leakage tests. The samples represented a wide domain of time-moisture history and storability (Marks and Stroshine 1995). Results are presented, not as comprehensive calibrations, but rather as a first attempt to assess the relationship between electrolyte leakage and storability. For the purpose of this analysis, the estimated initial slopes (SLOPE72) from the CO_2 evolution rate curves were used as an index of storability. The acceptability of SLOPE72 as an indicator of storability was asserted previously by Marks and Stroshine (1995); this conclusion was based on a good correlation ($r > 0.82$) with total CO_2 evolution after 200 hr of accelerated storage, which is an indicator of grain damage and mold development.

Electrolyte Leakage Tests

Several researchers demonstrated that electrolyte leakage from soaking seeds increases with temperature (Shull and Shull 1924, Tao 1978, Pandey 1988, Duke et al 1983). The results of the cur-

rent study (Fig. 1) confirmed that electrolyte leakage, using our test method, increases with temperature. Consequently, as described above, a water bath (25°C) was used to control test temperature.

Several researchers also have shown that the initial moisture content of seeds affects electrolyte leakage. No definitive relationship between initial moisture and leakage rates has been proposed; however, the general consensus is that seeds with lower initial moisture contents leak electrolytes at higher rates than do seeds with higher initial moisture contents (Simon and Raja Harun 1972, Halloin 1975, Simon and Wiebe 1975, Parrish and Leopold 1977, Tao 1978). Because no mathematical model has been reported in the literature to relate electrolyte leakage to initial moisture content, we evaluated the relationship further with tests on samples of three hybrids at several initial moisture contents.

For P3377, electrolyte leakage was greater at lower initial moisture contents (Fig. 2), which was consistent with the literature. However, the magnitude of this phenomenon was different among hybrids (Fig. 3). Initial moisture content had a greater effect on P3377 than on B73 \times Mo17 and had no effect on FR35 \times FR20. Regression models for this relationship yielded slopes of -24.7 , -11.2 , and 1.7 (S/m) and R^2 values of 0.93, 0.97, and 0.03 for P3377, B73 \times Mo17, and FR35 \times FR20, respectively (Marks 1993). The negative slopes indicate that greater initial moisture content corresponds to decreased electrolyte leakage. Interestingly, P3377 consistently had the lowest storability among these three hybrids (Friday et al 1990, Marks and Stroshine 1995), was most sensitive to initial moisture content in electrolyte leakage testing, and had the most leakage. In contrast, FR35 \times Mo17 typically had the best storability, was least affected by initial moisture content, and had the least leakage. These data imply that P3377 membrane integrity may have been more sensitive to desiccation, which in addition to other physicochemical factors might contribute to greater mold susceptibility.

Relating Electrolyte Leakage to Storability

To determine whether electrolyte leakage testing relates well to storability, correlation analyses were conducted that compared SLOPE72 and conductivity values at four times (COND5, COND10, COND15, and COND20 for 5, 10, 15, and 20 min, respectively). These soak times were selected on the basis of the literature review and inspection of the data (Marks 1993), which indicated that longer test times (from 20 min to 24 hr) did not yield better results in other applications.

Most previous electrolyte leakage research has focused on prediction of viability, with fair but inconsistent success. In the current study, the correlation coefficients (r) between germination per-

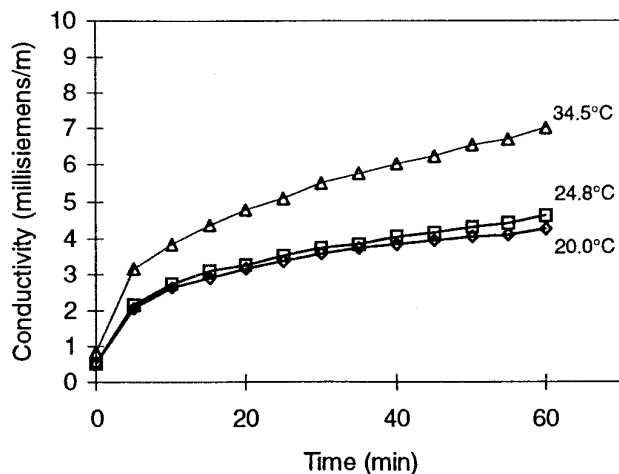


Fig. 1. Electrolyte leakage from three subsamples of recently harvested B73 \times Mo17 shelled corn grown in 1992, each tested at a different temperature.

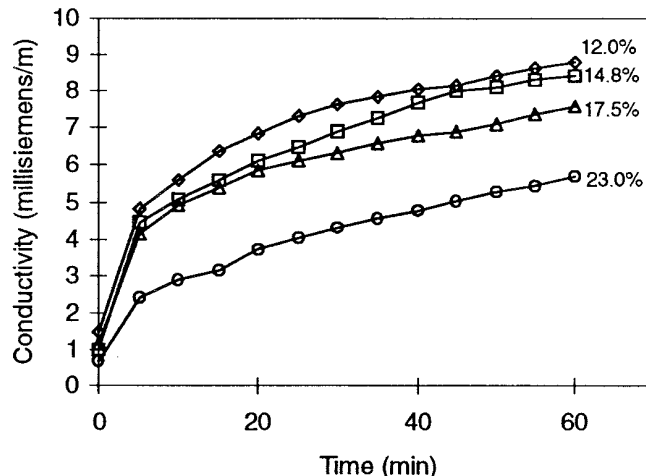


Fig. 2. Electrolyte leakage from four subsamples of recently harvested Pioneer P3377 shelled corn grown in 1992, each tested at a different initial moisture content.

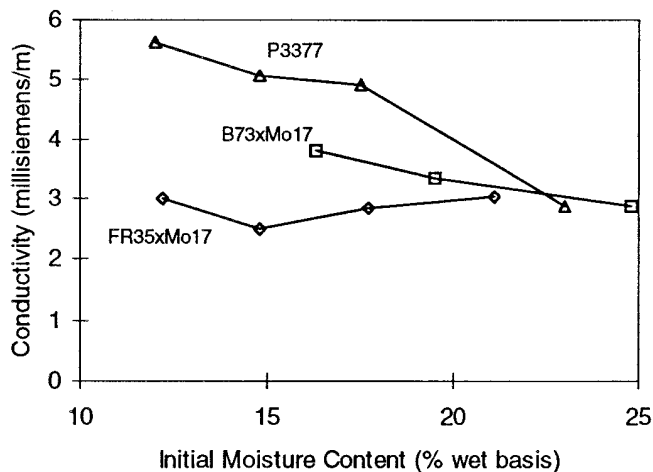


Fig. 3. Conductivity at 10 min, as a function of initial moisture content, for three shelled corn hybrids grown in 1992.

cent and four conductivity values ranged from -0.43 to -0.48 , implying that electrolyte leakage during the first 20 min of soaking was not well related to germination for the corn samples tested. The sample set included corn with a range of viability from 0 to 96% and a wide range of storage ages.

The conductivity values, however, were better correlated with SLOPE72, a measure of future storability. The correlation coefficients for the four conductivity values (COND5, COND10, COND15, and COND20) versus SLOPE72 were 0.783, 0.790, 0.788, and 0.786, respectively. Fig. 4 is a plot of SLOPE72 versus COND10 (the best correlation).

The correlation coefficient of 0.790 was achieved without any data pretreatment. Because initial moisture content affected electrolyte leakage, an attempt was made to transform the data to an arbitrary reference moisture content (15.0%), using the regression models previously mentioned for initial moisture content versus conductivity (Marks 1993). However, the correlation coefficient with transformed data was actually slightly less than for the raw data. This may have been because the moisture versus conductivity regression models were based on only a few samples of fresh (recently harvested) corn, which were not sufficiently representative of the complete sample population. Also, the data point marked as an outlier in Fig. 4 represented an extreme in previous storage history (i.e., 77 months at 14.5% mc).

In general, the correlation between COND10 and SLOPE72 was consistent with the sequence of degradative processes proposed by Heydecker (1972). Based on that model, membrane deterioration increases during the entire storage life of the grain. The same factors that influence storability (e.g., hybrid, drying method, and previous storage history) also should affect the degradative processes, which in turn affect membrane permeability and electrolyte leakage.

To evaluate the feasibility of a predictive storability model based on rapid tests, SLOPE72 was considered an acceptable measure of storability, and a stepwise linear regression was conducted. If conductivity tests were to be conducted routinely in a commercial environment, it would be difficult, if not impossible, to test all samples at a single moisture content, damage level, and soak temperature. Consequently, in this stepwise regression the possible independent variables were COND10, temperature of soak water, initial moisture content, DI, and germination percent. As previously mentioned, temperature, moisture content, and germination have an impact on electrolyte leakage. Tao (1978) and Keys (1982) also demonstrated that mechanical damage can influence electrolyte leakage. Therefore, damage and germination values were included, even though they were determined by slower laboratory procedures. The only significant ($\alpha = 0.15$) variable entered into

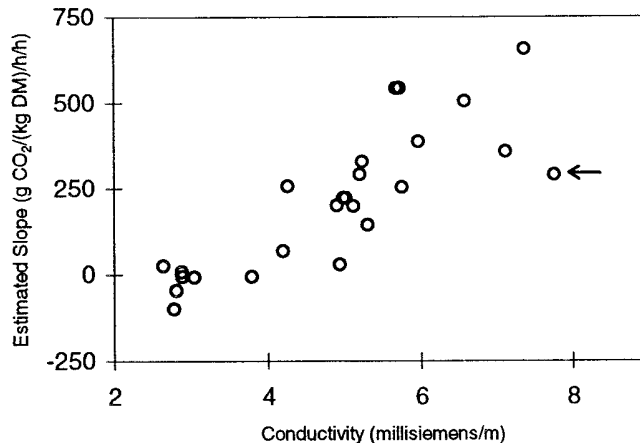


Fig. 4. Estimated initial CO_2 evolution rate slope versus soak water conductivity after 10 min of soaking. The marked outlier had been stored previously for 77 months at 14.5% moisture content.

the regression model was COND10. The R^2 of the model was 0.55, and the F value of the COND10 variable was 28.7. Although the fit of the linear model was not outstanding, the regression indicates that COND10 alone accounted for a significant portion of the variability in SLOPE72.

SUMMARY

Electrolyte leakage of shelled corn was related to storability, as evaluated by CO_2 evolution (SLOPE72) during accelerated storage. Conductivity after 10 min of soaking corn in deionized water (COND10) was correlated ($r = 0.79$) with SLOPE72, indicating that much of the variability in storability can be accounted for by differences in electrolyte leakage. Future research is needed to further establish the statistical relationships between storability and rapid physicochemical test methods and to better establish whether additional measurements (e.g., temperature and initial moisture content of grain) are needed to correct or adjust test results. Additionally, investigation of what postharvest conditions (e.g., extremely long storage history) cause failure of the testing model is needed. Although the SLOPE72 data in this study represented a wide range of storability, a larger data set should improve the statistical conclusions. Also, an automated procedure for electrolyte leakage testing of bulk samples might reduce variability in the conductivity data.

ACKNOWLEDGMENTS

This material is based on work supported by a National Science Foundation Graduate Research Fellowship. Additional support was provided by the Anderson Research Fund.

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[Received August 20, 1997. Accepted May 28, 1998.]