GLUTAMIC ACID DECARBOXYLASE ACTIVITY AS A MEASURE OF DAMAGE IN ARTIFICIALLY DRIED AND STORED CORN[1]

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ABSTRACT

Glutamic acid decarboxylase activity (GADA), as determined by Sandstedt and Blish pressuremeters, is introduced as an index of deterioration of artificially dried and stored corn. The method is rapid and simple compared with previous techniques used. The high positive correlation between viability and log GADA ($r = +0.949^{***}$; 60 d.f.) was significantly higher (at 0.1% level) than that involving fat acidity ($r = -0.433^{***}$; 60 d.f.).

Corn generally is harvested at 20 to 25% moisture to reduce picking losses. Because corn of such high moisture would deteriorate rapidly, artificial drying is necessary; this introduces one additional factor in an investigation of the quality of stored corn. Drying corn at too high temperatures will reduce the viability of the embryos and result in corn inferior for milling purposes, the critical drying temperature decreasing with an increase in the original moisture content (6). It is therefore no wonder that effective universal methods to estimate the degree of deterioration have long been sought. Semeniuk and Gilman (14) have published an extensive review on the relationship of fungi to the deterioration of corn in storage. Biological, physical, and biochemical changes during storage received early attention, and have been reviewed by Zeleny (16,17) and by Milner and Geddes (10).

It has long been known that the action of such enzymes as lipases, proteases, and phytase results in an increase in titratable acidity during storage of corn at elevated moisture levels, and a number of methods have been suggested for the determination of acidity as a measure of

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corn soundness (15,17,18). However, Bottomley *et al.* (4) were unable to estimate deterioration accurately by measuring any one of the biochemical changes they investigated, including fat acidity. The enzymatic reduction of 2,3,5-triphenyltetrazolium chloride (TTC) to formazan was suggested by Baird *et al.* (3) as a means of detecting the percentage of dead kernels in corn before purchase, yet some obviously dead but heavily molded kernels may also give a positive TTC-test (9,13).

No doubt a measure of the extent of damage to enzyme protein would indicate deterioration, but the measurement of enzyme activity should be relatively independent of the presence of fungi, as well as free from errors resulting from operators' visual judgments. Recent work in this laboratory has shown log glutamic acid decarboxylase activity (GADA) of wheat to have a high positive correlation with germination percentage (7,8). A simple and rapid manometric method was developed to determine GADA (7). Since preliminary experiments had indicated the presence of glutamic acid decarboxylase in corn[3], the new techniques were applied to establish the relationship between log GADA and viability of this cereal.

Materials and Methods

Sixty-two samples were investigated — 58 yellow and four white dent corn samples, at various stages of deterioration.

Germination percentage was determined as previously described (8).

Fat acidity was determined by a modified benzene extraction method (1). Wheat of approximately 10% moisture content was ground 2 minutes in a Waring Blendor just prior to the determination. Duplicate aliquots of 20 g. each were weighed in glass-stoppered Erlenmeyer flasks, and 50 ml. benzene were added. The contents were mixed by means of a magnetic stirrer for 15 minutes. After standing 5 minutes, the mixtures were filtered, the funnel being kept covered with a watch-glass. A 25-ml. aliquot of the filtrate was mixed with 25 ml. of 0.04% phenolphthalein in 95% ethanol and titrated with 0.0164*N* potassium hydroxide. Fat acidity was reported as mg. of potassium hydroxide required to neutralize the free fatty acids in 100 g. of ground material.

Glutamic acid decarboxylase activity (GADA) was determined as described by Linko (7), using Sandstedt and Blish (12) pressuremeters filled instead of mercury with ethyl lactate (EL) colored with crystal violet. Carbon dioxide evolution during 30 minutes at 30°C. by 30 g. of ground wheat (2 minutes with a Waring Blendor) from 15 ml. of 0.1*M* glutamic acid in 0.067*M* phosphate buffer at pH 5.8 was re-

[3]Linko, Y.-Y., Linko, P., and Milner, M. Unpublished results, Department of Flour and Feed Milling Industries, Kansas State University, Manhattan.

corded, and the reading, in mm. EL, plus 100 was taken as a measure of GADA.

Results and Discussion

Figure 1 illustrates the relationship between log GADA and germination percentage of 62 corn samples. The correlation coefficient

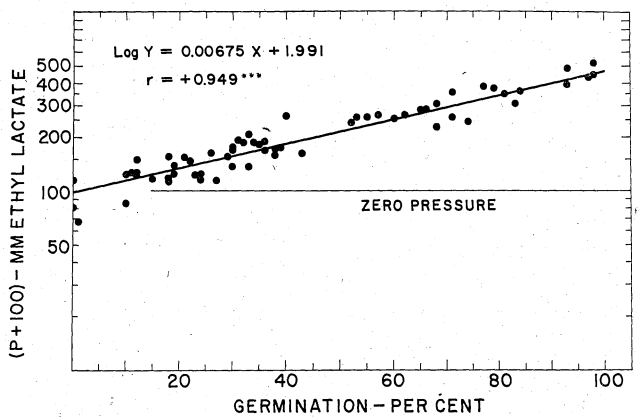

Fig. 1. Relation between log glutamic acid decarboxylase activity and germination percentage of 62 corn samples.

($r = +0.949^{***}$; 60 d.f.) was as high as previously obtained with wheat ($r = +0.928^{***}$; 58 d.f.) (7). The enzyme activities of corn were generally somewhat higher than those of wheat within the same viability range, but the slopes of the regression lines obtained from populations with similar viability distribution were surprisingly near to being identical. The regression coefficient for wheat was +0.0068 (7), and for corn +0.00675. The slope tends to increase somewhat with an increase in the number of high-viability, high-protein samples in the population. The higher rate of glutamic acid decarboxylation with corn may partially explain the early observations by Bailey (2) and Olafson *et al.* (11) that the respiratory rate of corn, as indicated by carbon dioxide evolution, exceeds that of wheat at the same moisture level. Although in the present study the number of high-viability corn samples was limited, Fig. 1 shows that all corn samples possessing a germination percentage above 75 developed a pressure increase above 200 mm. EL (300 in Fig. 1).

Figure 2 shows the relationship obtained between fat acidity and germination percentage of corn. The correlation coefficient ($r = -0.433^{***}$; 60 d.f.) is significantly lower (at 0.1% level) than that

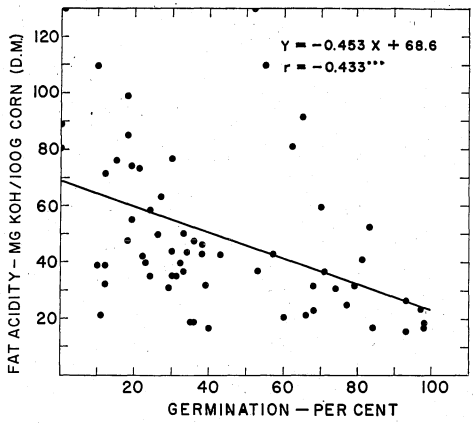

Fig. 2. Relation between fat acidity and germination percentage of 62 corn samples.

involving log GADA and viability. Although the five samples above 90% germination possessed low fat acidity values, also several samples of very low viability showed little if any increase in fat acidity. Furthermore, some samples having a germination percentage as high as 70 to 80 showed fat acidity values from 30 to 60. Although Zeleny and Coleman (18) had shown fat acidity to be more reliable as a measure of the degree of soundness than any other test then available, Bottomley *et al.* (4) found a low correlation ($r = +0.20$) between mold count and fat acidity. Under anaerobic conditions fat acidity did not exceed a value of 40; it was later shown that in nonaerated corn samples fat acidity may remain relatively constant despite a decrease in viability (5).

The present techniques for measuring GADA provide a quick and reliable way to estimate storage deterioration of corn. The method will also detect damage caused to proteins by operations such as drying at excessively high temperatures. In addition to protein denaturation, drying at high temperatures also seems to decrease the amount of pyridoxal phosphate, the coenzyme of glutamic acid decarboxylase (9). One sample may be analyzed in approximately 45 minutes, or a series of 10 in about 90 minutes, in contrast to the TTC-test which takes a minimum of 4 hours (3). In addition, the method is free of such human errors as may be encountered in the visual inspection required by the TTC-test.

Acknowledgment

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