THE EFFECT OF EXCESSIVE HEAT DURING ARTIFICIAL DRYING OF CORN ON REDUCING SUGAR CONTENT AND DIASTATIC ACTIVITY[1]

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ABSTRACT

Among the chemical and physical properties of the corn grain examined as possible indices of damage by high temperatures during artificial drying, diastase activity was the property having the greatest differential between unheated samples and samples dried at 200°F. Diastase activity showed an inverse linear correlation with increasing drying temperatures, significant at the 1% level. Diastase activity was more sensitive to inactivation by heat than esterase activity or dehydrogenase activity as measured by tetrazolium salts, but varietal differences, time of harvest, and differences in growing conditions appear to make it not accurate enough for predicting heat-damage in the samples. Diastase activity of dried grain was not greatly affected by grain moisture in the range of 7 to 21%. High drying temperatures and early harvest favored accumulation of reducing sugars in some samples tested.

Recent trends toward increased artificial drying of corn have introduced new problems to the corn processing industries, particularly when drying temperatures have been excessive. Overheated corn gives reduced yields of clean starch and lower oil yields when wet-milled (8,15,16,17,19,20). Excessive drying temperatures result in increased friability of corn (17,19). Breakage of corn during handling lowers the grade of the corn, which is a problem for handlers, shippers, and the dry-milling industry. Reduced nutritional value of overheated corn has also been reported (8,10,12,17). Overheated corn, of course, germinates very poorly, if at all.

Corn that has the ability to germinate does not possess these undesirable properties. Thus, viability tests based on germinability have been proposed as tests for heat-damage. Such a test was proposed by Baird *et al.* (1) using 2,3,5-triphenyltetrazolium chloride (TTC) as a viability indicator. Later, Schenk *et al.* (18) compared TTC results with germinability and found that some heat-damaged corn stained with TTC, yet did not germinate. The staining of the ungerminable grains in this instance was attributed to fungal activity. MacLeod (14), studying heat-damaged barley, found that germination was much more sensitive to heat damage than were several enzyme systems, including

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the dehydrogenases responsible for tetrazolium reduction. In a certain narrow range of temperature and grain moisture, MacLeod (13) found that TTC greatly overestimated actual germination values. Bishop (2) found similar results in heat-damaged barley using iodo-nitro tetrazolium. Grabe (9) has mentioned seed-treatment injury and fumigation injury as not being detected by tetrazolium tests.

French (7) found, in artificially heat-damaged barley, that starch formation in excised embryos floated on water for 8 hr. was a better index of germinability than the TTC test. Such starch formation, if it occurs in corn, is obscured by large quantities of starch already in the embryo.

Foster and Thompson (5) more recently observed an increase in the number of stress cracks in artificially heat-damaged corn. The number of cracks increased markedly from ambient room temperatures to 140°F., and increased further with increased temperatures to 240°F. The authors noted reduced germination with increasing numbers of cracks. The procedure at the time of publication had not yet been evaluated for use as a test for heat-damage.

Watson and Sanders (21) studied the steeping characteristics of thin sections of the horny endosperm of corn grains and found that heat-damaged sections retained more starch, presumably because the protein matrix containing the starch grains was denatured by heat and hence less soluble in the sulfur dioxide steep liquor.

The ultimate objective of this research was the development of a quick test for heat-damage in artificially dried corn. This paper summarizes the progress made toward the development of such a test. None of the work mentioned above has been found adaptable.

Materials and Methods

Early efforts in this research to develop a quick, reliable test for heat-damage in artificially dried corn consisted of a survey of many physical and chemical properties of the corn grain in the search for one measurable and reliable characteristic which is significantly increased or decreased by heat. Hopefully, this one characteristic would not be greatly influenced by water content of the grain.

All exploratory investigations were made on a series of samples prepared in the laboratory by heating corn in a forced-air oven at 120°, 140°, 160°, 180°, and 200°F. Samples were also prepared by heating corn under an infrared dryer until the grain reached the temperatures listed above. The corn used for the exploratory studies was a high-quality lot of feed corn received soon after harvest at 28% moisture. Several series of samples were obtained from laboratories studying other aspects of grain drying and heat-damage. Samples were obtained from James Dickens, North Carolina State University, and H. W. Schroeder, Texas A. & M., Market Quality Research Division; from George Foster at Purdue University, Transportation and Facilities Research Division; and from Stanley Watson, Corn Products Company, Argo, Illinois. These were used for the more detailed studies of reducing sugars to be described later.

The properties investigated included: properties associated with protein, compounds generated or destroyed by heat, and changes in physical properties.

Experimental Results

Properties Associated with Protein. The denaturation of protein, zein content, starch release from the protein matrix during bisulfite steeping of purified horny endosperm particles, and enzymatic activity were studied. Most investigations made were of types which required a minimum of laboratory procedure and time, and could thus be modified for a quick test. One of the most sensitive properties of the extracts was thought to be enzymatic activity. Enzymes are catalysts and proteinaceous, therefore should be doubly sensitive to inactivation by heat; the catalytic properties may be destroyed by heat, or the solubility of the proteinaceous enzyme could be destroyed by heat. Esterase activity, using indoxyl acetate as substrate, was relatively simple to measure and resulted in a colored end product, and thus was possibly adaptable to such a quick test. French (6), studying purified esterase extracts from corn seedlings, found evidence for the existence of more than one esterase enzyme. The esterase extract rapidly lost activity at 136°F., a temperature below the range where one would expect to encounter extensive heat-damage. Esterase activity, however, did not correlate well with grain-drying temperature. Esterase activity in the dry seed, like dehydrogenase activity, proved to be fairly resistant to damage by heat.

Zein content of 70% ethanol extracts, measured by the method of Craine *et al.* (4), did not vary appreciably with heat-damage. A modified type of starch release test was carried out on particles of horny endosperm which could be rather easily purified by washing away contaminating particles under a stream of tapwater. The horny endosperm particles were suspended in bisulfite and the starch release measured by the starch iodide reaction. No linear relationship to grain temperature was found.

Compounds Generated or Destroyed by Heat. A study of compounds generated or destroyed by heat included the following: direct colorimetric tests run on bisected grain for the presence or absence of organic acids, amino acids, phenolic compounds, aldehydes such as furfural, sulfhydryl compounds, and reducing substances. None of the reagents used was capable of clearly distinguishing corn damaged at 180° and 200°F. from the other samples.

Changes in Physical Properties. Water extracts of milled corn, being easily and rapidly prepared, were used for most of the investigations. For routine analysis, 50 or 100 whole, clean, and apparently disease-free seeds were selected and ground once in a Labconco mill.[3] The corn flour was then suspended in water or extracted in various solvents for analysis. The chromatographic behavior of water-soluble starch fractions on glass paper revealed some differences due to heat-damage, but the method was not adaptable to a quick test.

No change in the color of grain due to heat damage in the range up to 200°F. could be detected by the Hunter color difference meter. Turbidity of extracts varied markedly with grain temperature, but was also found to be greatly influenced by moisture content. For this reason the technique was discarded.

Reducing Substances. The reduction of permanganate, dichloroindophenol, and tetrazolium showed some promise.

Reducing substances were first noted in corn extracts by the tendency of starch iodide complexes to fade. Later, reducing substances were detected by dichloroindophenol and by alkaline tetrazolium salts. It should be mentioned that at certain concentrations, and under alkaline conditions, TTC can function as a very sensitive reagent for the detection of reducing substances. This has been discussed by Cheronis and Stein (3). This is a chemical reduction of tetrazolium, carried out at high pH, in contrast to the enzymatic reduction which takes place in seeds near neutrality. With alkaline TTC, water extracts of ground corn showed a progressive decrease in reducing substances with increasing drying temperature. A very marked difference in degree of redness was noted between control and 200°F. samples, the latter being a very light pink in color. This was the most promising lead toward the development of a quick colorimetric test for heat-damage, and therefore optimum conditions for the extraction of TTC reducing substances were studied.

When 1 g. of milled corn was shaken with 25 ml. distilled water at room temperature for periods up to 30 min., a gradual increase in

[3] Use of trade names in this paper is for identification only, and does not imply indorsement by the U.S. Department of Agriculture.

TTC reducing substances was noted. Longer extraction periods were considered impractical for a quick test. Temperature of extraction influenced yield of reducing substances markedly. Optimum yield of reducing sugars was obtained by ultrasonic extraction at 50°C. for 20 min. Six samples were treated as a unit for ultrasonic extraction and centrifugation. Extracts of the laboratory heat-damaged samples gave decreased TTC values with increasing temperature, with as much as tenfold differences in optical density noted between the unheated control sample and the 200°F. sample.

While the alkaline TTC procedure appeared promising, it was found that the TTC reaction was difficult to standardize. For any one series of samples, the ranking according to degree of redness was usually the same. However, the intensity varied considerably upon repeated analysis. Analysis of the water extracts by chromatographic procedures revealed that most of the reducing substances were the reducing sugars fructose, glucose, and maltose. One of the reasons for poor reproducibility of the alkaline TTC test is believed to be the fact that reduction of TTC is not linear with reducing sugar concentration. While alkaline TTC is ruled out as a quantitative reagent for reducing sugars, nevertheless it may be found useful for some form of quick color test for heat-damage.

Diastatic Activity. Once the reducing substances were identified as reducing sugars, Benedict's reagent, common for measuring reducing sugars, was used for analysis. With this reagent, color development is linear with reducing sugar concentration. With this reagent it was found necessary to remove interference from colloidal material in the water extracts. This was accomplished by the use of the Somogyi-Shaffer-Hartmann zinc hydroxide procedure (11). Time of addition of zinc hydroxide indicated that enzymatic production of reducing sugars increased upon addition of water, and ceased upon addition of zinc hydroxide. Reducing sugar yield was 2.25 mg. per g. milled corn (reported as glucose) when zinc was added at zero time. The value increased to 5.8 mg. when zinc was added at 20 min. Both samples received the same exposure to ultrasonic radiation. Since the specific enzymatic activity was not known, it was termed diastase activity, including amylases and invertase activity leading to the production of maltose, glucose, and fructose.

Crude diastatic activity could be measured rather easily without great modification of procedure, and this was done. Extracts were made in the ultrasonic generator at 50°C. for 20 min., zinc hydroxide was added at zero time to one set, and at the end of 20 min. to the other. The reducing sugar value at zero time was subtracted from the value obtained when zinc was added at the end of 20 min. This diastatic reducing sugar value was determined for all the samples and was, essentially, a measure of the activity of the diastase enzymes.

The studies with the zinc hydroxide procedure revealed that the major portion of the reducing sugar present in the extracts after 20 min. arose from enzymatic production. As shown in Fig. 1, initial re-

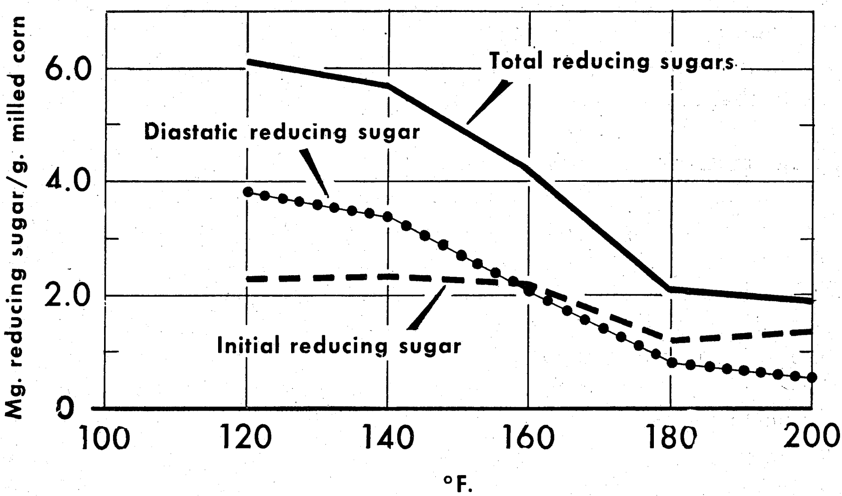

Fig. 1. Effect of temperature on reducing-sugar content of standard laboratory samples.

ducing sugar content in the laboratory series was not greatly influenced by heat, although the 180° and 200°F. samples contained less than the others. Total reducing sugars (zinc hydroxide added after 20 min.) decreased markedly in the samples receiving the greatest amount of heat. The difference between total reducing sugar concentration and initial reducing sugar content represents the amount of reducing sugars generated by diastatic activity, and appeared to be the reducing sugar value most sensitive to heat. The diastatic values reported are as measured by the method specified above, and should not be confused with diastatic values determined by other methods.

It is well known in the field of corn drying that grain tempered to a higher moisture is not the same as corn that has never been dried from the higher moisture. Since moisture in some of the earlier tests influenced the extent and direction of some of the reactions, an experiment was run to investigate the effect of grain moisture on reducing sugar content and diastatic activity. Samples of a good-quality dried field corn were placed in an atmosphere of 100% r.h. for various lengths of time up to 4 days. Percent moisture was determined, and ranged from 8 to 21%. Portions of the samples were ground for the determination of reducing sugars and diastatic activity. The results showed little effect of percent moisture on initial reducing sugar content or on reducing sugar concentration produced by diastatic activity, indicating that dried corn could vary appreciably in percent moisture without affecting the results of a quick test based on this principle. This does not imply that moisture content changes might not affect microbiological activity during storage, or that moisture due to immaturity would not affect the results.

All of the available samples were analyzed for diastatic activity. As with laboratory samples, diastatic activity decreased with temperature of the heated grain in the Indiana, Illinois, and Texas samples. That the total reducing value and initial reducing sugar value were not useful in themselves was apparent in the series of samples from North Carolina. Total reducing sugars for the North Carolina samples are not shown, but the values indicated little difference throughout the heated range. Initial reducing sugars (Fig. 2) indicated an inverse

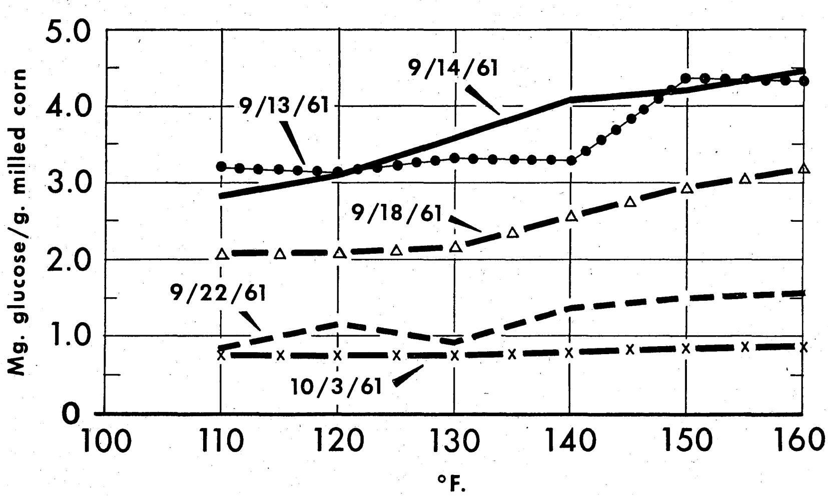

Fig. 2. Initial reducing-sugar content of North Carolina samples.

relationship with drying temperature, an unusual situation where increased drying temperature seemed to favor higher reducing sugar content. It would be of interest to know if such grain with high reducing sugar content would be more susceptible to microbiological attack during storage. This aspect was not within the scope of this investigation.

In the North Carolina samples the earliest harvested samples were very high in reducing sugars, and concentration increased in the more highly heated samples. Although the grains did not appear to be immature, they were apparently high in free sugars at time of harvest. At the lower drying temperatures, the sugar was probably converted through normal ripening processes to stored carbohydrate. With the more damaging drying temperatures, the normal enzymatic conversion to stored reserves was blocked, thus giving the high reducing sugar values. Since this situation might arise in any early harvest situation, the measurement of initial reducing sugar content alone as an index of heat-damage could not be used. Diastase activity, shown in Fig. 3, rather generally decreased with increased drying temperature.

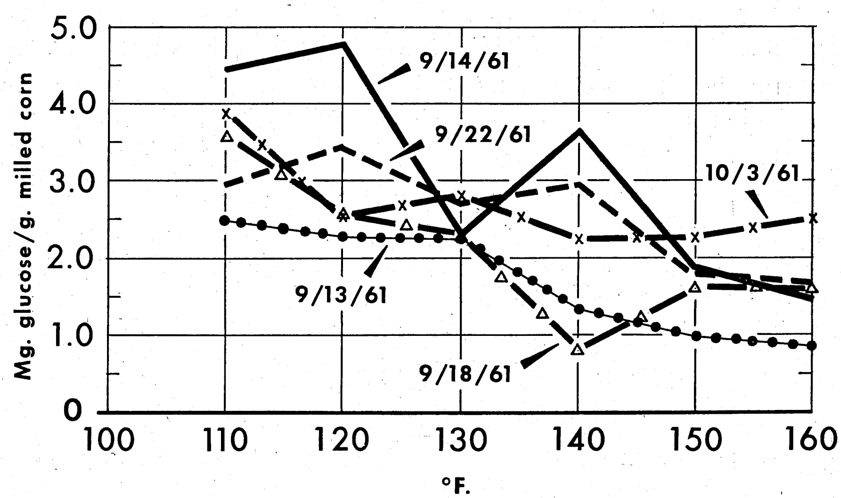

Fig. 3. Diastatic activity of North Carolina samples.

In all, some 93 samples of heat-damaged corn of different types and from different sources were studied. Different methods of preparation were used. Batch and continuous methods were used, using heated air. Some samples were dried with infrared radiation. Some of the corn, as indicated by analysis for initial reducing sugar content, appeared to be immature. High-quality hybrid corn lines were used, as well as a high-quality batch of feed corn of unknown parentage. Samples were all from experimental drying tests and operations; from Texas, Illinois, North Carolina, Indiana, and Maryland.

The diastatic activity values for all samples were plotted against the available grain temperatures during heating. The results are shown in Fig. 4 and Table I. The calculated linear regression curves for groups and for all samples is shown. The value of the correlation coefficient for all samples was −0.55, significant at the 1% level, but nevertheless a low correlation. With all groups of samples studied,

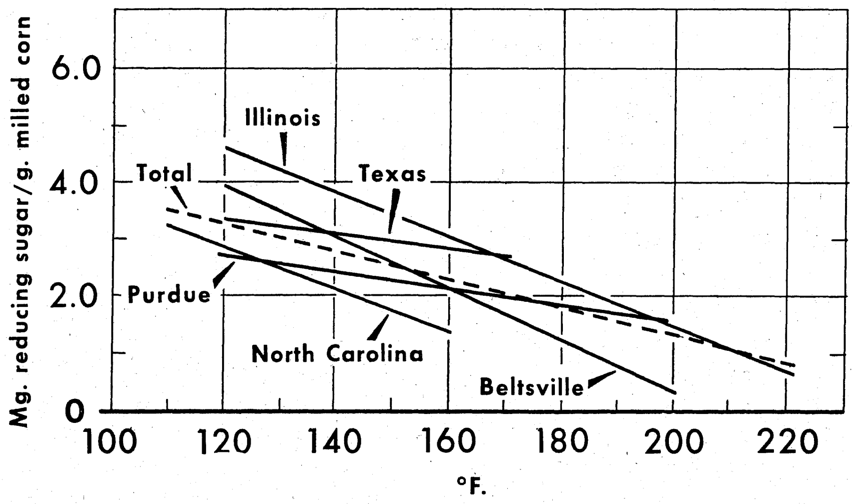

Fig. 4. Calculated linear regression curves for production of diastatic reducing sugars for all samples and for Purdue, Illinois, Texas, North Carolina, and Beltsville samples.

TABLE I

ANALYSIS OF GROUPS OF CORN SAMPLES FOR EFFECTS OF DRYING TEMPERATURE ON PRODUCTION OF DIASTATIC REDUCING SUGAR

Sample Series	N	Temperature Range (°F.)	$\bar{X}$	$\bar{Y}$	S^2 yx	S y.x	r	b	F
North Carolina	30	110-160	135	2.29	0.724	±0.851	−0.62	−0.038	17.04**
Illinois (Corn Products)	29	120–221	165	2.86	0.006	±0.075	−0.80	−0.040	47.25**
Purdue samples	13	119–195	150	2.29	0.288	±0.537	−0.59	−0.014	5.79*
Texas samples	16	120–171	138	3.08	0.153	±0.391	−0.48	−0.013	4.24
Beltsville (IR-2)	5	120–200	160	2.12	0.116	±0.341	−0.98	−0.045	70.64**
Total	93	110–221	148.2	2.59	0.963	±0.981	−0.55	−0.024	38.85**

$\bar{X}$ = mean of temperatures, °F.
$\bar{Y}$ = mean of mg. reducing sugar produced by diastatic activity.
r = correlation coefficient.
b = slope.

diastase activity tended to decrease with temperature of drying. The slopes indicate that the several groups do not belong to the same population, or are not identical. The diastase activity values obviously cannot be used at this stage of development to estimate the temperature corn grain has received during drying. The diversity in the different groups is not surprising in view of the different sources and different treatments the various groups received. The North Carolina samples, for example, were harvested very early, and drying temperatures did not exceed 160°F. The Texas samples also did not receive extreme heat, yet both groups showed a perceptible decrease in diastatic activity. The Illinois samples, although dried 6 and 10 years prior to testing, showed a marked decrease in activity, as did the more recent samples prepared at Beltsville.

It should be pointed out that the air-dried samples (controls) were also studied, and were usually higher in diastatic activity than the 120°F. samples. Since the results were compared on a temperature basis, it was difficult to assign a meaningful temperature value to the air-dried controls. Assignment of a 70°F. temperature to such samples to compare them with the heated samples would probably increase the statistical significance of the correlation, yet would not help one to distinguish a sample dried at 140°F. from one dried at 160°F.

While diastase activity was found to decrease substantially with increasing drying temperatures over the range 110° to 220°F., including temperatures damaging to milling quality, varietal, cultural, and harvest-time differences of corn samples appear to be so large that the usefulness of diastase activity as an index of heat-damage is greatly reduced. Some type of test for measuring extreme heat-damage, based on diastatic activity, might yet be devised and be of some use. Although the diastase activity value, at its present stage of development, is not a reliable test for heat-damage, it does represent a property of the corn grain which is appreciably sensitive to heat, and which is not greatly influenced by moisture content (over the range studied). It is an enzymatic activity which in corn appears to be more sensitive to heat than is esterase activity or dehydrogenase activity as measured by tetrazolium salts.

Conclusions

Of the many chemical and physical properties of milled corn grain examined as possible indices of heat damage by artificial drying, diastase activity appeared to be the most sensitive.

Diastase activity, measured by the production of reducing sugars, decreased regularly with increasing drying temperatures. Statistical analysis of all samples showed a highly significant correlation of diastase activity vs. temperature reached in drying. However, variability among corn lots with different cultural and handling histories indicated that diastase activity, as run in these tests, would not be of use in predicting heat-damage.

Diastase activity was found to be more susceptible to heat-damage than esterase activity, or dehydrogenase activity as measured by tetrazolium salts in seed-viability tests. Diastase activity of dried corn was not greatly influenced by seed moisture content in the range from 7 to 21% moisture.

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