NOTE

Applicability of the Colorimetric Alpha-Amylase Assay to Evaluate Sprouted Sorghum[1]

P. R. MATHEWSON,[2] C. H. FAHRENHOLZ,[3] and Y. POMERANZ[2]

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ABSTRACT

A colorimetric α-amylase assay for evaluating sprout damage in sorghum is reported. A standard curve relating α-amylase content to absorbance at 620 nm was used to evaluate sorghums germinated in the laboratory. Increase in visual sprouting generally resulted in higher absorbance at 620 nm. The modified colorimetric procedure differentiated sound from visually sprouted sorghum and detected incipient damage.

We recently developed a modified colorimetric method for the determination of α-amylase in wheat (Mathewson et al 1982). The procedural changes incorporated in this method resulted in a simple and rapid test for evaluating large numbers of samples as required for screening tests by plant breeders or routine analyses of grain in elevators. The availability of a commercial instrument designed specifically to perform this test led us to investigate further applications of this method.

Sprout damage in sorghum is defined as "kernels or pieces of kernels of sorghum in which the sprout definitely protrudes from the germ" (USDA 1980). In 1973, heavy rains in Kansas caused extensive sprouting in the sorghum crop. The crop was heavily discounted, resulting in substantial losses to producers. Although this discounting was justified in terms of the definition of sprout damage (or at least because of the susceptibility of the sorghum to microbial damage in storage), the lack of an identified quality index led to arbitrary, nonstandard discounting practices. In many other areas of the world, Asia and Africa in particular, sorghum serves as a primary food source, and an objective assay reflecting end-use quality would be of value. α-Amylase is an important factor in end-use quality, but acrospire length is not necessarily an accurate index of α-amylase activity. A test reflecting this enzymatic activity would therefore provide a truer test of quality than visual inspection.

The present visual method of measuring sprouting in sorghum does not differentiate among samples that vary in degree of sprouting and cannot detect incipient damage in which α-amylase has been produced in the grain but the acrospire is not visible. In addition, the acrospire will often be broken off due to grain handling procedures, thus destroying the basis for evaluation.

MATERIALS AND METHODS

Field samples of sorghum were hand-harvested and shelled in 1977 from stands grown by the Department of Agronomy, Kansas State University, Manhattan. They included the following hybrids: ACCO R920, GR-1018, R-1029A, DeKalb B-38, C-42Y, Funk's G-404, Northrup-King 2778 and 2022, and Pioneer 8272. These samples showed various degrees of sprouting from none visible to visible sprouting with acrospires up to 3 cm long. The Agronomy Department also furnished six samples of sound commercial sorghum (Funk's G499 and G623, DeKalb 42A and 61, Asgrow Colt, and Warner W851) and four samples of public release hybrid sorghum (NB691, RS610, RS626, and RS702) from the 1979 crop.

Two samples of damaged commercial sorghum (as determined by official grain inspection) were received from D. W. Fulk, Marketing Standards Branch, Federal Grain Inspection Service, U.S. Department of Agriculture, Kansas City, MO.

Two additional sound samples (Pioneer 8311 and a mix of sorghums grown in Garden City, KS, in 1972) were obtained at this laboratory from L. M. Seitz.

Samples were identified as either sound or obviously sprouted on the basis of the presence or absence of an acrospire. The sorghum was considered obviously sprouted if the acrospire was more than 2 mm long.

Sound sorghum samples were rinsed in deionized water to remove debris and steeped in deionized water for 13-20 hr at 25°C to ~40% moisture. The water was drained and the grain germinated until the acrospire was about 3 mm long. During this time, the grain was rinsed with fresh deionized water two or three times daily. Fifty-gram subsamples were removed at the end of the steep and at subsequent intervals of 4-6 hr until the end of germination. Each sample was weighed and oven-dried at 45°C until no further weight loss was recorded. The samples were then ground in a Udy Cyclone mill (with a 1-mm screen) and analyzed for α-amylase.

Cibacron-blue amylose substrate powder was received from Hoffmann-LaRoche, Inc. (Nutley, NJ 07110). The powder was pressed into 60-mg tablets. Substrate is now available in tablets containing buffer and $CaCl_2$.

The Alpha-Amylase Analyser was manufactured by D&S Instrument, Ltd., Pullman, WA 99163.

The modified procedure for α-amylase determination was as described by Mathewson et al (1982). α-Amylase activity was also determined according to the ASBC international α-amylase method (ASBC 1976).

RESULTS AND DISCUSSION

Standard Curve

Field samples having gross differences in degree of visual sprouting were ground and assayed for α-amylase by the modified colorimetric procedure. Absorbance at 620 nm generally increased as the severity of visual sprouting increased. Waxes, tannins, and pigments present in sorghum do not appear to alter the linear relationship between α-amylase content and the absorbance at 620 nm resulting from the test procedure.

To establish a standard curve relating the α-amylase content of the sorghum to absorbance at 620 nm, three samples of sound sorghum (Pioneer 8311, the Garden City blend, and a sample from D. Fulk, having 0.1% total damage) were germinated in our laboratory as described. The malts were dried at 45° C, ground, and analyzed for α-amylase activity according to the ASBC method.

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[2]Respectively, research chemist and director, U.S. Grain Marketing Research Laboratory, Agricultural Research Service, USDA, Manhattan, KS 66502.

[3]Research assistant, Department of Grain Science and Industry, Kansas State University, Manhattan 66506.

Each malt was blended with sound sorghum to produce mixtures containing 0.25–1.50 millidextrinizing units per milligram in increments of 0.25 millidextrinizing units. Each series of samples was analyzed for α-amylase activity by the modified colorimetric procedure. The results from all three malts were combined to produce the standard curve regression equation. Its correlation coefficient (0.9901) was highly significant ($P<0.001$), and the standard deviation was 0.020. Thus, we could relate the color produced by testing a sample to the extent of sprouting in the sample, using α-amylase as the sprouting index. We determined that the optimal conditions for the colorimetric assay of α-amylase in sorghum were pH 5.5 and 50°C. The commercial substrate is buffered to give a pH of 6.20 in deionized H_2O. In the interest of simplicity, we used the commercial tablets developed for the assay of α-amylase in wheat. Although not maximized for the sorghum α-amylase, the absorbance response is adequate for differentiating different degrees of sprouting.

Laboratory-Germinated Samples

Sound samples of commercial and public release hybrid sorghums were germinated and oven-dried in the laboratory, with subsamples taken as germination progressed. Samples of the sound and germinated sorghum were analyzed for α-amylase activity by the colorimetric procedure. An obvious difference was found in the absorbance at 620 nm for sound and visually sprouted sorghum (Table I). Although this distinction may be made by visual inspection, the advantage of the colorimetric technique is in providing a meaningful numerical value (whether or not the acrospire is still attached), which could be used as the basis for objective grading.

Of perhaps greater significance is the finding that a range of absorbance exists between sound and obviously sprouted sorghum, representing sprouting that has progressed sufficiently to elevate the α-amylase content of the grain but not sufficiently to make the acrospire discernible. This is the condition of incipient sprouting, not obvious to visual inspection. Previously this condition would have gone undetected, but the colorimetric α-amylase assay allows for its detection, again by an objective measure that is directly related to sprouting.

Several points noted in our studies could prove relevant to grading sorghum. For sprouting to occur, the moisture content must have increased after maturity to about 40%. This can enhance microbial damage yet be undetected by visual inspection. The α-amylase content, even in obviously sprouted sorghum, may vary widely (Table I). Some hybrids (such as NB691) that appear to be badly sprouted may contain relatively small amounts of α-amylase and therefore may still be suitable for a particular end-use.

TABLE I
Absorbance (620 nm) of Sound and Laboratory-Germinated Sorghums

Variety	Visually Sound	Visually Obvious Damage[a]
RS		
610	0.217	0.597
702	0.145	0.488
626	0.130	0.714
NB 691	0.174	0.354
Funk's		
G623	0.142	0.448
G499	0.134	0.805
Asgrow Colt	0.060	0.481
Warner W851	0.072	0.783
DeKalb		
42A	0.270	0.771
61	0.047	0.515
Mean	0.139	0.595
SD	0.070	0.161
	Mean + SD 0.209	Mean − SD 0.434

[a] Acrospires of at least 2 mm.

We conclude that the modified colorimetric procedure can provide a simple rapid method for evaluating sprouting in sorghum. We suggest, based on the data in Table I, that absorbance values of 0.200 or below indicate sound sorghum; from 0.200 to 0.500, incipient sprouting; and 0.500 and above, sorghum significantly sprouted to have had a protruding acrospire at one time. Our tests with other grains, ie, oats, rye, millet, and triticale[4] suggest that the same principle can be applied to the evaluation of sprouting in most cereal grains.

[4] Unpublished data.

LITERATURE CITED

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