COMMUNICATION TO THE EDITOR:

Rapid Nephelometric Determination of Alpha-Amylase Activity in Sprouted Wheat Kernels[1]

To the Editor:

Measurement of α-amylase activity as an assessment of sprout damage in wheat has received considerable attention since the 1968 embargoes of U.S. wheat shipments to Japan due to sprout damage. The report by the Pacific Northwest Grain Export Association (1978) states that the United States, during this 10 year period, lost an estimated $18 million in sales. The problem of accurate and objective assessment of sprout damage in wheat is widely recognized. A rapid, simple, and reliable test for α-amylase determination is a prerequisite to a solution of monitoring sprout-damaged wheat in marketing channels and for accurate and reliable wheat inspection.

This is the first communication on a semiautomated nephelometric determination of α-amylase activity in wheat kernels. The decrease in turbidity of an amylopectin suspension when acted upon by α-amylase is measured nephelometrically. The rate of nephelos change is used as a measure of α-amylase activity.

Approximately 35 g of wheat is ground in a Udy-Cyclotec grinder using a 1.0 mm round-hole mesh sieve. A 1-g sample of the ground grain is weighed into a 60 ml screw-cap plastic bottle, 20 ml of 0.5 percent NaCl solution containing 200 mg/L of $CaCl_2 \cdot 2H_2O$ is added, and the material is shaken for 5 min with a React-R-Shaker (Unitec Model 1350). The extract is filtered through Whatman No. 2V filter paper.

The instrument used is a nephelometer with two built-in analytical cycles designed and developed for our analysis. The instrument is a redesigned Amylase-Lipase Analyzer Model 91 (GAA-191) (Perkin-Elmer Corporation, Coleman Instruments Division, Oak Brook, IL) that is used primarily for clinical analyses in hospitals. The analyzer we used includes a dry bath incubator set at 37°C, containing 12 compartments to hold spectrophotometric disposable cuvettes. The substrate is amylopectin buffered to pH 5.1. The substrate is prepared by adding 75 ml of distilled water to the predetermined amount of stock substrate (Perkin-Elmer Corporation) supplied in individual bottles. The amylopectin substrate is shaken and then boiled for 10 min in a water bath to give a uniform and stable suspension. Three ml of amylopectin substrate is pipetted into a cuvette and preincubated in the dry bath for 20 min. Then 200 μl of the filtrate is pipetted into the cuvette using an automatic pipette with disposable tips. The cuvette is capped and the suspension mixed by inverting the cuvette four times. The amylase reading is taken when the cycle light flashes on the precalibrated instrument.

In an experiment to compare the GAA-191 and falling number methods, two samples of white wheat were chosen: one sound sample of falling number (FN) 350 and one sprouted sample of FN 150 were blended in proportions to form 10 samples of different α-amylase levels. On each of the 10 samples, six measurements were made by FN and GAA-191 methods.

The activity of α-amylase decreased curvilinearly with increase in FN values. Similar relationships have been reported for other methods (Barnes and Blakeney 1974, Carr and Spillane 1969, Tipples 1969).

The sensitivity ratio described by Mandel (1954) was used to compare the methods of measurement statistically. This ratio involves both the standard deviation of each method and the rate of change in readings by each method as the underlying quantity being measured changes. It is important to include this latter factor because one method could have a smaller standard deviation than another simply because it does not respond to small changes in the quantity being measured.

For two methods of measurement M and N, the sensitivity ratio is defined as:

$$\frac{\psi_M}{\psi_N} = |K| \frac{\sigma_N}{\sigma_M}$$

where K is the slope of the relation of M to N in the region of interest, and σ_M and σ_N are the standard deviations of M and N, respectively. A sensitivity ratio greater than 1 is evidence that method M is a more sensitive measurement than method N. The square of the sensitivity ratio may be interpreted as the number of repeat measurements by method N of which the average would have the same precision as a single measurement by method M.

Transforming FN to liquefaction number (LN) by use of the equation: LN = 6,000/(FN − 50) resulted in a linear relationship between the readings of LN and the GAA-191. This transformation of the scale of measurement of the FN method does not change the estimate of the sensitivity ratio. The slope of the linear relationship was estimated by a regression method described by Madansky (1959), which takes into account the fact that both methods under consideration provide measurements that are subject to error. The estimates of the standard deviations are the pooled standard deviations from the 10 samples.

For our data, the ratio of the sensitivities was estimated to be:

$$\frac{\psi_{GA}}{\psi_{FN}} = \frac{\psi_{GA}}{\psi_{LN}} \Big| = \Big| \frac{2.5}{5.1} = 2.2$$

The square of the sensitivity ratio, in this case $4.8 \doteq 5$, is the estimate of the number of repeat measurements by FN that must be averaged to obtain approximately the same precision as a single measurement by the GAA-191 for estimating the underlying α-amylase level.

Because it is not known precisely how the sensitivity ratio is affected by the statistical variability in the readings of the measurement methods used, no definite statements concerning statistical significance can be made. It is also not known how measurements made by FN and GAA-191 equipment other than those used in the experiment might affect the estimate of relative sensitivity. Nevertheless, there is sufficient evidence to conclude that the GAA-191 method is, at the very least, comparable to FN in terms of sensitivity and should be evaluated by an end-use study as a possible substitute for the FN method in current inspection procedures.

The measurement of α-amylase activity requires about 1 1/2 min per assay, excluding grinding and extraction, and therefore has potential application for grain grading and marketing. However, if samples are run in batches, the time per assay, including grinding

[1]Published with the approval of the Director, Standardization Division, FGIS, USDA, Washington, DC 20250.

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and extracting, is about 1 1/2 min. The relation of nephelometric method to amylograph and FN methods will be established as well as its accuracy and precision, and the results will be reported.

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Acknowledgments

We thank John R. Smeaton, Stat Engineering, Wayne, IL 60184, for the development of substrate and Coleman Instrument Division, Perkin-Elmer, Oak Brook, IL, for providing the Grain Amylase Analyzer.

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[Received August 18, 1978. Accepted October 25, 1978]