A RAPID PROCEDURE FOR ALPHA-AMYLASE DETERMINATION IN MALT[1]

D. G. MEDCALF, E. E. TOMBETTA, O. J. BANASIK, AND K. A. GILLES[2]

ABSTRACT

The falling number procedure, developed by Hagberg for the determination of alpha-amylase activity in flour, has been adapted to the direct determination of alpha-amylase activity in malt. Dry malt or malt extract was mixed with a standard unmodified starch and the FNV determined. A straight line relationship was observed between FNV's and 20° Units of alpha-amylase activity (standard American Society of Brewing Chemists method) when the range of FNV's was not great. With a wide range of FNV's, the relationship was curvilinear and a straight line was obtained by plotting on semilog paper. Both linear and semilog standard curves were made comparing alpha-amylase units to FNV's for dry malt and malt extract. The regression equations for these curves were used to convert FNV's to alpha-amylase units for 60 samples of dry malt and malt extract having a wide range of amylase activity. Correlations between alpha-amylase units determined directly by the standard method and values obtained with either the linear or nonlinear regression equations were quite high. However, the relationships were much more linear for values calculated with the nonlinear regression equations. The falling number procedure is simple and rapid, requires no special reagents, and has the advantage of being applicable to both dry malt and malt extract.

Hagberg's falling number test (1,2) was developed as a rapid and convenient method for the determination of alpha-amylase activity in wheat and rye flours. Falling number values were curvilinearly related to alpha-amylase activity. Later Perten (3) obtained a linear relationship to alpha-amylase activity by using an empirical formula and converting falling number values (FNV's) to liquefaction number values.

Perten (3) showed that the amount of barley malt added to wheat or rye flour was related linearly to the liquefaction number. However, no report appears to have been published on the use of the falling number method for the direct determination of alpha-amylase activity in barley malt. The present paper describes such a procedure.

Materials and Methods

Starches. Unmodified corn starch was obtained from Clinton Corn Processing Company, Clinton, Iowa. Unmodified wheat starch was obtained from General Mills, Inc., Keokuk, Iowa.

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[2]Assistant Professor, Rockefeller Fellow, Associate Professor, and Professor and Department Chairman, respectively, Department of Cereal Technology, North Dakota State University, Fargo.

Enzymes. Enzymes used were: Malt Diastase, Special for Analytical Purposes; Alpha-Amylase, Special for Analytical Purposes; Beta-Amylase, free from alpha-amylase. All were obtained from Wallerstein Laboratories, New York, N.Y

Barley Malts. Malt samples were obtained by experimentally malting (4) a variety of barley samples grown on plots at North Dakota Agricultural Experimental Station during the 1965 crop year.

Alpha-Amylase, Standard Method. The alpha-amylase activity of the various malts was determined by the standard American Society of Brewing Chemists (ASBC) method (5). In this modified Wohlgemuth procedure (6), the results are expressed as 20° Units of alpha-amylase activity. The 20° Units are similar to SKB units (7).

Falling Number Procedure. The apparatus[3] used for this procedure was described by Hagberg (1,2). With the exception of sample size, the test procedure was also as described by Hagberg (2). The size of the starch sample used was adjusted so that the range of FNV's encountered would be generally between 75 and 200 sec. For corn starch, 6.0 g. (d.b.) was used and for wheat starch, 5.5 g. (d.b.). In general, 25 ml. of distilled water was added. However, where enzyme solutions or malt extract was used, the amount of water was reduced so that the total volume of liquid in each test was 25 ml.

Preliminary experiments were conducted using commercial enzyme preparations. Malt Diastase powder was added directly to the starch in the precision test tube. Alpha-Amylase was added using aliquots of a 1% solution. Beta-Amylase was added to a mixture of starch and 0.1 g. of a standard dry malt using aliquots of a 1% solution.

Dry Malt. Malt samples (5 g.) were ground on a MIAG malt mill (set for fine grinding) and sifted by hand through a No. 43 SS screen. A portion of the material passing through the screen, 0.1 g., was weighed on an analytical balance and added directly to the starch in the precision test tube.

Malt Extract. Five-gram samples of malt were extracted according to the standard ASBC procedure (5). A portion of the filtered extract (10 ml.) was diluted to 100 ml., and 25 ml. of this diluted solution was added to the starch for the falling number test.

Results and Discussion

Figure 1 shows a semilog plot of FNV's *vs.* alpha-amylase concentration with wheat starch used as the substrate. The correlation coefficient for these data was -0.963^{**}. A similar curve for malt diastase with corn starch used as the substrate is shown in Fig. 2. The correla-

[3] Obtained from Falling Number AB, Norrlandsgaten 16, Stockholm, Sweden.

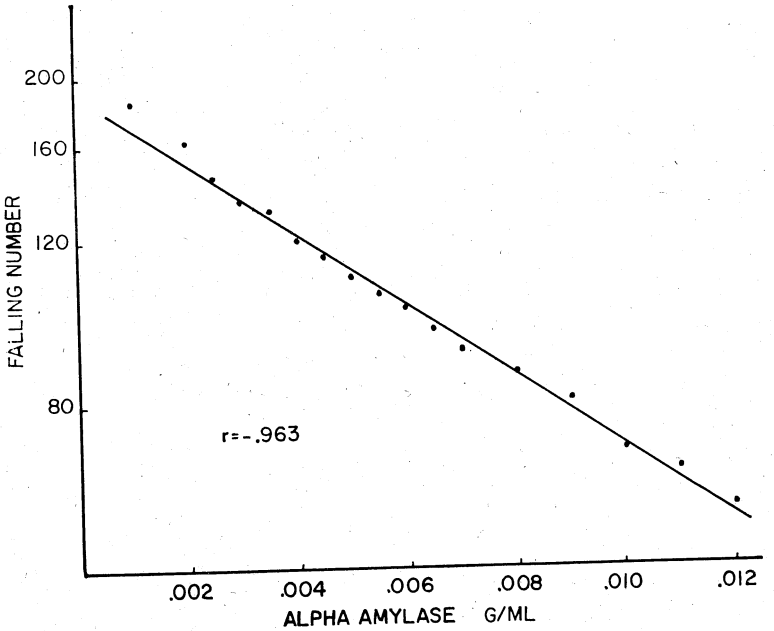

Fig. 1. Semilog plot of alpha-amylase concentration *vs.* falling number values using wheat starch as substrate.

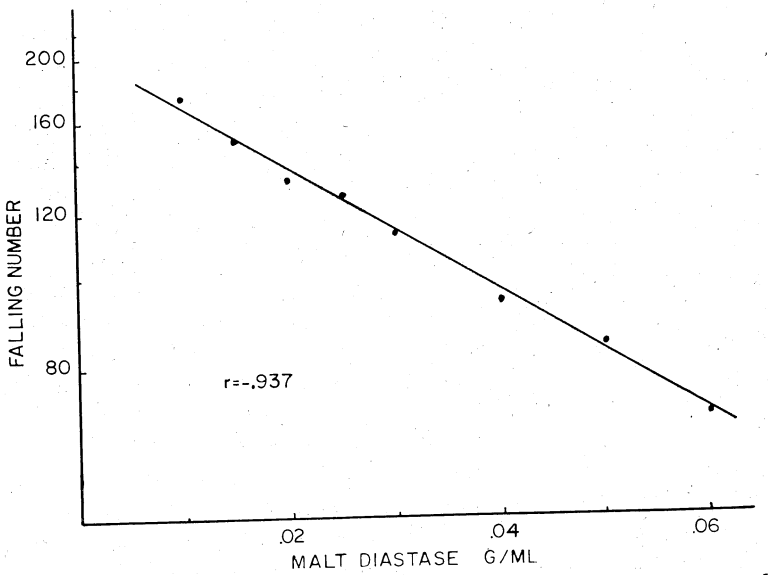

Fig. 2. Semilog plot of malt diastase concentration *vs.* falling number values using corn starch as substrate.

tion coefficient for these data was −0.937**. In both experiments, a straight line relationship was observed when the data were plotted on semilog paper. These preliminary experiments indicated that the falling number method, when modified to use starch as a substrate, should be applicable to the determination of alpha-amylase activity in barley malt.

Perten (3) indicated that beta-amylase did not affect FNV's appreciably. To confirm this under the conditions used in the present work, various concentrations of beta-amylase were added to corn starch containing a quantity of a standard dry malt. Results are shown in Table I.

TABLE I

EFFECT OF BETA-AMYLASE ON FALLING NUMBER VALUES FOR STARCH-MALT MIXTURES

BETA-AMYLASE ADDED	FALLING NUMBER
mg.	*sec.*
none	142
1.0	140
2.0	139
3.0	146
5.0	146
6.0	144
7.0	144
8.0	140

Beta-amylase concentrations up to 8 mg. did not alter FNV's beyond the experimental error of the method. This concentration of beta-amylase is approximately double that which ordinarily would be found in the amount of dry malt or malt extract used in this work.

The first experiments, on a series of 18 malt samples, compared FNV's with 20° Units of alpha-amylase activity. For dry malt, both corn and wheat starches were used as the substrate. With these two substrates, FNV's were similar but not identical. For malt extract, corn starch was used as the substrate. For this series, the range of FNV's was relatively small (90–150). In all cases, a straight line relationship was observed between FNV's and alpha-amylase units. Correlation coefficients for the dry malts were −0.971** for wheat starch and −0.978** for corn starch. For the malt extracts, r was −0.975**.

It was concluded from these data that either starch could be used for this procedure. However, because different starches do not give identical values, each laboratory must use a single source of starch for its standard curves and for subsequent determinations where a specific standard curve is used. In this work, corn starch was used in all subsequent determinations.

It was apparent also from the above experiments that dry malt and malt extract did not give identical FNV's by the described procedure. It was necessary, therefore, to prepare a standard curve which compared alpha-amylase units to FNV's for both dry malt and malt extract.

Forty malt samples were chosen for preparation of standard curves. Alpha-amylase activities were determined for these samples by the standard ASBC procedure, and FNV's were determined for both dry malt and malt extract with corn starch as the substrate. Linear plots of these data are shown in Fig. 3, and semilog plots in Fig. 4. Correla-

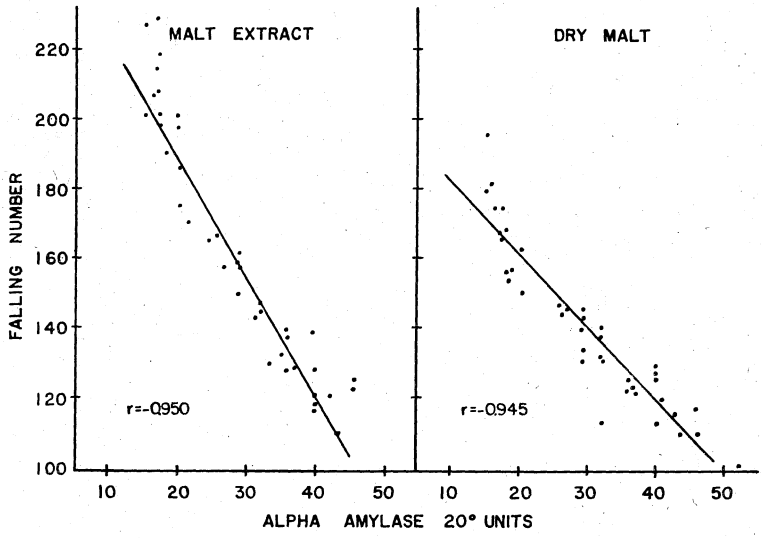

Fig. 3. Linear plot of 20° Units of alpha-amylase activity *vs.* falling number values (Standard Curves).

tion coefficients were high in both cases. However, the data appeared to give a better straight line relationship when plotted on semilog coordinates.

From the above data, linear and nonlinear regression equations were determined for both dry malt and malt extract. These equations then were used to convert FNV's to alpha-amylase units for a second series of malt samples. The relationship of alpha-amylase units determined directly to those calculated from FNV's using the linear regression equations is shown in Fig. 5. Figure 6 shows the same relationship for values calculated using the nonlinear regression equations. Correlation coefficients were high in all cases. However, the r values were slightly higher when the nonlinear equations were used; it is

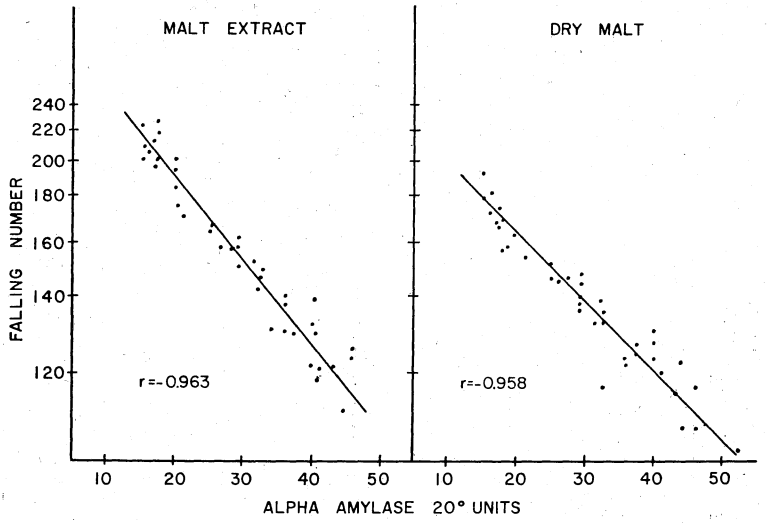

Fig. 4. Semilog plot of 20° Units of alpha-amylase activity *vs.* falling number values (Standard Curves).

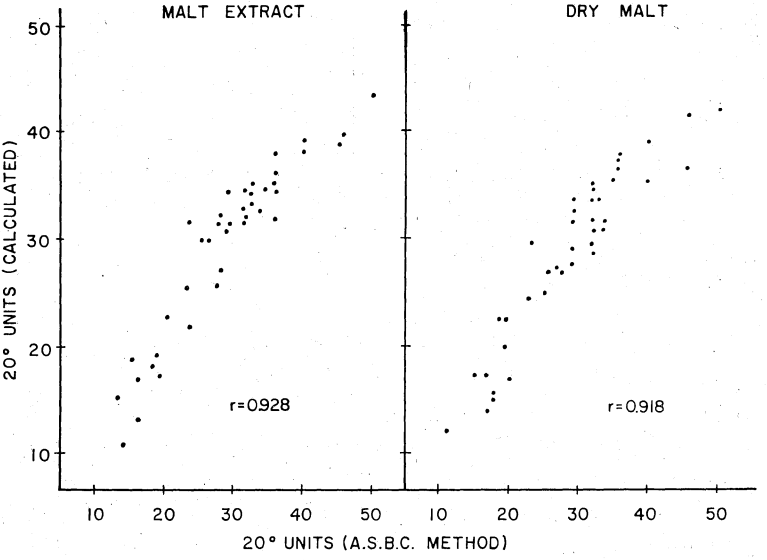

Fig. 5. Scattergrams of 20° Units determined directly *vs.* 20° Units calculated from falling number values using the linear regression equations.

apparent from the scattergrams that better straight line relationships were obtained using the nonlinear equations.

These data indicate that the falling number procedure can be used to determine alpha-amylase activity in barley malts. The values obtained correlate very well with the standard ASBC method for alpha-amylase activity. Standard errors of estimate were 3.18 and 2.96 20° Units for dry malt and malt extract, respectively, using the linear regression equations, and 2.94 and 2.62 20° Units, respectively, using the nonlinear regression equations. For a relatively small range of

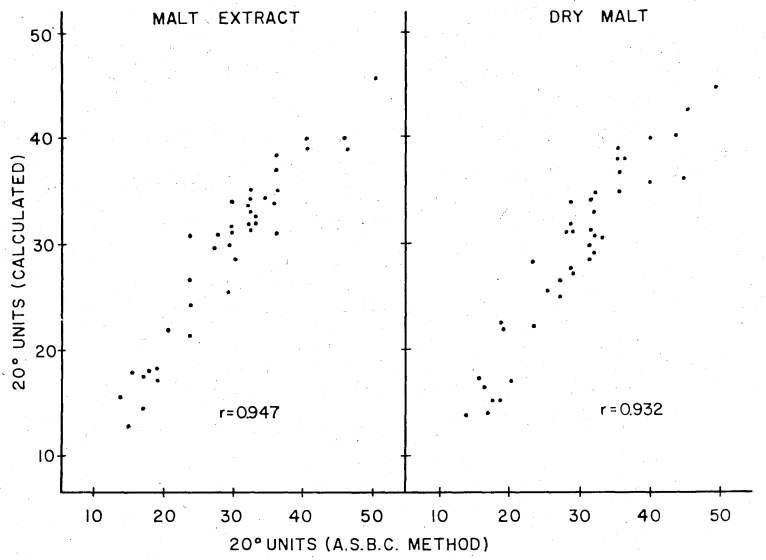

Fig. 6. Scattergrams of 20° Units determined directly *vs.* 20° Units calculated from falling number values using the nonlinear regression equations.

activity, a linear standard curve would be adequate. Where a wide range of activity is encountered, a nonlinear regression equation should be used to convert FNV's to alpha-amylase units. The precision of the method is quite good. Standard error of a single determination for a series of 15 samples tested in duplicate was 4.7 sec. for dry malt and 2.3 sec. for malt extract.

The falling number procedure is simple and rapid, and requires no special reagents. It can be used to determine the alpha-amylase activity in either dry malt or malt extract. The two procedures do not give identical FNV's for the same sample. However, FNV's for dry malt and malt extract do correlate well with each other. The correlation coefficient for this relationship for the 40 malt samples used to prepare the standard curves was 0.89**.

Acknowledgments

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