Comparison of the Grain Amylase Analyzer with the Amylograph and Falling Number Methods[1]

B. L. D'APPOLONIA, L. A. MacARTHUR, W. PISESOOKBUNTERNG, and C. F. CIACCO,[2] Department of Cereal Chemistry and Technology, North Dakota State University, Fargo 58105

ABSTRACT

Cereal Chem. 59(4): 254–257

The Perkin-Elmer model 191 grain amylase analyzer was used to analyze five different series of samples, and the results were compared to falling number and amylograph peak viscosity values. One sample series contained a sound sample of wheat flour to which various amounts of barley malt were added. Two series each of wheat samples grown during the 1979 and 1980 crop years were used. The 1979 crop material contained very little sprouted wheat, whereas the 1980 crop samples showed a wide range in sprouting. Highly significant correlations were found between grain amylase analyzer results and falling number or amylograph peak viscosity values. The correlation coefficient values were highest with those samples having a wide range in amylase activity.

Sprouting in wheat is a condition that occurs when weather at harvest is wet, which causes moisture content of the grain to increase to a level at which germination takes place and the α-amylase increases. Changes also take place in other enzymes and biochemical components. Measurement of the amount of amylase enzyme present, however, has normally been the method used to assess the level of sprouting in wheat.

Sprouting affects the grade of wheat. Grain inspectors determine the amount of sprout damage in wheat by visual examination of a sample. Such a procedure is subjective, and changes known as incipient sprouting often can occur in the wheat before visual damage is detectable.

Various methods have been used to determine α-amylase in cereals. Some of these methods were reported by Kruger et al (1979) and Campbell and Ranum (1980). Two methods more commonly used with wheat are the falling number and amylograph procedures.

A technique using the model 191 grain amylase analyzer (GAA) and based on nephelometry was recently introduced (Perkin-Elmer Corp., Oak Brook Instrument Division, Oak Brook, IL) to measure α-amylase. The theory and operating principles have been discussed by Campbell (1979) and Campbell and Ranum (1980). Results obtained with the model 191 GAA using amylopectin as substrate were discussed by Kruger et al (1979) and Prasad et al (1979a, 1979b).

The present study was undertaken to compare results obtained with the model 191 GAA, using β-limit dextrin as substrate, with the results of viscometric falling number and amylograph procedures.

MATERIALS AND METHODS

Samples
Five sets of samples were used to conduct this study.

Set 1 consisted of various known amounts of barley malt, added to a sound sample of hard red spring wheat flour.

Set 2 consisted of 51 samples of hard red spring wheat grown during the 1979 crop year. Each sample tested represented a composite of 10 different commercial samples collected for each of 51 counties in North Dakota.

Set 3 consisted of 84 samples of hard red spring wheat and represented 12 named varieties, experimentally grown, at seven locations throughout North Dakota.

Set 4 consisted of 48 samples of hard red spring wheat grown during the 1980 crop year. Each sample represented a composite of 10 different commercial samples collected for each of the 48 counties in North Dakota.

Set 5 consisted of 18 samples of hard red spring wheat grown during the 1980 crop year. Each sample was a composite of several samples collected for each of the 18 districts in North Dakota, South Dakota, Montana, and Minnesota.

Model 191 Grain Amylase Analyzer Method
The procedure for determining amylase with the model 191 GAA was as described by Campbell and Ranum (1980). Results were based on an average of three determinations and reported as GAA units per 4 g of material. Measurement was performed on the ground whole wheat and on the Buhler-milled flour.

Amylograph Procedure
This determination was performed on a Brabender Visco Amylograph using 65 g of flour derived from the Buhler Mill in place of 100 g (AACC 1969) for evaluation of sample sets three and five. For amylograph determination on sample set one, 100 g of flour was used.

Falling Number Procedure
The falling number was determined on 7.0 g of ground whole wheat or Buhler-milled flour (AACC 1969).

Milling of Samples
Grinding of samples (approximately 50 g) for whole wheat analysis was performed on a Udy Cyclone Mill. Analysis was on flour obtained from a Buhler experimental mill.

TABLE I
Effect of Barley Malt Addition on Amylograph Peak Viscosity, Falling Number, and Grain Amylase Analyzer Values of Sound Wheat Flour

Malt Addition (mg)	Amylograph Peak Viscosity (BU)	Falling Number (sec)	Grain Amylase Analyzer Units
0	3,400	523	20
50	1,165	323	110
100	650	253	213
150	455	210	253
250	325	197	440
400	240	170	810
600	185	152	1,305
800	155	135	1,661
1,000	130	127	2,222

RESULTS AND DISCUSSION

Evaluation of Sample Sets

Set 1. Table I shows the results obtained on the sound wheat flour with the addition of various amounts of barley malt. Amylograph peak viscosity values ranged from 3,400 to 130 BU, falling number values from 523 to 127 sec, and GAA values from 20 to 2,222 units.

Figures 1–6 show the results obtained using the three different methods to measure amylase activity.

The results obtained with the GAA showed a curvilinear relationship with either the falling number or amylograph methods. Similarly, falling number and amylograph peak height exhibited a curvilinear relation. When the $\log_e$ of the results were plotted, however, highly significant correlations of 0.997, −0.985, and −0.988 were obtained between falling number and amylograph peak height, falling number and model 191 GAA units, and amylograph peak height and model 191 GAA, respectively. The regression equations obtained are given also on Figs. 2, 4, and 6.

Set 2. Sample set 2 consisted of 51 samples of hard red spring wheat grown during the 1979 crop year. With this set of samples, the model 191 GAA was used to measure amylase activity in the wheat ground on a Udy Cyclone Mill. The falling number was determined on an aliquot of the same ground sample. Hard red spring wheat grown during the 1979 crop year was mainly sound; sprouting was not a problem (Table II). With set 2, more than 90% of the samples had falling number values over 300 sec.

Although the data indicated a highly significant correlation between the $\log_e$ of the falling number and GAA values (Table III), the correlation was not as high as that noted with the malted samples (Fig. 4). One possible reason is that the bulk of the samples
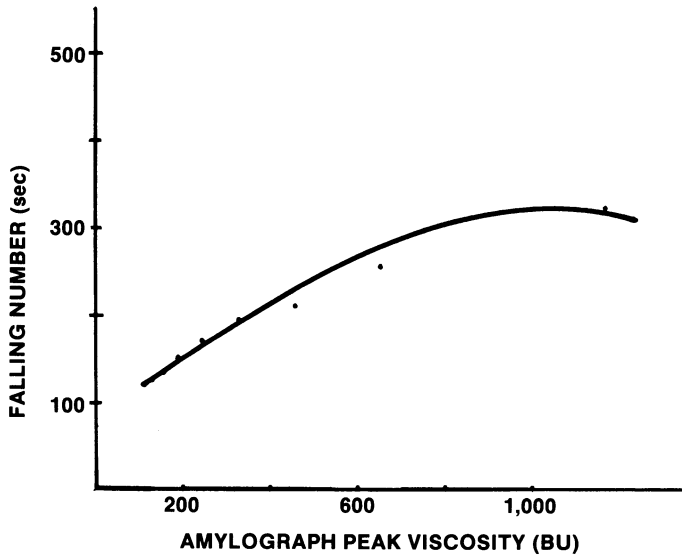

Fig. 3. Curve showing relation between falling number and model 191 grain amylase analyzer units on malted flour samples.

Fig. 4. Curve showing relation between $\log_e$ falling number and $\log_e$ model 191 grain amylase analyzer units on malted flour samples.

Fig. 1. Curve showing relation between falling number and amylograph peak height on malted flour samples.
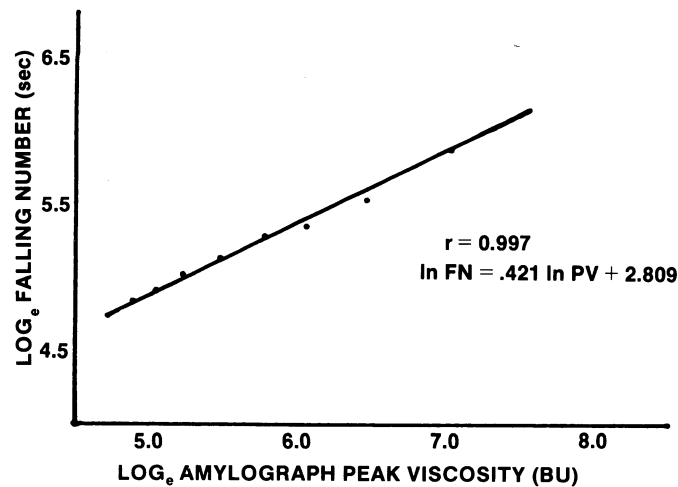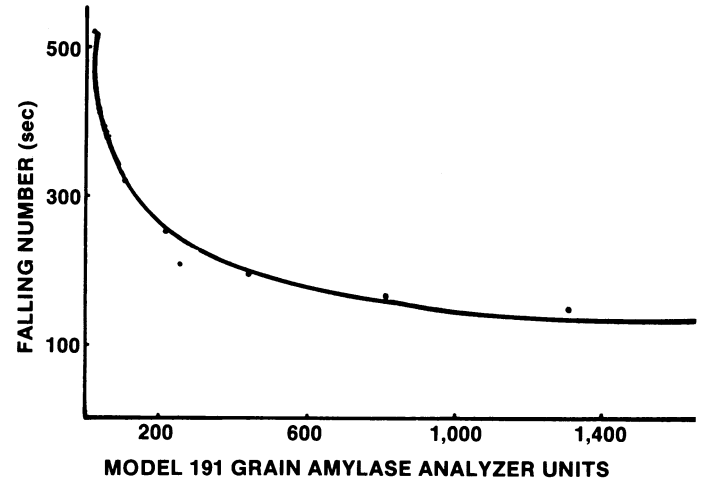

Fig. 2. Curve showing relation between $\log_e$ falling number and $\log_e$ amylograph peak height on malted flour samples.

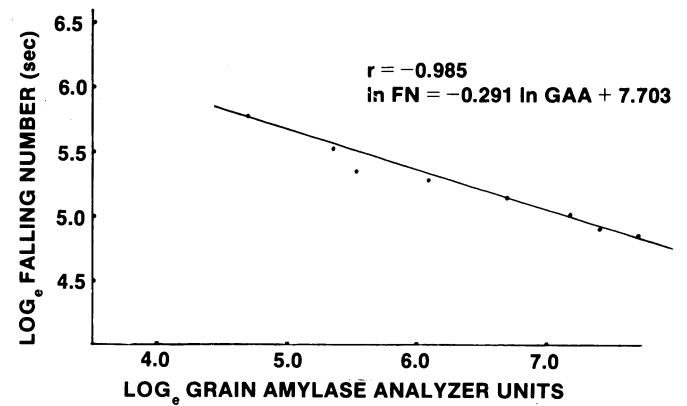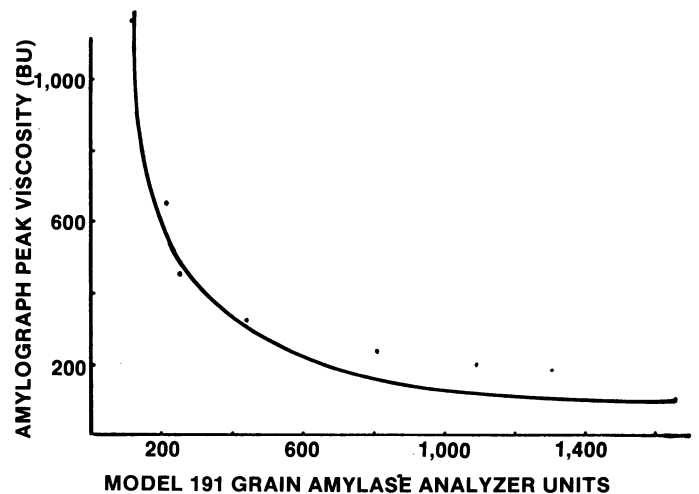**Fig. 5.** Curve showing relation between amylograph peak height and model 191 grain amylase analyzer units on malted flour samples.

were sound and contained very little α-amylase. The GAA is a more direct measurement of α-amylase than the falling number method, which is also dependent upon the nature of the starch itself. This is particularly true with samples containing low levels of α-amylase.

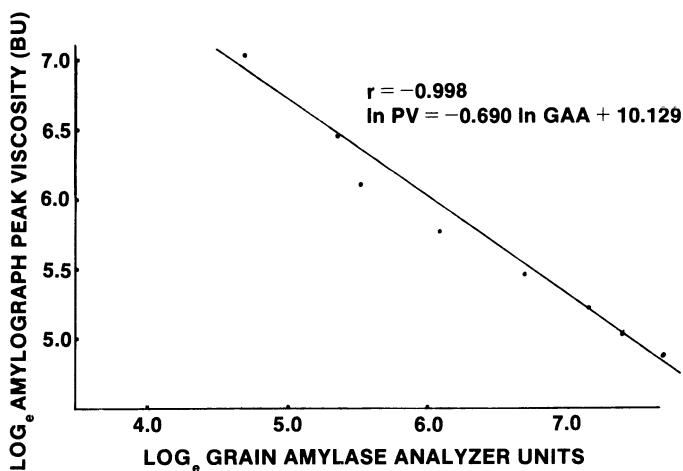*Set 3.* This set of samples (84) consisted of 12 named varieties of

Fig. 6. Curve showing relation between $\log_e$ amylograph peak height and $\log_e$ model 191 grain amylase analyzer units on malted flour samples.

hard red spring wheat, experimentally grown at seven different locations throughout North Dakota. As with the first set of samples, amylase activity was measured on the wheat after grinding in a Udy Cyclone Mill, using the GAA and the falling number methods. A portion of the samples was milled on a Buhler experimental flour mill, and amylase activity in the straight grade flour was measured using the GAA, falling number, and amylograph methods.

Once again, most of the samples were sound, as determined by the falling number method.

Table IV shows the various correlation coefficients obtained with this set of samples. In this instance the correlation between wheat falling number and wheat GAA, $\log_e$ values was -0.794, which was similar to the value found in set 2. The correlation coefficient obtained with the Buhler-milled flour between the ln of falling number and ln of GAA, although highly significant, was considerably lower (-0.391). Similarly, correlation coefficients between ln amylograph peak viscosity and ln grain amylase analyzer values, although highly significant, were low, and were lowest when the determinations were performed on the flour.

Of particular interest also with this set of samples was the possible effect of variety and location on amylase activity. Location had a highly significant effect on the GAA and falling number values, whereas variety did not. Both location and variety had a highly significant effect on amylograph peak viscosity values.

Evaluation of Sample Set Four

As noted in Table II, the samples used in this instance showed a much wider range in falling number values. Much higher correlation coefficients were obtained than were found in sample sets 2 or 3 between falling number and GAA values (Table V).

Evaluation of Sample Set Five

As was true for set 4, a wide range in falling number values was noted for this series of samples (Table II). Likewise, Table VI indicates high correlation coefficients not only between falling number and the GAA values but also between the GAA values and amylograph peak viscosity values.

Evaluation of Samples With a Particular
Range of Falling Numbers

From the samples evaluated in sets 2–5, we arbitrarily selected 35 with wheat falling number values below 249, 35 samples between 250 and 349, and 35 samples above 350. Using $\log_e$ values, correlation coefficients between the falling number and the GAA values were determined on each set of 35 samples.

Correlation coefficients of 0.94, 0.64, and 0.30 were obtained with the sets of samples having falling number values below 249, between 250 and 349, and above 350, respectively.

These results again indicate a better correlation with those samples having higher amylase activity.

SUMMARY

The results of this study indicated that a highly significant correlation exists between falling number and grain amylase analyzer values or between amylograph peak viscosity and grain

TABLE II
**Percentage of Wheat Samples Within a Particular Range
of Falling Numbers**

Falling Number Range (sec)	Sample Set			
	2 (%)	3 (%)	4 (%)	5 (%)
Below 150	···	···	27.1	22.2
150–199	···	···	12.5	5.6
200–249	2.0	2.4	16.7	5.6
250–299	7.8	6.0	6.3	11.2
300–349	15.8	13.1	14.6	11.2
350–399	23.5	33.3	18.8	11.2
400–449	33.3	38.1	4.2	33.3
450–499	17.6	7.1	···	···

TABLE III
Correlation Coefficients for Sample Set Two[a]

	WFN	ln WGAA	ln WFN	ln FFN
WGAA	−0.702	0.752	−0.753	−0.608
WFN		−0.784	0.994	0.711
ln WGAA			−0.772	−0.651
ln WFN				0.716

[a] All values are highly significant. WGAA = wheat grain amylase analyzer, WFN = wheat falling number, FFN = flour falling number.

TABLE IV
Correlation Coefficients for Sample Set Three[a]

	WGAA	WFN	FGAA	FFN	Amylo P.V.	ln WGAA	ln WFN	ln FGAA	ln FFN	ln Amylo P.V.
WGAA		−0.655	0.641	−0.461	−0.547	0.762	−0.701	0.378	−0.509	−0.609
WFN			−0.577	0.715	0.702	−0.789	0.994	−0.384	0.737	0.721
FGAA				−0.518	−0.613	0.538	−0.631	0.726	−0.597	−0.744
FFN					0.613	−0.601	0.698	−0.341	0.993	0.616
Amylo P.V.						−0.636	0.713	−0.417	0.652	0.971
ln WGAA							−0.794	0.363	−0.626	−0.643
ln WFN								−0.400	0.729	0.748
ln FGAA									−0.391	0.462
ln FFN										0.671
ln Amylo P.V.										

[a] All values are highly significant. WGAA = wheat grain amylase analyzer, FGAA = flour grain amylase analyzer, WFN = wheat falling number, FFN = flour falling number, Amylo P.V. = amylograph peak viscosity.

	WGAA	WFN	FGAA	FFN	ln WGAA	ln WFN	ln FGAA	ln FFN
WGAA		−0.739	0.913	−0.731	0.784	−0.861	0.676	−0.826
WFN			−0.794	0.962	−0.971	0.970	−0.926	0.952
FGAA				−0.808	0.817	−0.896	0.756	−0.902
FFN					−0.949	0.937	−0.955	0.981
ln WGAA						−0.958	0.920	−0.951
ln WFN							−0.890	0.968
ln FGAA								−0.929
ln FFN								

[a] All values are highly significant. WGAA = wheat grain amylase analyzer, FGAA = flour grain amylase analyzer, WFN = wheat falling number, FFN = flour falling number.

	WGAA	WFN	FGAA	FFN	AMYLO P.V.	ln WGAA	ln WFN	ln FGAA	ln FFN	ln Amylo P.V.
WGAA		−0.943	0.816	0.870	−0.828	0.920	−0.973	0.790	−0.898	−0.914
WFN			−0.796	0.924	0.936	−0.972	0.992	−0.830	0.936	0.971
FGAA				−0.853	−0.755	0.781	−0.822	0.850	−0.907	−0.870
FFN					0.932	−0.944	0.915	−0.950	0.991	0.962
Amylo P.V.						−0.938	0.905	−0.870	0.922	0.967
ln WGAA							−0.960	0.880	−0.942	−0.960
ln WFN								−0.820	0.934	0.964
ln FGAA									−0.944	−0.901
ln FFN										0.975
ln Amylo P.V.										

[a] All values are highly significant. WGAA = wheat grain amylase analyzer, FGAA = flour grain amylase analyzer, WFN = wheat falling number, FFN = flour falling number, Amylo P.V. = amylograph peak viscosity.

amylase analyzer values. Samples that were primarily sound gave correlations that were highly significant but lower in value than samples that contained a wide range in amylase activity.

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