Effect of Wheat Variety on the Relationship Between Falling Numbers and Alpha-Amylase Activity

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ABSTRACT

For 12 wheat varieties standard curves were established that related α-amylase activity to falling number values, and a method was developed that accurately quantifies α-amylase activity by using only falling numbers. The method essentially eliminates the genetically controlled factors, other than α-amylase, that affect falling numbers.

The falling number (FN) test is a relatively simple and rapid method to estimate α-amylase activity in grain flours (Hagberg 1960, 1961). The method relies on the ability of α-amylase to liquefy gelatinized starch so it is thereby a predictive measurement of bread crumb quality since "the interval between the gelatinization of starch (55–65° C) and the inactivation of the enzymes during baking (75–80° C) is a decisive factor in determining bread crumb quality" (Perten 1964). Perten obtained a linear relationship between α-amylase activity and liquefaction numbers (LN). Medcalf et al (1966) adapted the test, using unmodified corn starch to estimate α-amylase content of barley malts and their extracts.

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Medcalf et al (1966) and Greenaway and Neustadt (1967) reported methods to control experimental factors that affect experimental error in the FN test. Perten (1967) also studied factors that influence FN values and stressed the importance of finely ground, homogenous sample preparation and showed that FN values increase directly with degree of flour fineness. Perten (1967) and later Tipples (1971) clearly demonstrated the importance of grinding no less than 300 g of grain for the FN test to avoid sampling error. Greenaway and Neustadt (1967) and Perten (1967) reported that variation in the temperature of the boiling water bath is the major source of error in the test. Barometric pressure, which varies with elevation, and gross differences in mineral content of the water are the source of that error. Lorenz and Wolt (1981) corroborated those results.

Using the Youden-diagram method to evaluate interlaboratory collaborative testing (Woolcott 1973, Gertstenkorn and Brummer 1974, Möttönen 1976) reported that the FN test is plagued with systematic as well as random error and that, during long FN times, bubbles form that can merge, move inconsistently, cause the stirrer to reduce its downward motion, and thereby cause both systematic and random error (Möttönen 1977, 1978). Modifying the FN method to incorporate 8 g of flour and 30 ml of water eliminated some of the random error caused by bubbles.

As a tool for predicting wheat flour α-amylase activity, the FN method has been compared to the amylograph technique (Patterson and Crandell 1967, Kruger and Tipples 1981, D'Appolonia et al 1982). Both methods reasonably predict amylase activity but both are limited in predictive value by factors other than α-amylase, such as starch damage, the nature of the starch and its susceptibility to enzyme attack, and flour particle size distribution. Those factors are controlled by genetic mechanisms as well as by agronomic and milling conditions. Medcalf et al (1968) reported that both varietal and environmental factors influence FN values of six "sound" hard red spring wheat varieties. Using fractionation and reconstitution techniques, they showed that differences in both starch susceptibility and amylase activity contribute to the FN values.

Today there continues to be a need for a simple but quick, accurate, and relatively inexpensive α-amylase assay. Among a few methods being considered is the FN test. Reported here are results indicating that a modified FN test can be used to accurately quantitate α-amylase in wheat flour. Also reported are results that illustrate why even the modified FN test is unsuitable for the grain trade.

MATERIALS AND METHODS

For the first phase of this study, eight of the 12 wheats used were composites from three to six crop years between 1972 and 1978. Arthur 71 was a single seed lot grown in 1978, and the remaining three cultivars (Cheney, Newton, and Eagle) were hard red winter wheat composites grown in 1978 at several locations. For the second and third phases of this study, single seed lots of 41 wheat cultivars grown in 1981 at Lind or Pullman, WA, were used.

Wheat Germination

Wheats were steeped for 12 hr at 23 ±1° C in excess, continuously aerated water. After steeping, seeds were germinated 36 hr (total, 48 hr) in a cabinet with controlled temperature (23 ±0.5° C) and humidity (100% rh) that provided automatic rinsing and draining every 2 hr, as designed and built by Y. S. Hsu and described by Hsu et al (1980).

Grinding

All wheats were ground on a Udy cyclone mill to pass through a 0.5-mm mesh screen. The germinated wheats were dehydrated at 45 ±1° C in a forced-air oven for 12 hr and then ground by the Udy mill. Flour samples were stored at −20° C throughout the study except when subjected to analysis.

Analytical Procedures

Moisture and protein contents of wheat (N × 5.7) were determined by AACC methods 44-15A and 46-12, respectively. Wheat α-amylase was determined by AACC method 22-06. AACC falling number method 56-81B was used unless otherwise stated.

RESULTS AND DISCUSSION

A standard curve was constructed that plotted absorbance from the α-amylase analyzer (Demaray Scientific Instruments, Pullman, WA) vs. known α-amylase content of a control straight grade bakers flour spiked with increments (0.2 DU/g, 20° C) of α-amylase (Fig. 1, $r = 0.998$). α-Amylase activity of the 12 germinated

TABLE I
Alpha-Amylase Content of Wheat Variety Composites Diluted 1:9 with Bakers Flour

Variety	Absorbance[a]	Alpha-Amylase DU/0.1 g	Alpha-Amylase DU/g
Cheyenne	0.154	0.250	2.50
Fielder	0.080	0.130	1.30
Fortuna	0.040	0.065	0.65
Nugaines	0.325	0.527	5.27
Paha	0.072	0.117	1.17
Peak	0.147	0.238	2.38
Wanser	0.110	0.178	1.78
Yamhill	0.151	0.245	2.45
Arthur 71	0.278	0.451	4.51
Cheney	0.169	0.274	2.74
Newton	0.149	0.241	2.41
Eagle	0.329	0.533	5.33

[a] Average of three samples.

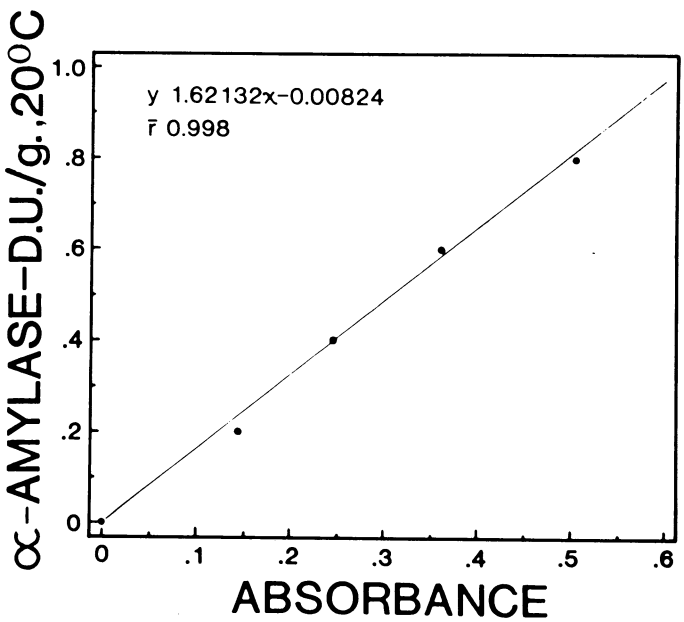

Fig. 1. Standard curve relating absorbance to α-amylase activity of control bakers flour spiked with increments of malted barley flour.

TABLE II
Falling Numbers vs. Ratio of Germinated Whole Wheat Flour to Ungerminated Whole Wheat Flour

Variety	1/4:6 3/4 (sec)	1/2:6 1/2 (sec)	1:6 (sec)	2:5 (sec)	3:4 (sec)	4:3 (sec)	5:2 (sec)	6:1 (sec)	7:0 (sec)
Cheyenne	337	242	191	121	81	68	62	61	61
Fielder	288	236	182	121	94	93	71	65	63
Fortuna	563	410	330	238	185	145	128	109	97
Nugaines	222	155	103	66	61	60	60	60	60
Paha	384	308	245	180	138	117	103	94	85
Peak	345	260	196	130	93	73	69	64	62
Wanser	363	274	225	145	107	89	73	66	64
Yamhill	274	227	149	103	78	65	63	61	60
Arthur 71	255	213	144	91	69	61	61	60	60
Cheney	314	247	168	105	80	64	63	61	60
Newton	328	261	201	135	96	73	63	62	61
Eagle	267	193	131	75	63	61	60	60	60
r value	−0.736	−0.814	−0.833	−0.838	−0.785	−0.760	−0.661	−0.622	−0.605

wheat variety composites was quantified by combining one part of each flour with nine parts of the control flour. α-Amylase activity was assayed spectrophotometrically and expressed in dextrinizing units per gram, (20° C), using Figure 1 as the standard curve (Table I). Fortuna had the least α-amylase activity, and Eagle had the greatest, with 0.065 and 5.33 DU/g, respectively.

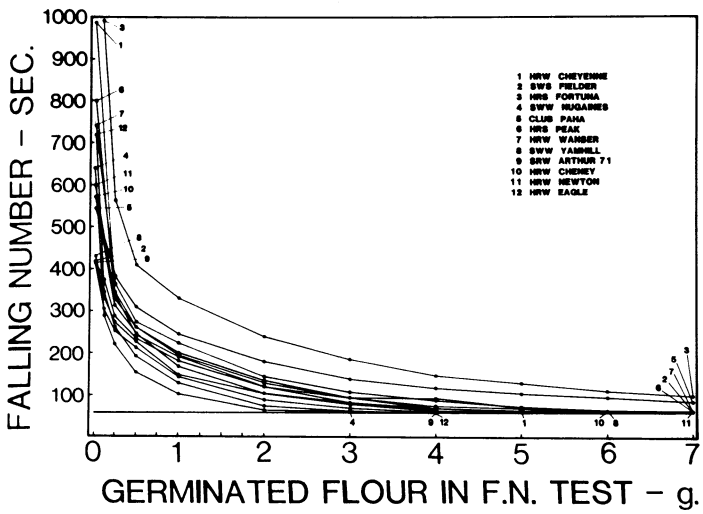

Fig. 2. Standard curves for 12 wheat variety composites relating falling numbers to the ratio of germinated to ungerminated whole wheat flours.

FN Values of Germinated Wheats

The FN values for 7 g of germinated wheat flour ranged from 97 to 60 sec, with 10 of 12 flours producing 64 sec or less, indicating that flours contained appreciable quantities of α-amylase. Therefore, FN values were determined on each of the 12 germinated wheat flours at proportions of germinated to ungerminated flours varying from 0.25:6.25 to 7:0. Correlation coefficients relating α-amylase content and FN values increased from $r = -0.605$ to $r = -0.838$ as germinated flour content decreased from 7 to 2 g but then decreased to $r = -0.736$ as germinated flour content was decreased from 2 to 0.25 g (Table II).

Estimate of α-Amylase from FN

The FN data from Table II was graphed to form distinct curves for each of the 12 germinated wheat varieties (Fig. 2). Next, increments (0.5–3 DU/g, 20° C) of water-extracted malted barley α-amylase were added to the 12 ungerminated whole wheat flours and FN values were determined. In effect, 12 standard curves relating α-amylase to FN values were produced, each of which preserved and reflected inherent varietal differences that affect FN values, such as susceptibility of starch to α-amylase hydrolysis and diffusion in pasting (Fig. 3).

Data from Figures 2 and 3 combined were used to estimate the α-amylase content of each of the 12 germinated wheat flours. Figure 2 establishes the grams of germinated wheat flour per 7 g of

TABLE III
Alpha-Amylase Content Estimated from Falling Number Values[a] Divided by the Amount of Germinated Wheat Required to Produce a Falling Number of 150, 200, 250 or 300[b]

		Falling Number											
		150			200			250			300		
No.	Variety	Amylase Required by Wheat (DU)	Germinated Wheat (g)	Estimated Amylase (DU/g)	Amylase Required by Wheat (DU)	Germinated Wheat (g)	Estimated Amylase (DU/g)	Amylase Required by Wheat (DU)	Germinated Wheat (g)	Estimated Amylase (Du/g)	Amylase Required by Wheat (DU)	Germinated Wheat (g)	Estimated Amylase (DU/g)
1	Cheyenne	2.87	1.58	1.82	1.88	0.91	2.06	1.13	0.48	2.34	0.90	0.35	2.57
2	Fielder	1.63	1.52	1.07	0.96	0.83	1.15	0.74	0.44	1.70	0.49	0.24	2.09
3	Fortuna	2.75	3.90	0.71	1.68	2.73	0.62	1.12	1.88	0.59	0.91	1.33	0.69
4	Nugaines	2.32	0.55	4.21	1.33	0.34	3.96	0.96	0.20	4.80	0.77	0.13	6.16
5	Paha	2.47	2.68	0.92	1.32	1.68	0.79	0.94	0.96	0.98	0.74	0.57	1.31
6	Peak	2.75	1.68	1.64	1.60	0.96	1.66	1.05	0.58	1.83	0.87	0.39	2.26
7	Wanser	2.47	1.93	1.28	1.42	1.29	1.10	0.99	0.75	1.31	0.82	0.43	1.93
8	Yamhill	2.47	0.98	2.52	1.41	0.68	2.09	0.84	0.38	2.24	0.59	0.19	3.08
9	Arthur 71	2.97	0.96	3.09	1.72	0.59	2.93	0.97	0.27	3.66	0.71	0.14	5.22
10	Cheney	2.67	1.28	2.09	1.56	0.80	1.95	1.03	0.49	2.12	0.85	0.30	2.84
11	Newton	2.80	1.76	1.59	1.72	1.00	1.72	1.11	0.58	1.93	0.84	0.35	2.39
12	Eagle	3.01	0.83	3.63	2.10	0.48	4.42	1.30	0.30	4.33	0.93	0.19	4.89

[a] Data for 150, 200, 250, or 300 falling number values from Figure 2.
[b] Data shown in Figure 3.

TABLE IV
Falling Numbers vs. Ratio of Germinated Whole Wheat Flour to Straight Grade Flour

Variety	1/4:6 3/4 (sec)	1/2:6 1/2 (sec)	1:6 (sec)	2:5 (sec)	3:4 (sec)	4:3 (sec)	5:2 (sec)	6:1 (sec)	7:0 (sec)
Cheyenne	279	232	171	127	100	83	77	75	61
Fielder	306	272	211	153	127	99	86	77	63
Fortuna	360	315	268	223	189	162	150	134	97
Nugaines	226	176	128	82	67	61	60	60	60
Paha	329	279	234	184	150	129	112	102	85
Peak	293	238	189	134	106	92	...	...	62
Wanser	306	254	217	153	125	99	91	79	64
Yamhill	282	236	175	122	98	79	63	62	60
Arthur 71	236	191	140	96	78	64	61	60	60
Cheney	276	230	175	122	97	81	63	61	60
Newton	282	242	190	138	111	88	80	66	61
Eagle	220	184	128	86	71	61	60	60	60
r value	−0.969	−0.967	−0.946	−0.913	−0.892	−0.845	−0.765	−0.706	−0.605

total flour required to produce a given FN value (e.g., 200 sec); Figure 3 establishes the amount (in dextrinizing units per 7 g, 20° C) of α-amylase required to produce that FN value. The amount of germinated flour was computed from Figure 2 and the amount of α-amylase was computed from Figure 3, which was required to produce four arbitrary FN levels—150, 200, 250, and 300 sec—and that data was tabulated (Table III). Estimates of the α-amylase activity at the four FN levels were comparable, but systematic changes in those estimates appeared as FN values increased. The correlation coefficients between α-amylase activity and FN at the four arbitrary FN levels were between −0.97 and −0.98.

So far I have illustrated how factors other than α-amylase activity affect wheat flour FN values and have shown, by establishing a standard curve for each variety, how to accurately quantify α-amylase activity using only FN values. The next phase of the research determined the effect a reduction in those factors would have on a correlation between α-amylase and FN values. Once again that reduction was accomplished by reducing the amount of unknown flour assayed in the FN test. The FN values were determined, again on samples from 6 to 0.25 g. In this case, however, those sample sizes were combined with 1 to 6.75 g, respectively, of the sound, straight-grade bakers flour, thereby eliminating genetic differences in parent flours (Table IV). Decreasing the amount of germinated flour from 7 to 0.25 g progressively increased the negative correlation between α-amylase and FN values from $r = -0.605$ to $r = -0.969$ (Table IV).

α-Amylase vs. LN

A curvilinear relationship exists between FN values and α-amylase activity. Perten (1964) demonstrated how to convert that essentially logarithmetric function into a straight-line function by converting the FN values into LN values according to an empirically derived formula. The FN values from Table II were converted to LN values according to Perten's equation. At all substitution levels the LN values correlated positively and more highly with α-amylase values than did the FN values (Fig. 4). Whereas the correlation with FN maximized when 5 g of parent whole wheat flour was substituted for the germinated wheat flour, the LN correlation values maximized when only 3 g of flour was substituted. The LN values correlated with a 0.15–0.22 higher coefficient than did the FN values when between 0 and 4 g of whole wheat flour was substituted. However, when 5–6.75 g of the whole wheat flours were substituted for the germinated wheat flours, a conversion from FN to LN values improved the correlation with α-amylase by only about 0.05 to 0.1 (Fig. 4).

The FN values (Table IV) were also converted to LN numbers by Perten's conversion. At all straight-grade flour substitution levels, the LN values correlated positively and more highly with α-amylase activity than did the FN values (Fig. 4). When bakers flour was substituted, both FN and LN correlations with α-amylase activity continued to increase with increased substitution levels. Substitution with a common straight-grade flour resulted in a much higher correlation with α-amylase than did substitutions with the respective 12 parent whole wheat flours. Most likely those continuous improvements in correlations were due to the increased elimination of the factors other than α-amylase that affect FN and LN values.

The high correlations between FN or LN values and α-amylase activity depended on the amount and range of the α-amylase in the 7 g of germinated wheat flour assayed. When 0.25 g of germinated wheat flour and 6.75 g of sound, straight-grade bakers flour were assayed, the α-amylase activity of the 12 germinated wheats ranged between 0.162 and 1.342 DU per 7-g assay. Judging from Figures 2 and 3, that range in α-amylase activity would be well suited for α-amylase estimation by the FN technique because relatively small increases in α-amylase resulted in relatively great decreases in the FN values. That the LN values correlated only slightly higher with α-amylase activity than did the FN values is also understandable, because the relationship of FN values to α-amylase activity in that range was essentially linear, so that Perten's conversion to LN values would have much less impact on correlations.

Why were correlations between α-amylase activity and FN and LN values maximized at levels of 5 and 3 g, respectively, when parent whole wheat flour was substituted rather than progressively relating more highly with increasing substitution levels up to 6.75 g as they did when the sound, straight-grade bakers flour was

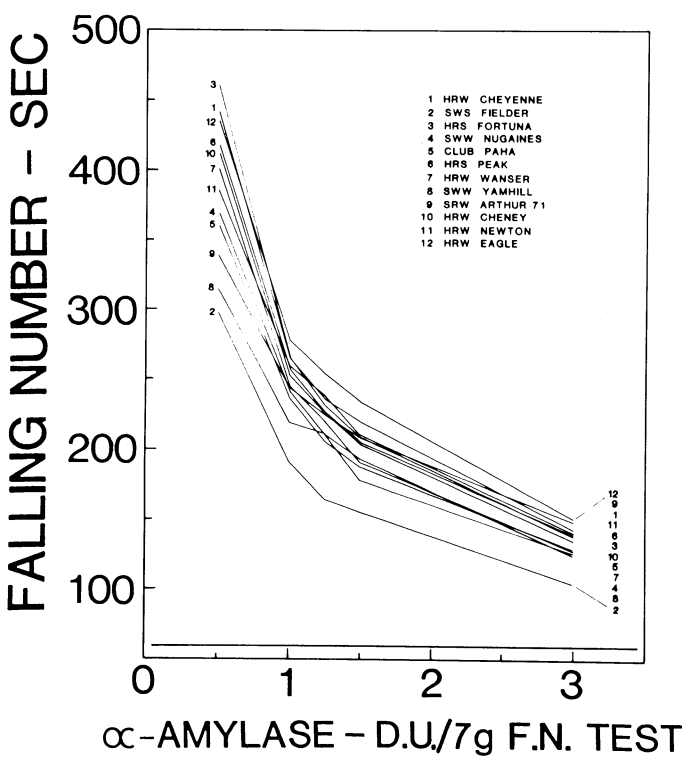

Fig. 3. Standard curves for 12 wheat variety composites relating falling numbers to α-amylase activity of the 12 wheat varieties spiked with increments of malted barley.

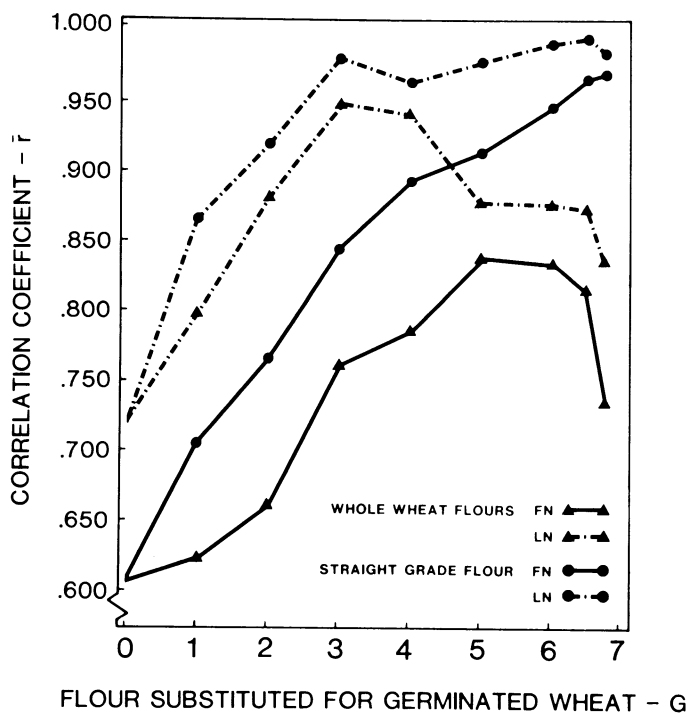

Fig. 4. Correlation coefficients relating α-amylase activity of 12 germinated wheat flours combined with proportions of the parent ungerminated whole flours or of one straight-grade flour vs. falling numbers (FN) or liquefaction numbers (LN).

substituted? Figure 4 shows that the change in range of α-amylase activity in the 12 wheat flours with increasing substitution levels is not responsible for reducing the correlations. Instead, as more sound, parent whole wheat flour is substituted beyond 3 g, the varietal differences in factors other than α-amylase that affect falling numbers outweigh the advantages in reducing the α-amylase in the 12 ungerminated and germinated wheat flour samples. Why those same factors have less effect on FN values after the wheats have been germinated cannot be explained at this time.

Since the LN correlation values of both parent whole wheat flour and the sound, straight-grade bakers flour reached near maximum with 3-g substitution levels (Fig. 4), α-amylase activity from 4 g of the germinated wheat flour was being assayed, or about 2.60–21.32 DU/7-g FN test (i.e., four times 0.65 and 5.33 DU/g for Fortuna and Eagle, respectively, Table I).

The LN graphs (Fig. 4) also indicate that, at higher α-amylase activity levels (i.e., with only 0–2 g substituted), genetically controlled factors other than amylase affecting FN and LN values were less important than at lower amylase levels (i.e., with 5–6.75 g substituted). Which factors and why they are so important when determining falling numbers of samples that contain relatively low levels of α-amylase remains inexplicable.

CONCLUSIONS

α-Amylase activity can be accurately quantified from falling numbers by 1) establishing a standard curve that relates known spiked α-amylase activity to the falling numbers of the unknown flour, 2) substituting various quantities of known sound wheat flour for the unknown sample, and 3) relating the quantity of unknown flour required to produce the same falling number as a given amount of spiked α-amylase produced. The method eliminates the genetically controlled factors other than α-amylase that affect falling numbers. Obviously, this modified FN procedure is an impractical way for rural and export elevators to evaluate degree of sprout damage in wheat lots because of the hours needed to establish standard curves, and it would be impossible to accomplish when samples of sound, parent wheat varieties are unavailable or unknown.

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