

Effects of Falling Number Sample Weight on Prediction of α -Amylase Activity

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ABSTRACT

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Reports vary on the effects of falling number (FN) sample weight on test precision, reproducibility, and predictability of α -amylase activity. Straight grade flours of 200 samples (25 cultivars \times 2 locations \times 2 N₂ levels \times 2 repetitions) were assayed for α -amylase activity and FN. Location significantly affected α -amylase activity and FN values. The coefficients of variation (CV) for the FN tests were 5.75, 2.12, 1.93, 1.72, 4.27, and 14.47%, when assayed with sample weights of 7, 6, 5.5, 5, 4.5, and 4 g, respectively. The FN test with the greatest reproducibility

between sample replicates (lowest LSD and highest ratio of range/LSD) was also produced using the 5-g sample weight. By reducing FN sample weight from 7 to 5 g, FN values that averaged 350 sec, considered essentially sound, averaged 215 sec, thus shortening the FN test time by an average of 2 min and 15 sec when assaying sound wheat flour. The results suggest a review of the 7-g stipulation of AACC Approved Method 56-81B for FN in favor of reduced sample weight.

The falling number (FN) determination is commonly used to estimate the degree of field-sprouting and α -amylase activity in wheat meal and flour. Today, Approved Method 56-81B of the American Association of Cereal Chemists (AACC 2000) recommends a 7-g sample weight, without qualification. However, when the FN method was first introduced for analysis of low diastatic flours, a sample weight of ≤ 5 g was recommended; for analysis of high diastatic flours, a sample weight of >5 g was recommended (Hagberg 1960). Later, without explanation, the FN sample weight was increased from 5 to 7 g (Hagberg 1961). Perten (1964) referenced both Hagberg (1960, 1961) articles but used the 5-g sample size as originally recommended to compare the FN test with other methods to estimate degree of field-sprouting and α -amylase activity. Perten (1964) expressed the curvilinear relationship between flour FN and α -amylase activity by a more nearly straight line function, $r = 0.975$ ($r^2 = 0.951$), after converting the FN into a "liquefaction number" by dividing 6,000 by FN – 50 sec. Since the FN test was introduced, others have recommended sample sizes of <7 g (Greenaway and Neustadt 1967; Medcalf et al 1968; Meredith 1970). In a study involving 14 collaborators, the AACC Falling Number Subcommittee concluded that FN results from 6-g and 7-g tests were essentially the same when the FN values from the 6-g test were multiplied by 7/6 (Greenaway 1969). Finney (1985) reported an FN method that accurately quantified α -amylase activity that essentially eliminated the genetically controlled factors, other than α -amylase, that affect FN values. Tara and Bains (1976) believed that precise and conclusive information was lacking on 1) the relation between sample weight FN values, and 2) how well the FN test predicts α -amylase activity. Based on studies of 126 wheat cultivars grown in India, they reported that the FN method became less sensitive as the sample weight for the test was reduced to <7 g.

Today, grain handlers from producers to millers segregate grain according to critical quality parameters, one of which is degree of field-sprouting as expressed by α -amylase activity. There are a number of ways to accurately estimate α -amylase activity, including the FN test. However, there continues to be a need to reduce test time without materially reducing precision and accuracy, particularly

at the point of initial sale of grain when trucks are backed up at country grain elevators or at mills. In a survey of the industry, we documented that the FN test is used by most U.S. elevators and mills during grain receiving. Most of those surveyed indicated that an average reduction of FN testing time of 1 to 2 min would be important to elevators and millers, as well as to grain producers with a limited truck fleet, who haul grain to the elevator or mill as it is harvested.

This study was designed in response to the wheat industry's desire to minimize FN test time and because of the discrepancy in the literature documenting the effects of FN sample size on test precision and predictability of field-sprouting. The main purpose of this research was to thoroughly study the effects of FN sample size on FN test time, on the precision of the FN test results, and on how accurately the FN values correlate with α -amylase activity.

MATERIALS AND METHODS

Samples

Twenty-five wheat cultivars were sown in a randomized block split-plot design with two nitrogen levels and two replicates at Lexington and Princeton, KY, in Fall of 1994. The 25 cultivars included 85C-031-06, 90W, Caldwell, Cardinal, Clark, Clemens, Coker-9474, Coker-9543, Coker-9803, Elkhart, FFR-525, FFR-555W, Glory, Jackson, Madison, Patriot, Patterson, Pioneer-2510, Pioneer-2552, Pioneer-2580, Pioneer-2628, Pioneer-2643, Pioneer-2684, Verne, and Wakefield.

TABLE I
Effect of Production Location on Test Weight, α -Amylase, and Milling Properties

Location	Test Weight (lb/bu)	SE (%) ^a	Flour Yield (%)	α -Amylase (DU/g)
Lexington, KY	63.2	55.7	70.2	0.098
Princeton, KY	59.9	56.5	68.8	0.145

^a Softness equivalence.

TABLE II
Effect of Sample Weight on Falling Number Values, Pooled Standard Deviation (SD), and Coefficient of Variation (CV)

Sample Wt (g)	Falling Number (sec)				Pooled SD	CV (%)
	High	Low	Range	Mean		
4.0	147	63	84	87	12.7	14.47
4.5	250	123	127	195	8.3	4.27
5.0	300	186	114	249	4.3	1.72
5.5	356	202	154	291	5.6	1.93
6.0	395	203	192	324	6.9	2.12
7.0	644	260	384	495	28.5	5.75

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Milling

After removal of extraneous matter and highly shriveled kernels by aspiration, 2-kg sample lots were tempered to 15% moisture, stored overnight (22–23°C), then milled on a revised Allis-Chalmers laboratory mill (Yamazaki and Andrews 1982). Milling quality traits were expressed as percentages of straight-grade flour and break flour. The flours were kept in airtight containers at ≈3°C and tested after equilibration at room temperature.

Additional Analyses

Wheat test weight, flour protein ($N \times 5.7$), and flour FN values were determined according to Approved Methods 55-10, 46-12, and 56-81B, respectively (AACC 2000). Moisture was determined according to Approved Method 44-15A, except that the samples were dried at 140°C for 30 min. α -Amylase activity was determined according to Approved Method 22-06. Minimally, duplicates were completed on all analyses.

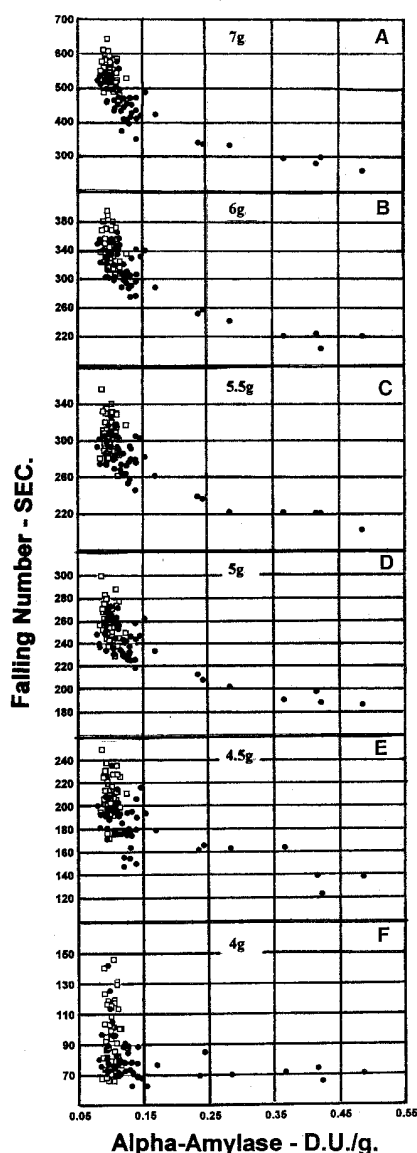


Fig. 1. α -Amylase activity (dextrinizing units [DU]/g, 25°C) vs. falling number (FN, sec) for 25 eastern U.S. commercial wheat cultivars grown in replicate at two locations, Lexington (\square) and Princeton (\bullet), KY, with two levels of nitrogen application ($25 \times 2 \times 2 \times 2 = 200$ samples). **A–D**, FN determinations completed in duplicate using six sample weights (7, 6, 5.5, 5, 4.5, and 4 g, respectively).

Statistical Analyses

Statistical analyses of the data were accomplished using the descriptive statistics and correlations of QuatroPro or Statistica (Novell). For significance of differences within cultivars and mean values of the cultivars, analysis of variance (ANOVA) and least significant differences (LSD, $P = 0.01$) were computed.

RESULTS AND DISCUSSION

Grain Condition

Two hundred 1994-95 Kentucky-grown wheat samples were recognized as valuable for this study because half were sound and most of the other half were variably field-sprouted. That is, the Lexington samples were grown essentially without rain between physiological maturity and harvest, while the Princeton samples received a few days of intermittent rain and sunshine during the month before harvest. The rainy periods at Princeton reduced average test weight, increased average grain softness equivalence (SE), and increased average α -amylase activity compared with the Lexington samples (Table I).

α -Amylase Activity

The rainy conditions at Princeton also increased average α -amylase, which reduced average FN values for all sample weights (Fig. 1). All 100 Lexington samples were sound, not field-sprouted, and without elevated α -amylase activities.

Most of the Princeton samples varied from zero to moderately field-sprouted, with materially elevated α -amylase activity in 14 of the 100 flour samples (Fig. 1). Those 14 flour samples with materially elevated α -amylase activities included both nitrogen treatments and both field replicates for the three cultivars (Pioneer 2684, Cardinal, and Glory) and both field replicates of the high nitrogen treatment for Coker 9474. Those four wheat cultivars with elevated α -amylase activities have been previously documented (by this laboratory) to be inherently more prone to field-sprouting than most popular soft red winter wheat cultivars grown in the eastern half of the United States (data not shown). α -Amylase activities of 70 of the remaining 86 samples grown in Princeton varied from 0.01 to 0.058 DU/g (dextrinizing units per gram) greater than corresponding samples grown in Lexington. The α -amylase activity of the remaining 16 samples grown in Princeton were within experimental error of the corresponding samples grown in Lexington and included both replicates of both nitrogen treatments of the four cultivars 85C-031-06, Caldwell, Elkhart, and Wakefield ($4 \times 2 \times 2 = 16$ samples). Thus, based on this study, those four cultivars likely have some inherent degree of resistance to field-sprouting. The differences in nitrogen application did not significantly affect α -amylase activities of samples grown at either location.

FN Sample Size vs. α -Amylase

As discussed above, 14 of the 100 wheat samples grown in Princeton were definitely field-sprouted based on α -amylase activities of 0.200 to 0.486 DU/g, 25°C (Fig. 1). When 4 g of flour was assayed, the FN values of more than half of the remaining 86 other samples from Princeton fell into the same FN range (61–78 sec) as those 14 samples (Fig. 1F). In fact, FN values of 10 of the 100 samples from Lexington also fell into that range. Thus, the 4-g FN test did not separate the samples with elevated α -amylase from those with zero (or near zero) activity. Compared with the 4-g sample size, the use of the 4.5-g sample size better separated elevated from normal α -amylase. However, with the 4.5-g sample size, the FN values of 16 samples from Princeton improperly fell into the field-sprouted FN range (112–170 sec) as defined by the 14 Princeton samples with elevated α -amylase activity (Fig. 1E). With the 4.5-g FN sample size, the FN value of only one sample from Lexington improperly fell within that range, defined by elevated α -amylase activity. Thus, although the 4.5-g FN sample size improved α -amylase differentiation (Fig. 1) and improved the test

coefficient of variation (Table II) compared with the 4-g sample, its poor correlation with α -amylase clearly illustrates that neither the 4-g nor 4.5-g FN test could be of practical use to quantitatively segregate field-sprouted samples.

When FN sample sizes of 5, 5.5, 6, and 7 g were used, FN values of the 14 Princeton samples with elevated α -amylase activities were accurately segregated from 86, 84, 86, and 85 of the 86 remaining samples from Princeton, respectively, and were accurately segregated from all 100 of the Lexington samples (Fig. 1).

Effect of Sample Size on FN Values

The effects of flour sample weights of 7, 6, 5.5, 5, 4.5, and 4 g on FN values were compared. The coefficients of variation (CV) when using FN sample sizes of 7, 6, 5.5, 5, 4.5, and 4 g were 5.75, 2.12, 1.93, 1.72, 4.27, and 14.47%, respectively (Table II). Based on CV, a 5-g FN determination approaches the optimum.

FN Assay Time

A reduction of FN sample size from 7 to 5 g reduced mean Lexington and Princeton FN values of 545 and 445 to 260 and 238, respectively, an average reduction in assay time of more than 50%. The percent reduction in time would be somewhat less for those who routinely stop the 7-g FN test after 350 sec, the minimum FN believed by many to be sound wheat. In this study, a comparable cut-off point for sound wheat flour was \approx 215 sec when a 5-g sample was used. Thus, reducing the FN test weight from 7 to 5 g reduced the FN time from \approx 350 to 215 sec, an average reduction of 2 min and 15 sec for sound samples.

CONCLUSIONS

Two hundred samples consisting of 25 commercial wheat cultivars grown at two locations were used to study the effects of α -amylase activity when assayed using variable test sample sizes. α -Amylase activity confirmed weather data showing that essentially no rain fell at one of those locations (Lexington) between grain physiologically maturity and harvest. α -Amylase activity also confirmed that samples grown at the other location (Princeton) had received intermittent rain just before harvest because both replicates of seven samples from Princeton contained elevated α -amylase (all four field replicates of Pioneer 2684, Cardinal, and Glory, and the two field replicates of the high nitrogen treatment for Coker 9474). In addition to those 14 samples, the remaining 86 Princeton samples

varied from fair to poor overall condition when compared with the Lexington samples, indicated by moderate to intermediate grain puffing and concomitant elevated α -amylase activity, average lowered test weight/bushel, elevated grain softness, and reduced flour yield. In spite of those physical and biochemical changes caused by the rainy period near harvest, the α -amylase activity of both field replicates of both nitrogen treatments of four cultivars (85C-031-06, Caldwell, Elkhart, and Wakefield) grown in Princeton were not elevated. It is likely that those four cultivars possess some degree of resistance to field-sprouting.

The effects of sample size on FN precision (CV), reproducibility (LSD), and accuracy in predicting α -amylase activity were studied. As sample sizes were increased from 4, 4.5, 5, 5.5, and 6 to 7 g, a parabolic relationship among CV was found, with the minimum CV from a 5-g sample. The FN test with the greatest reproducibility (lowest LSD) was also produced using the 5-g sample size. By reducing FN sample size from 7 to 5 g, a FN value of 350 sec, which is considered essentially sound by many who routinely use the FN method, was reduced to 215 sec, shortening test time by 2 min and 15 sec.

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