A Note on Sample Size Error in the Falling Number Test[1]

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The Hagberg falling number test (1) has, for a number of years, enjoyed widespread favor as a rapid means of determining alpha-amylase activity in whole wheat. Both the American Association of Cereal Chemists and the International Association of Cereal Chemistry have adopted the procedure as one of their "Official Methods."

The AACC Official Method (2) specifies that sample size taken for grinding should be 100 g. with the Labconco or Kamas-Slago mill and a minimum of 25 g. with the Weber pulverizer or Udy cyclone mill.

This is in sharp contrast to the instructions given in the ICC Standard Method for the falling number test (3), which states: "Approximately 300 g. of the grain should be prepared for grinding. If less than 200 g. of grain is prepared misleading results may be obtained."

The object of this paper is first to present a theoretical basis for selecting a suitable sample size for grinding in the falling number test, and secondly to illustrate with experimental data the magnitude of error inherent in the determination for various sample sizes.

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THEORETICAL CONSIDERATIONS

In any consideration of wheat sample size to be taken for alpha-amylase activity determinations, it is essential to grasp three basic points:

1) For a given sample size containing a small percentage of sprouted kernels, the number of sprouted kernels actually sampled will always be a whole number (although the mean number per sample may not be). For example, if a large, well-mixed sample contains 0.5% sprouted kernels and all kernels weigh the same, a 1,000-kernel sample should, on average, contain 5 sprouted kernels, whereas a 10,000-kernel sample will contain an average of 50 sprouted kernels.

2) If a series of samples of a given size is taken at random, the number of samples containing 0, 1, 2, 3, 4, etc., sprouted kernels will be (statistically) in accordance with a Poisson distribution.

3) One feature of a Poisson distribution is that the standard deviation (standard error of a single determination) is equal to the square root of the mean number (of sprouted kernels). Therefore, in a series of 1,000-kernel samples with a mean of 5 sprouted kernels, the actual number of sprouted kernels would be expected to vary by $\pm\sqrt{5}$ and approximately 70 times out of 100 should fall within these limits. Since the number of sprouted kernels must be a whole number, most of the 1,000-kernel samples would be expected to contain from 2 to 8 sprouted kernels. Similarly, we would expect a series of 10,000-kernel samples to contain $50 \pm\sqrt{50}$ = 42 to 58 sprouted kernels; and samples of 100,000 kernels would contain $500 \pm\sqrt{500}$ = 477 to 523 sprouted kernels. Hence, for a series of 1,000-kernel samples, the standard error in terms of number of sprouted kernels sampled would be ±60% of the mean number; for 10,000-kernel samples the standard error would be ±16% of the mean number; and for 100,000-kernel samples the standard error would be ±4.6% of the mean number.

Because alpha-amylase activity of a sprouted kernel may be 100 times or more greater than that of a sound kernel (4), the effect of sampling error becomes readily apparent. If 25-g. samples (approximately 1,000 kernels for HRS wheat) are taken for grinding, then, as explained above, one would expect to sample from 2 to 8 sprouted kernels if the mean number is 5. If the alpha-amylase activity of the sound kernels = 1 arbitrary unit per weight unit, and the activity of the sprouted kernels = 100 units, then at a level of 0.5% sprouted kernels the mean activity of the sample will be 1.495 units. However, if only 2 kernels are sampled the activity would be 1.198 units, whereas if 8 kernels are sampled the activity would be 1.792 units. In this situation, therefore, taking a sample size of 25 g. immediately introduces a standard error of ±20% of the mean alpha-amylase activity.

The actual error expected will vary with sample size, with percentage of sprouted kernels, and with the actual degree of difference in activity between sprouted and sound kernels. In practice there is more likely to be a range of activity found among kernels, particularly among kernels of varying stages of sprout development. Figure 1 illustrates the hypothetical situation where kernels are considered to be either sprouted (with 100 units of alpha-amylase activity) or sound (with 1 unit of alpha-amylase activity). The standard error (theoretical, based on plus or minus the square root of the mean number of sprouted kernels in a given sample size) decreases with increasing sample size and with increasing percentage of sprouted kernels, with a maximum error at 0.5 to 1% sprouted kernels. Based on the actual alpha-amylase activity value, to obtain a standard error less than ±10%, a 65-g.

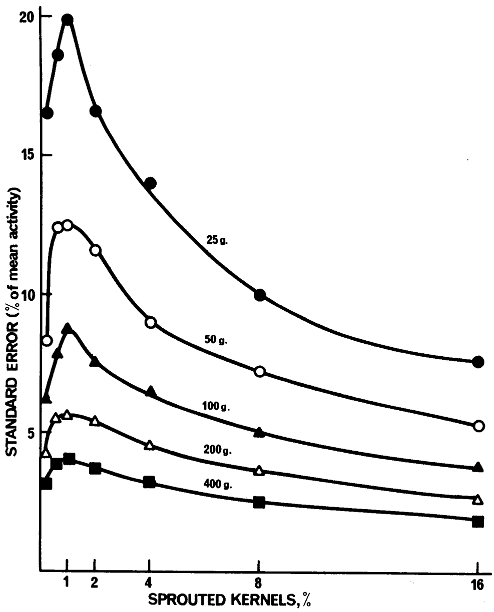

Fig. 1. Theoretical errors (based on mean alpha-amylase activity) due to sample size in hypothetical mixtures of sound and sprouted kernels.

sample would be required in this hypothetical case, whereas a 250-g. sample size would be required to reduce the error to ±5%. Obviously, if there were no sprouted kernels and the sample was composed entirely of sound kernels of uniform activity, sample size would introduce no error into the determination.

EXPERIMENTAL

To demonstrate the effect of sample size in the falling number test, two thoroughly mixed average-grade samples of Canadian HRS wheat were tested. The first was a No. 3 Manitoba Northern which contained a low percentage (0.12%) of sprouted kernels. The second was a No. 2 Manitoba Northern with no sprouted kernels.

Using the Udy cyclone mill, the No. 3 Northern was used to prepare eight individual 25-g. grinds, eight 50-g. grinds, four 150-g. grinds, and four 250-g. grinds. Each ground sample was thoroughly mixed and the falling number test was carried out on duplicate 7-g. portions of each grind. Results are shown in Table I. Mean falling number values ranged from 245 to 410 sec. for the 25-g. samples; from 255 to 388 sec. for the 50-g. samples; from 305 to 350 sec. for the 150-g. samples; and from 292 to 313 sec. for the 250-g. samples. For each set of grind-size results, the "error of sample size" and the "error of a single determination" were calculated. These values are shown in Table II. There was a dramatic increase in error – from ±14 to ±79 sec. – as sample size taken for grinding was reduced from 250 to 25 g. Within grinds the error of a single determination was reasonably constant. In most

TABLE I. FALLING NUMBER RESULTS FOR A SAMPLE OF NO. 3 MANITOBA NORTHERN WHEAT CONTAINING 0.12% SPROUTED KERNELS

Grind Sample Size	Falling Number		
	1	2	Mean
g.	sec.	sec.	sec.
25	245	246	245
25	412	408	410
25	302	283	290
25	293	296	295
25	334	338	336
25	353	368	360
25	383	398	390
25	296	300	298
50	270	273	270
50	336	351	345
50	308	312	310
50	253	257	255
50	334	349	342
50	381	396	388
50	323	315	319
50	361	376	368
150	353	349	350
150	328	342	335
150	305	313	310
150	306	307	305
250	288	297	292
250	306	321	313
250	310	309	310
250	312	314	313

TABLE II. ERRORS CALCULATED FROM DATA GIVEN IN TABLE I

Sample Size g.	Error of Sample Size sec.	Error of a Single Determination sec.
25	±79	±7.4
50	±64.5	±7.9
150	±30.3	±5.9
250	±14.0	±6.2

laboratory tests it is generally acceptable when the error due to sampling is around twice the error of a single determination.

For the sound No. 2 Manitoba Northern sample, ten 200-g. grinds and ten 25-g. grinds were prepared with the Udy cyclone mill. Results of duplicate falling number determinations showed sampling errors of ±10.6 and ±10.3 sec., respectively, for the 200- and 25-g. series. Error of a single determination was ±7.2 sec. for the 200-g. series and ±8.7 sec. for the 25-g. series. Thus, for the sound sample, containing no sprouted kernels (mean falling number 411 sec.), there was no difference between taking 200 g. for grinding and 25 g., in sharp contrast to falling number tests carried out on the sample with 0.12% sprouted kernels.

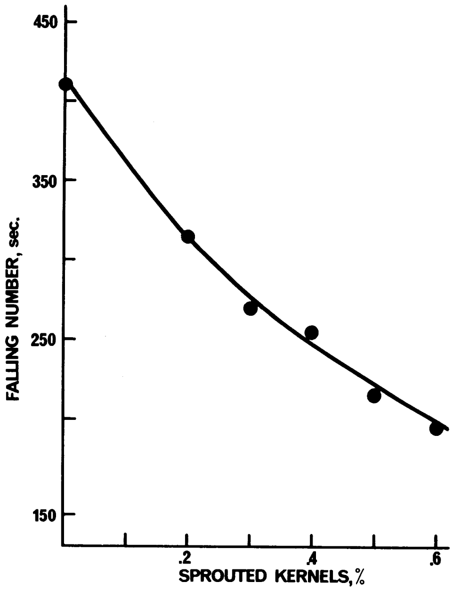

Fig. 2. Effect on wheat falling number of addition of field-sprouted kernels to sound No. 2 Manitoba Northern wheat.

To further illustrate the effect of sprouted kernels on falling number determinations, additions of field-sprouted kernels (alpha-amylase activity = 133 VR units (4) per g.) were made to the sound No. 2 Manitoba Northern wheat (alpha-amylase activity = 0.83 VR units per g.) to give levels of 0.2, 0.3, 0.4, 0.5, and 0.6%. A 200-g. mixture of wheat kernels was made in each case and the entire sample was ground. Duplicate falling number determinations were made on the well-mixed meals. In terms of actual numbers of kernels, the 0.4% addition corresponded to 29 sprouted kernels in 200 g. Figure 2 shows how falling number decreased with increasing addition of sprouted kernels. From these data, based on the theoretical standard error involved, sample size errors were calculated as follows:

Sample Size g.	*Standard Error* sec.
25	±62
50	±43
150	±24
250	±19
800	±10

These figures are of the same order as those found experimentally and shown in Table II, and confirm the importance of taking a large sample (at least 250 g.) for grinding for wheat samples containing small proportions of sprouted wheat. This philosophy, of course, is applicable not only to the falling number test but to any measure of alpha-amylase activity such as the amylograph test. However, the actual size of sample taken for grinding by a laboratory will also be determined by a number of other factors, including size of sample available (this may be a significant factor for the plant breeder); a general knowledge of the type of material being tested (i.e., whether or not the alpha-amylase activity is fairly uniform for all kernels in the sample); convenience (it takes longer to grind a larger sample); and the magnitude of sample size error acceptable. Nevertheless, it seems obvious that the sample size recommended for grinding in the AACC method is too small in the area of greatest interest (i.e., where the sample is neither completely sound nor heavily sprouted). Therefore, it is recommended that sample size be increased to a minimum of 250 g. Increasing the number of replicates of a smaller sample grind size would also effectively reduce the error, but would involve considerably more work.

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