Collaborative Evaluation of a Rapid Nephelometric Method for the Measurement of Alpha-Amylase in Flour

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ABSTRACT

An interlaboratory investigation of the Perkin-Elmer model 191 grain amylase analyzer was conducted in the United Kingdom. The instrument was calibrated against the Farrand method for bread flours on the amylase-2 scale only; the conversion factor was 17 amylase-2 units to 1 Farrand unit. The calibration was stable with time and between three laboratories. The reproducibility was excellent, with a standard deviation of 0.035P at P amylase-2 units. An α-amylase level could be obtained in 10 min compared to 2–5 hr for the Farrand method.

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The measurement of α-amylase activity is an important aspect of the quality control of bread flours because it permits activity to be adjusted to the desired level, either by blending flours or by supplementation with malt flour or a fungal α-amylase preparation. In the United Kingdom, fungal amylase preparations are widely used for the diastatic supplementation of bread flours, and thus a measurement method is required that is sensitive to both fungal and cereal α-amylases. Many procedures such as the Hagberg falling number (ICC 1968) or the amylograph (AACC 1980) involve the measurement of the viscosity of wheat or flour suspensions at elevated temperatures. These methods cannot be used to measure fungal amylase because of its low thermal stability. This objection also applies to the AACC cibacron-blue method. Although the amylograph has been modified (Ranum et al 1978) so that fungal amylase can be measured, this technique still suffers from the drawback that the result is influenced by many factors other than α-amylase activity.

The established U.K. method, which is a direct measurement of both cereal and fungal α-amylase, measures the amount of a specially prepared β-limit dextrin substrate consumed in a specified period of time at 37°C. The measurement principle is the colorimetric determination of the remaining substrate with iodine (ICC 1968). Unfortunately, this method is time-consuming and operator-sensitive.

However, a recently developed instrumental technique has been used for the measurement of cereal and fungal amylase (Campbell 1980a). This instrument measures the rate of change of light scattered from the substrate suspension as the reaction follows zero-order kinetics during an automatically timed cycle. The procedure is known as nephelometry and the rate of decrease in scattered light (nephelos) is a measure of the amount of α-amylase present. This method is much more rapid than the Farrand and thus may be useful for flour quality control.

The nephelometer has been evaluated for its suitability in determining α-amylase levels in sprouted soft white wheat (O'Connell et al 1980) and in wheats and flours containing low to moderate levels of the enzyme (Kruger and Tipples 1981). Linear relationships were found between increase in concentration of enzyme and rate of decrease in nephelos per minute, measured either with a recorder or with automatically timed cycles (the latter up to 720 machine units of activity). Calibrations were obtained for Hagberg falling numbers, amylograph viscosities, and liquefaction numbers.

The purpose of the present work was to study, by interlaboratory evaluation, the possible application of a commercial nephelometer to the measurement (in Farrand units) of α-amylase in U.K. bread flours. We wished to establish a stable calibration between the nephelometer and the Farrand method and to compare the within-laboratory and between-laboratory precision of the two methods.

MATERIALS AND METHODS

The Instrument

The model 191 grain amylase analyzer (Perkin-Elmer Corp., Oak Brook, IL) was evaluated in this study. A detailed description of the instrument, substrate, and calibrator is given by Campbell (1980b).

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Samples

To test the reproducibility of the nephelometric method and its relationship to the Farrand method, two sets of samples were prepared. The samples were devised so as to include blended flours and flours supplemented with fungal amylase to the range of Farrand values that may be encountered for U.K. bread flours.

Series A. English flour and Canadian Western red spring (CWRS) flour were blended to give a range of flours with Farrand α-amylase activities of approximately 3–25 units. The five flours consisted of CWRS, English, and three blends of CWRS/English (75:25, 50:50, 25:75).

Series B. To a commercial flour with a naturally low level of cereal α-amylase activity, four levels of fungal amylase were added to give four flours with total activities similar to those of the samples in series A that contained English flour.

Ring Test

The two series of samples were analyzed on different instruments by three laboratories using duplicate extraction and duplicate readings on each extraction on four separate occasions during a one-month period.

Methods

α-Amylase activities were determined on the nine samples using the Farrand method (Farrand 1964). The calibration and analysis procedure for the nephelometer was followed exactly as described by Campbell (1980b) and in the handbook for the Perkin-Elmer grain amylase analyzer (Anonymous 1979).

RESULTS AND DISCUSSION

Farrand Method

The Farrand values (Table I) for each flour were based on a number of replicates (five on the average) performed by different operators at different times at one laboratory. From the replicates, estimation of the within-laboratory variability of the Farrand method was possible. The variance of replicates appeared to be proportional to the Farrand value (F), giving a standard deviation of approximately $0.6\,(F)^{1/2}$, eg, 1.3 at 5 Farrand units and 2.7 at 20 Farrand units. Reexamination of historical data (1964–1979) of interlaboratory testing of the Farrand method confirmed that this relationship is valid also for between-laboratory variation. The data is based on 11 trials involving between five and 11 U.K. laboratories. This result is not surprising because the Farrand method is operator-sensitive and therefore the variation between different operators in one laboratory would probably be much the same as that between different operators in separate laboratories.

TABLE I
Farrand Values of Samples

Sample			Amylase Activity
Number[a]	Series	Containing[b]	(Farrand units)
1	A	CWRS/E, 25:75	13.5
2	A	CWRS/E, 75:25	6.0
3	B	Fungal amylase	8.4
4	B	Fungal amylase	23.0
5	A	E	17.4
6	A	CWRS	2.8
7	B	Fungal amylase	18.1
8	B	Fungal amylase	13.8
9	A	CWRS/E, 50:50	10.8

[a]Numbered in random order.
[b]CWRS = Canadian Western red spring flour, E = English flour.

Nephelometric Method

With the Farrand variability for comparison, the same nine samples were subjected to nephelometric assay using the standardized procedure (Campbell 1980b).

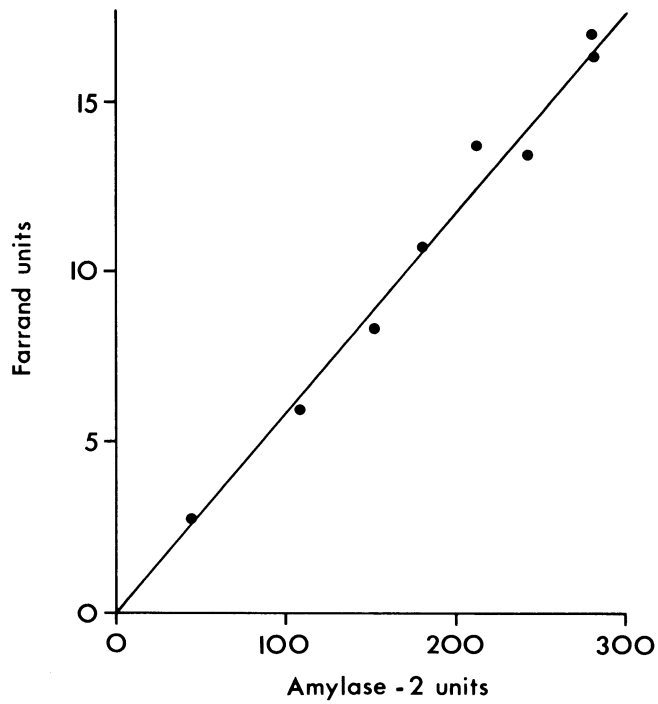

Fig. 1. Plot of Farrand α-amylase values against readings on the amylase-2 scale of the grain amylase analyzer for eight flour samples.

TABLE II
Interlaboratory Evaluation[a] of Model 191 Grain Amylase Analyzer

	Instrument 1				Instrument 2				Instrument 3			
	First Extraction		Second Extraction		First Extraction		Second Extraction		First Extraction		Second Extraction	
Samples	1	2	1	2	1	2	1	2	1	2	1	2
Read on cycle 2												
1	225	250	242	238	202	208	213	204	258	268	282	293
2	90	95	110	121	87	90	96	98	112	124	102	102
3	142	154	137	149	139	146	115	111	228	203	155	160
4[b]	127	125	128	117	...	...	...	...	...	...	...	...
5	278	275	295	297	284	291	272	282	...	...	...	...
6	41	38	36	35	30	37	42	47	...	...	...	...
7	275	279	>300	290	...	...	...	...	...	...	...	...
8	212	198	212	212	165	174	197	215	250	262	267	283
9	150	185	165	150	152	162	159	158	224	220	189	178
Read on cycle 1												
4	...	...	...	...	441	460	515	530	463	433	449	400
5	...	...	...	...	...	...	...	...	442	430	477	458
7	...	...	...	...	349	358	332	350	391	427	440	474

[a]In units read off the cycle 2 scale (amylase-2 units) or the cycle 1 scale (amylase-1 units).
[b]Extract used was 50 μl instead of 200 μl.

Six of the samples were read on the amylase-2 cycle using 200 μl of sample in all cases. The other three samples (Table I, samples 4, 5, and 7) gave readings close to or off the top of the scale when read in this way and consequently usually received different treatment, either the use of the amylase-1 cycle or reduction of injected sample to 50 μl, as recommended in the instrument handbook. The results are based on the six samples read 288 times in total. A typical set of results is shown in Table II.

The variability between replicate readings was similar for each of the three instruments, with an average replicate standard deviation for the six samples of 6.9 units on the amylase-2 scale. This standard deviation showed some tendency to increase with higher readings and decrease with lower ones. The variability between extractions was also similar for each of the laboratories. The average standard deviation between extractions (each extraction being the mean of two replicates) was 11.7 units on the amylase-2 scale, again with a tendency for variability to be greater for higher readings.

Analyses of the mean results for each determination (two replicates of two extractions) suggests that the standard deviation is proportional to the mean, with a value at P amylase-2 units of 0.024P or roughly 2.5% of the mean for either amylase-2 or Farrand units. If a single extraction were used, still with duplicate readings, the 2.5% would increase to 3.5%, which is still better than the Farrand method in the 1–20 Farrand unit range.

No evidence was found of trends with time or of excess variability between days over and above that expected from the variability between extractions. However, evidence (significant at the 1% level) was found that instrument 2 consistently gave results 10% lower than the other two. A further investigation of this problem was made.

The relationship between the Farrand scale and the grain amylase analyzer on the amylase-2 scale appears to be linear, as shown in Fig. 1, in which the average amylase-2 results have been plotted against Farrand units for the six samples. In addition, the highest two points on the graph are for samples 5 and 7, which were read on some occasions using the standard procedure even though this produced results very close to the top of the scale. The line corresponds to a conversion factor of 17 amylase-2 units to 1 Farrand unit. The deviations from the line are consistent with the variability described above and are probably mainly from errors in the Farrand measurements, for which far fewer replicates were available.

To confirm the 10% bias originally observed in instrument 2, three of the flour samples were analyzed repeatedly, using instruments 1 and 2 side by side in one laboratory (Table III). A three-way analysis of variance of the logarithms of the results showed no significant bias. To check for possible discrepancies caused by differences in the three calibrators supplied with each instrument, each of the three instruments was calibrated with its own calibrator and the other two were read (Table IV). The 10% bias originally observed in instrument 2 cannot be explained by variations in the calibrator solution as used at the three sites in the ring test. If this were the case, calibrator values for instrument 2 would be 10% lower than those of the other instruments. The pattern observed was not consistent with this but was consistent with random variation. Because the bias observed in the ring test was not observed when the instruments were studied side by side in one laboratory, it was probably either from differences between the syringes used or from operator variation.

Because the three flours that gave readings at the top of the amylase-2 scale were treated in different ways (Table II), data on which to base conclusions are insufficient. However, if results for which a 50-μl aliquot was used are multiplied by four, the resulting figures are too high (by about 30%). A similar discrepancy appears between the amylase-1 and amylase-2 scales that were calibrated using 200 and 50 μl of calibrator solution, respectively. This is clearly a problem that needs to be resolved, because the amylase-1 and amylase-2 scales have been reported to be continuous (O'Connell et al 1980), but it does not prevent the use of the technique.

The grain amylase analyzer has been in use for over a year with successful results. Periodic checks against the Farrand method have shown excellent agreement. High-activity flours have been successfully measured by dilution of the extract with extracting solution and by performing the assay with a 200-μl aliquot on the amylase-2 scale.

TABLE III
Repeated Analysis of Three Flour Samples to Check for Bias Between Two Instruments

	Samples on Instrument 1			Samples on Instrument 2		
Day	1	6	7	1	6	7
1	200	256	27	195	244	27
	207	247	41	202	251	33
2	189	241	42	218	254	37
	197	228	33	210	269	30
3	219	269	26	187	254	18
	224	286	26	198	254	29
4	186	241	31	189	242	38
	180	243	21	186	242	44
5	190	215	36	209	239	56
	170	229	61	231	278	66
Mean	196	246	34	202	253	38

TABLE IV
Comparison of Readings[a] from Three Calibrators on Three Instruments

		Calibrator		
Instrument	Replication	1	2	3
1	1	150	150	140
	2	180	165	151
	3	115	123	140
	Mean	148	146	144
2	1	138	154	175
	2	155	130	145
	3	158	140	135
	Mean	150	141	152
3	1	108	150	177
	2	141	159	151
	3	139	126	150
	Mean	129	145	159

[a] Values are in nephelos.

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