EVALUATION OF TWO INFRARED INSTRUMENTS FOR DETERMINING PROTEIN CONTENT OF HARD RED WINTER WHEAT

C. A. WATSON[1], D. CARVILLE[2], E. DIKEMAN[2], G. DAIGGER[1], and G. D. BOOTH[3]

Cereal Chemistry 53(2): 214-222

ABSTRACT

Two Grain Quality Analyzers (GQA), Model 1, and one Grain Analysis Computer (GAC), Model GAC-2, were evaluated for determination of protein content in hard red winter wheat. The instruments are based on infrared spectrophotometry and each has interference filters, photosensors, and a computer for analyzing the data. Method of grinding samples affected the results. The grinders sold with the instruments were unsatisfactory, so the Udy Cyclone Mill or the Weber Pulverizer was used. Moisture affected the results only slightly. Variance between duplicate readings on the same sample was small. Correlation coefficients between protein content determined on the instruments and by the Kjeldahl method were highly significant (0.98 to 0.99). The GQA had some electronic problems but was easier to operate and calibrate than the GAC.

In 1971 two electronic instruments were introduced to the grain trade for measuring the protein, oil, and moisture contents of grain and oilseeds. Both instruments use the principle of near-infrared spectrophotometry developed by Karl Norris of the Instrumentation Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture. The principle has been used to determine moisture content of grains and to determine reflectance and transmittance properties of grains (1,2,3). For near-infrared reflectance, the principle uses the difference in reflectance at two wavelengths (maxima and adjacent minima) of the material being analyzed. Protein, oil, and moisture exhibit different peaks of major absorbance in the near-infrared spectrum; however, each of these substances also absorbs appreciably at other wavelengths. By making reflectance absorbance measurements at all three maxima and at the adjacent minima on samples having a range of all three components similar to that in the unknowns, it is possible to solve equations using multiple linear regression analyses for constants, relating the absorbances to the content of protein, water, or oil. These constants are used in calibrating the instruments for the various constituents. The measuring system consists of interference filters to isolate selected wavebands of infrared energy, a photosensor and associated signal conditioning amplifier, and a computer for analyzing the data. We report here an evaluation of two commercial instruments and a brief description of problems and errors associated with the instruments and methods for analysis of protein in hard red winter wheat.

[1]Research Chemist and Agricultural Engineering Technician, North Central Region, Agricultural Research Service, USDA, U.S. Grain Marketing Research Center, 1515 College Avenue, Manhattan, KS 66502. Dr. Watson's present address is: Hard Red Spring and Durum Wheat Laboratory, ARS, USDA, North Dakota State University, Fargo, ND 58102.

[2]Research Assistants, Grain and Feed Science Department, Kansas State University, Manhattan, KS 66506.

[3]Mathematical Statistician, Agricultural Research Service, USDA, Iowa State University, Ames, IA 50010.

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MATERIALS AND METHODS

Instrumental Methods

The instruments tested were the Dickey-john Grain Analysis Computer, Model GAC-2 (GAC) (now named the "Infra Alyzer") and two of Neotec Corporation's Grain Quality Analyzers, Model 1 (GQA). Both instruments were developed for rapid measurement of oil, protein, and moisture in grain and measure the difference in reflectance from the surface of the sample of near-infrared energy at six selected wavelengths. The main difference between the two instruments is that the GAC has six interference filters to isolate precise wavelengths, whereas the GQA has three filters mounted in a rotating "paddle wheel." Each of the three filters passes a variable wavelength depending on the angle between the filter and the light source. An encoder attached to the "paddle wheel" permits selection of the six wavelengths to be used in the analysis from 30 precise wavelengths, 10 from each of the three filters. With both instruments, the reflected light is detected and the data produced are introduced into a built-in computer which reads out percentage of oil, protein, or moisture.

The wavelengths (nm) used by the GAC and the GQA are compared below:

GAC	*GQA*
1680	1867
1940	1920
2100	2118
2180	2167
2230	2250
2310	2297

The computation processes for the instruments differ slightly. The absorbances (A) (referred to as Log_1, Log_2, etc.) for the six wavelengths of the GAC are combined with a set of K_i values (constants) which are determined by a set of three equations as follows:

$$\% \text{ moisture} = K_{1m}L_1 + K_{2m}L_2 + K_{3m}L_3 + K_{4m}L_4 + K_{5m}L_5 + K_{6m}L_6 + K_{7m}$$

$$\% \text{ oil} = K_{1o}L_1 + K_{2o}L_2 + K_{3o}L_3 + K_{4o}L_4\ K_{5o}L_5 + K_{6o}L_6 + K_{7o}$$

$$\% \text{ protein} = K_{1p}L_1 + K_{2p}L_2 + K_{3p}L_3 + K_{4p}L_4 + K_{5p}L_5 + K_{6p}L_6 + K_{7p}$$

where Li = Log i.

The absorbances measured by the GQA at six wavelengths are combined to give three values of absorbance difference (ΔA) as follows:

$$\Delta A_m = A_{1920} - A_{1867}$$

$$\Delta A_o = A_{2297} - A_{2250}$$

$$\Delta A_p = A_{2167} - A_{2118}$$

These ΔA's, which are labeled on the GQA as "C" values, are then combined into equations to give the percentage of each constituent as follows:

$$\% \text{ moisture} = K_o + K_1 (\Delta A_m) + K_2 (\Delta A_o) + K_3 (\Delta A_p)$$

$$\% \text{ oil} = K'_o + K'_1 (\Delta A_m) + K'_2 (\Delta A_o) + K'_3 (\Delta A_p)$$

$$\% \text{ protein} = K''_o + K''_1 (\Delta A_m) + K''_2 (\Delta A_o) + K''_3 (\Delta A_p)$$

The K_i values determined from the above equation are set in the instruments by 10k potentiometers, seven for each constituent in the GAC and four for each constituent in the GQA. The "K" values are constants characteristic of the component being measured in the sample.

For determination of constituents, the instruments must be calibrated against other analytical laboratory results, *i.e.*, Kjeldahl protein content for protein. Calibration is based on analyses of about 50 samples covering the range of content of the constituents expected in the samples to be analyzed. Constituents (*i.e.*, moisture and lipid) affecting the constituent being analyzed (protein) must exhibit a range of that expected in the unknown; otherwise, constants for multiplying certain absorbances may be weighted. The samples are ground and analyzed to give chemical data with which the instrumental values may be compared. For each sample, absorbance values are read from the instruments at the wavelengths set by the filters. These values—six for the GAC and three for the GQA—along with the data from chemical analyses, are used to run a multiple linear regression analysis to obtain the proper constant values. These "K" values for the GAC are entered into the instrument by adding precision resistors to the grain board and setting the "K" value with potentiometers. (The "K" values for the InfraAlyzer are dialed in, using variable potentiometers.) The "K" constants for the GQA are entered by adjusting existing potentiometers to the correct "K" value. The adjustment is studied to determine whether "K" values times the respective "log" value yield the correct percentage within reasonable error. If not, the "K_7-value" is adjusted to correct to the desired percentage reading. The calibration is then ready to be checked with about 50 samples to determine the standard error of the method for the material being studied. When a different product is to be measured, the electronic circuit board for the calibration may be recalibrated or replaced with a new board to allow the reuse of each board.

In this study, the instruments were calibrated according to the manufacturers' instructions as outlined above for protein only, using Kjeldahl protein determined according to AACC Method 46-11 (4). Moisture of the sample was determined by AACC Method 44-15 (4). Protein contents of the 50 samples used for calibration ranged from 8.0 to 16.6% and averaged 12.3%. Moisture contents of the samples ranged from 8.9 to 13.1% and averaged 10.8%.

To determine the effect of grinders on particle size, samples from five classes of wheat were ground on the Udy Cyclone Mill (0.040 in. screen), the Udy-modified Weber Pulverizer (0.024 in. screen), and the Krups coffee mill. Also, five different Krups mills were used to grind a portion of the same sample of each class of wheat to determine the variation in amounts of a given particle size among the mills. Grinding time was 1 min. Ranges in particle size were determined by Standard Tyler sieves with openings of 210, 150, 125, and 105 μ. Two Carmichael cleaners were put in each sieve to assist in the separation. The samples were shaken on a Fisher Wheeler Sieve Shaker for 30 min and the percentage of material remaining on each sieve was determined. Proteins were read on the

GAC by using the hard red winter wheat calibration board for all classes of wheat to give a relative indication of particle-size effect on the instrument readings. The board had been calibrated by using samples ground on the Weber Pulverizer.

Samples and Experimental Design

A total of 88 samples of HRW wheat grown in Kansas in 1973 were analyzed. A 200-g portion of each sample was divided into 50-g portions. Two 50-g portions were tempered to about 10.0% moisture and two to about 14.5% moisture. Each 50-g portion was ground separately on the Weber Pulverizer and they were identified as grind 1, 2, 3, and 4. Approximately 1 and 2.5% moisture were lost during grinding from the low- and high-moisture samples, respectively. Duplicate determinations were made on each portion by the Kjeldahl and the three infrared instruments. Protein content of the samples ranged from 8.1 to 15.1% and averaged 12.0%.

RESULTS AND DISCUSSION

Data for the sieve analysis of wheat ground in the three mills are shown in Table I. Differences in fineness of grind were smaller between the Udy Cyclone and Weber Pulverizer than between these mills and the Krups. The Krups gave a much coarser particle size than the other two mills. Differences in particle size between classes of wheat were large regardless of the mill used. Previous use had shown that the bearings in the Udy Cyclone would not tolerate extended grinding of whole kernel corn. Because we wanted to use the one mill throughout our studies on all grains, we selected the Weber Pulverizer. We think that the Udy Cyclone would be satisfactory for grinding wheat, barley, oats, and other small grains and could be used for pre-cracked corn. A finer grind was possible with the smaller screen (0.020 in.), but the mill tended to clog when grinding soft wheats at the same feed rate as the hard wheats. In this study, particle size had a small effect on protein readings from the GAC. The protein readings tended to be slightly higher on samples ground in the Krups mill than on those ground in the other two mills.

Percentages of particles of a given size ($<150\ \mu$) and GAC protein readings for samples ground on five different Krups mills are shown in Table II. Percentages of $<150\ \mu$ particles varied considerably from mill to mill. Therefore, we decided not to use either the Krups or Moulinex (which is similar to the Krups) mill. Furthermore, for continuous grinding, several of these types of mills must be used because they get exceedingly hot.

Results from the analysis of variance (AOV) of the data from all sources are shown in Table III. Because all main effects and interactions were significant ($p < 0.01$), this AOV is not very informative. For more detail, the data for each moisture and grind combination were treated by AOV. A portion of the analysis is given in Table IV. The mean squares for duplicate readings, an estimate of the variance between duplicate readings on the same sample, were 0.02, 0.04, and 0.02% for the GQA-A, GQA-B, and GAC, respectively. The percentage of total variability due to duplicate determinations was small; however, those for the GAC were consistently lower. Coefficients of variation for duplicate readings were about equal for the GAC and GQA instruments. The mean protein contents were about equal for the three instruments; however, the mean protein content

TABLE I
Sieve Analysis and Protein Content (GAC) of Five Classes of Wheat Ground on the Weber Pulverizer, Udy Cyclone, and Krups Mills

Sample Number	Wheat Class	Moisture %	Grinder	Sieve Analysis						Kjeldahl Protein %	GAC Protein %
				>210 μ %	150–210 μ %	125–150 μ %	105–125 μ %	<105 μ %	<150 μ %		
1	Durum	11.1	Weber[a]	33.95	16.27	7.96	4.71	37.08	49.75	11.7	11.4
		11.1	Udy[b]	33.32	16.18	8.06	4.97	37.45	50.48	11.7	11.2
		11.1	Krups[c]	41.25	10.60	4.78	3.24	40.11	48.13	11.7	11.6
2	Durum	11.8	Weber	34.05	16.47	7.66	4.54	37.25	49.45	12.1	11.0
		11.8	Udy	29.84	15.69	8.58	5.43	40.45	54.46	12.1	10.9
		11.8	Krups	47.88	8.58	3.75	2.49	37.28	43.52	12.1	10.7
3	Hard red spring	10.7	Weber	18.42	12.48	7.76	5.46	55.86	69.08	11.8	12.2
		10.7	Udy	24.83	10.52	6.28	4.48	53.86	64.62	11.8	12.1
		10.7	Krups	38.42	11.22	5.75	4.87	39.71	50.33	11.8	12.3
4	Hard red spring	9.4	Weber	20.85	12.71	7.27	4.64	54.42	66.33	12.2	11.8
		9.4	Udy	26.37	12.17	6.42	4.43	50.59	61.44	12.2	12.2
		9.4	Krups	40.05	11.87	5.49	3.82	38.75	48.06	12.2	12.5
5	Hard red winter	10.6	Weber	20.95	12.20	6.78	4.06	55.99	66.83	12.2	12.1
		10.6	Udy	27.42	11.30	5.94	3.88	51.43	61.25	12.2	12.1
		10.6	Krups	46.80	10.31	4.68	2.78	35.40	42.86	12.2	12.5

TABLE I (*continued*)
Sieve Analysis and Protein Content (GAC) of Five Classes of Wheat Ground on the Weber Pulverizer, Udy Cyclone, and Krups Mills

Sample Number	Wheat Class	Moisture %	Grinder	Sieve Analysis >210 μ %	150–210 μ %	125–150 μ %	105–125 μ %	<105 μ %	<150 μ %	Kjeldahl Protein %	GAC Protein %
6	Hard red winter	10.6	Weber	23.80	13.20	7.02	4.46	51.52	63.00	12.2	12.2
		10.6	Udy	25.17	12.00	6.58	4.27	51.95	62.80	12.2	12.3
		10.6	Krups	48.53	10.66	4.81	3.09	32.88	40.78	12.2	12.6
7	Western white	8.9	Weber	13.40	9.52	5.50	3.43	68.13	77.06	12.5	12.7
		8.9	Udy	14.82	7.18	4.31	2.79	70.88	77.98	12.5	12.6
		8.9	Krups	32.77	8.79	4.35	2.24	51.82	58.41	12.5	12.9
8	Western white	9.4	Weber	14.57	8.77	4.64	3.01	68.99	76.64	12.0	12.0
		9.4	Udy	18.50	7.92	4.18	3.30	66.07	73.55	12.0	11.7
		9.4	Krups	20.78	8.01	4.17	2.48	64.45	71.10	12.0	11.8
9	Soft red winter	12.4	Weber	15.15	9.40	6.82	6.58	61.64	75.04	11.8	11.8
		12.4	Udy	18.76	7.86	5.50	5.20	62.66	73.36	11.8	11.9
		12.4	Krups	32.13	9.30	4.50	3.68	50.36	58.54	11.8	11.7
10	Soft red winter	12.8	Weber	11.86	20.33	5.90	6.36	55.52	67.78	11.4	11.3
		12.8	Udy	19.19	7.78	5.91	7.28	59.82	73.01	11.4	11.5
		12.8	Krups	32.60	9.28	5.19	5.30	47.54	58.03	11.4	11.3

[a]Weber Laboratory pulverizer, as modified by Udy (0.024 in. screen).
[b]Udy Cyclone grinder (1.0-mm screen).
[c]Krups coffee grinder (1 min grinding time).

for the GAC on the high-moisture samples, grind 4, was 0.5% lower than that of the GQA. If an instrument consistently gives slightly low or high results, it can be corrected very easily by a simple adjustment on the instrument. The mean protein content as determined by the Kjeldahl method was 12.0%.

Correlation coefficients between protein content for all samples as determined by the Kjeldahl method and the infrared instruments are highly significant (Table V). A plot of the data indicated slight differences between the low- and high-moisture samples; therefore, separate regression analyses were made for them. However, differences in the regression equations for the low- and high-moisture samples were very small and are not shown.

TABLE II
Variation in Percentage < 150 μ Particles and Protein Content (GAC) of Five Classes of Wheat Ground on Five Krups Mills

Krups Mills	Hard Red Winter		Hard Red Spring		Durum Wheat		Soft Red Winter		Western White	
	<150 μ %	Protein %	<150 μ %	Protein %	<150 μ %	Protein %	<150 μ %	Protein %	<150 μ %	Protein %
1	47.9	14.7	21.1	13.3	27.5	15.6	60.3	11.3	69.6	8.9
2	29.4	15.2	31.5	12.9	26.3	15.2	67.4	11.3	70.8	8.9
3	23.3	14.5	25.4	13.0	24.5	15.0	68.3	11.5	71.4	9.3
4	36.4	15.4	26.5	13.0	21.8	15.6	70.7	11.2	70.5	8.6
5	13.9	15.0	20.9	13.3	17.4	15.1	69.1	11.5	70.8	9.1
Average	30.2	15.0	25.1	13.1	23.5	15.3	67.2	11.4	70.6	9.0

TABLE III
Analysis of Variance on Percentage Protein for all Samples and Variables

Source	df	Mean Square	F[a]
Samples	87	38.591	977.31
Samples × moisture	87	0.097	2.46
Grind within samples × moisture	176	0.039	
Moisture	1	7.988	82.15
Samples × moisture	87	0.097	
Methods	3	11.532	74.36
Samples × methods	261	0.155	
Moisture × methods	3	3.296	163.78
Samples × moisture × methods	261	0.020	
Grind within samples × moisture	176	0.039	2.84
Samples × methods	261	0.155	11.15
Samples × moisture × methods	261	0.020	1.45
Moisture × grind within samples × moisture	528	0.014	

[a]All effects are significant at $P < 0.01$.

Because the infrared instruments tested are new, comments regarding their operation and performance are warranted. We found the GQA instrument easier to calibrate and operate than the GAC. The GQA cell for the sample has a cover glass with a tension spring that helped to obtain uniform surface and compaction. For the GAC, the sample cell is open and was filled, tamped, and smoothed off with a spatula. Experience is required to obtain continuous repeatable results. We have had considerable problems with the lamp burning out in the GQA which, in all but one case, required recalibration—a very time-consuming process. Preliminary studies showed that replacement of the lamp by another of the same lot number may avoid recalibration, because lamps with the

TABLE IV
Results of Analyses of Variance on Percentage Protein for Each Instrument at Each Moisture Level and Grind

	GQA–A	GQA–B	GAC
Low moisture: Grind 1[a]			
Mean square for duplicate determinations	0.021	0.049	0.013
Percentage variability[b]	1.19	2.35	0.48
Coefficient of variation (%)	1.18	1.79	0.95
Mean (%)	12.4	12.3	12.3
Low moisture: Grind 2			
Mean square for duplicate determinations	0.026	0.039	0.018
Percentage variability for duplicate determinations	1.34	1.86	0.68
Coefficient of variation (%)	1.30	1.60	1.09
Mean (%)	12.5	12.4	12.3
High moisture: Grind 3			
Mean square for duplicate determinations	0.022	0.042	0.017
Percentage variability for duplicate determinations	0.98	1.72	0.55
Coefficient of variation (%)	1.19	1.68	1.10
Mean (%)	12.4	12.2	12.0
High moisture: Grind 4			
Mean square for duplicate determinations	0.017	0.035	0.014
Percentage variability for duplicate determinations	0.81	1.51	0.46
Coefficient of variation (%)	1.07	1.54	1.01
Mean (%)	12.4	12.3	11.8

[a]Two 50-g portions of each sample were tempered to about 10% moisture (low moisture) and two to about 14.5% and ground separately.
[b]Percentage of total variability attributed to duplicate determinations.

TABLE V
Correlation Coefficients for Protein Content as Determined by the Kjeldahl and Infrared Instruments

	GQA–A	GQA–B	GAC
Kjeldahl	0.979	0.982	0.982
GQA–A		0.994	0.979
GQA–B			0.982

same lot number may accommodate the "C" values of the samples. Replacement of the lamp without recalibration might increase the standard error. We have had no electronic problems with the GAC. The above comments pertain only to the instruments we evaluated and may or may not be applicable to newer models.

Other data in our laboratory (not reported) show that variety and location effects, associated with wheat hardness, influenced the particle size of the ground sample. If this interaction could be eliminated or reduced, the accuracy of the instruments would be increased.

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[Received March 14, 1975. Accepted June 5, 1975]