EFFECT OF WHEAT CLASS ON NEAR INFRARED REFLECTANCE[1]

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ABSTRACT

Regression equations were developed, with five classes of wheat, for the relation between protein contents as determined by Kjeldahl and by near infrared reflectance (NIR) with hard red spring wheat as the calibration standard. The slope of the regression equation varied considerably and depended on the class of wheat. The effect of class on the regression equations could not be related directly to particle-size distribution of the ground samples. Correlation coefficients between Kjeldahl and NIR protein content were highly significant (0.93 to 0.99) for all classes of wheat. The class of wheat affected the log values of the NIR instrument and the log values were related to the general hardness class of the wheats.

The use of near infrared reflectance (NIR) spectroscopy has increased for the determination of protein, oil, and moisture contents in grains, oilseeds, and other products since it was introduced in 1971 (1). For protein and oil contents, high correlations have been shown between estimates by NIR and analyses by standard laboratory methods (2–6). Factors that affect NIR determinations and ways of reducing their effects are less documented.

Hymowitz *et al.* (2) showed that, for soybeans and oats, grinding time affected protein and oil contents as determined by NIR, and that for corn the grinding time × genotype interaction was significant for protein content. Williams (3) reported that grinding samples on seven different grinders affected NIR determinations, and that the accuracy of the calibration of the NIR instruments was affected by season and location of wheat production. Watson *et al.* (4), who determined particle-size distribution of five classes of wheat ground on three types of mills, reported that differences in particle size between classes of wheat were large regardless of the mill used. Type of grinder appeared to affect the NIR determinations, but particle-size distribution was not consistently related to NIR results. Pomeranz and Moore (5) showed no consistent differences due to variety or location in the particle-size distribution of ground-wheat samples. Popineau and Godon (7) showed that grinder and especially grinding time significantly affected NIR results. The effect of grinding time was greater for hard than for soft wheat.

Sample preparation, *i.e.*, grinding, is important in NIR determinations for protein, oil, and moisture. Size, shape, and uniformity of particles influence NIR results and all are affected by the grinding process. Handling and preparation of samples for NIR instruments have been reviewed (8).

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The purpose of this research was to study further the influence of wheat class and of particle size on NIR determinations.

MATERIALS AND METHODS

About 50 samples from each wheat class—hard red spring (HRS), hard red winter (HRW), durum (D), soft red winter (SRW), and white (W)—were ground in a Udy cyclone mill with a 0.5-mm round-hole screen.

The range in moisture content of the samples was limited to 9–11% in order to minimize its effect on NIR results. Moisture and Kjeldahl protein were determined by AACC Methods 44-15 and 46-10 (9). Protein content was determined by NIR with an InfraAlyzer (Technicon Instruments Corp., Tarrytown, NY 10591). The instrument was calibrated and operated according to the instruction manual. The glass-covered sample cell was used. Protein content by Kjeldahl and NIR was compared on an as-is moisture basis.

Composites in protein increments of 1% were made from the ground wheat. At

TABLE I
Kjeldahl Protein Range and Mean of Each Class of Wheat[a]

Wheat Class	n	Range %	Mean %
Hard red spring	45	11.4–17.7	14.5
Hard red winter	49	7.9–15.1	11.6
Durum	51	10.1–17.7	13.5
Soft red winter	52	9.4–12.8	11.3
White	49	10.4–15.6	12.6

[a]As-is moisture basis.

TABLE II
Correlation Coefficients between Protein Content Determined by Kjeldahl and InfraAlyzer

Wheat Class	n	r-Values[a] A	r-Values[a] B	Regression Equation[b]	Standard Deviation[c]
Hard red spring	45	0.99**	0.99**	Y = 1.015X − 0.304	0.205
Hard red winter	49	0.98**	0.98**	Y = 1.017X + 0.43	0.369
Durum	51	0.99**	0.99**	Y = 0.948X + 0.994	0.244
Soft red winter	52	0.93**	0.98**	Y = 1.318X − 3.121	0.363
White	49	0.98**	0.99**	Y = 1.224X − 1.258	0.277
Combined		0.96**			0.549

[a]A = InfraAlyzer protein content determined from K-constants and log values. B = InfraAlyzer protein content read from instrument calibrated against the HRS log values.

[b]For InfraAlyzer protein content as determined by Method B. Y = estimated Kjeldahl protein.

[c]Standard deviation of regression equation between Kjeldahl and InfraAlyzer protein content determined from log values.

least four composite samples were made for each wheat class. Sieve analysis was made on 20 g of the composite ground wheat with a Ro-Tap shaker and U.S. Standard Tyler sieves; shaking time was 5 min.

RESULTS AND DISCUSSION

The number of samples and ranges and mean of protein content for each class of wheat used appear in Table I. The samples were selected in each class to obtain a normal distribution of protein content.

Correlations between protein content determined by Kjeldahl and the InfraAlyzer for each class of wheat and for all classes combined were determined for two sets of InfraAlyzer data (Table II). For one set of data, the log values for the samples in each class were obtained and the protein content was determined by multiple regression analyses from these log values and the calculated K-constants (A in Table II). For the second set of data, the protein contents of the samples were read directly on the InfraAlyzer after it was calibrated to the HRS samples (B in Table II). The correlation coefficients were highly significant for both methods, but generally, were slightly higher with the protein content from

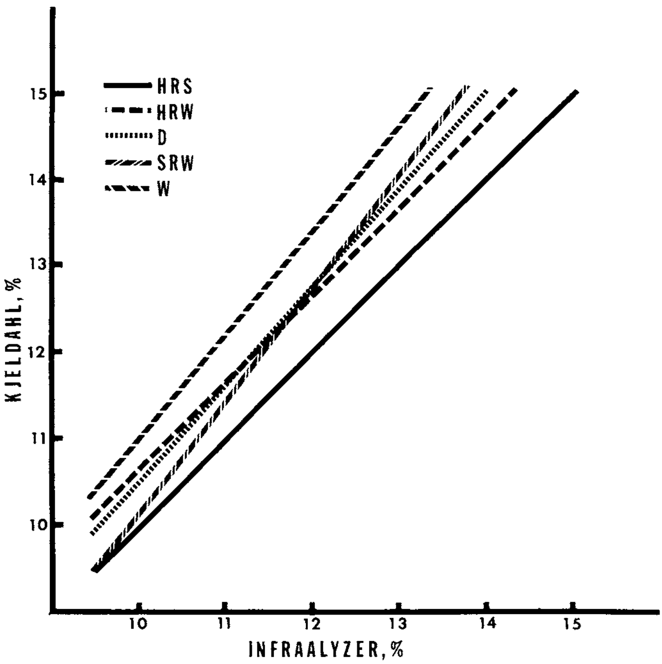

Fig. 1. Regression line for per cent protein as determined by Kjeldahl and by infrared methods with hard red spring wheat as the calibration standard. HRS = hard red spring; HRW = hard red winter; D = durum; SRW = soft red winter; W = white.

the instrument readings (B). This is because there were one to three samples in each case that were further from the regression line than the remainder of the samples. Calibration of the instrument to the "best fit" regression equation, as recommended in the instruction manual, minimizes these variations. When all classes of wheat were combined, a multiple regression of the log values gave a highly significant r-value of 0.96.

The correlation coefficients (Table II) do not show the accuracy of the two methods (instrument readings and log values). Therefore, the regression lines for each class of wheat are shown in Fig. 1, and the regression equations and standard errors of estimate in Table II. These regression lines and equations were from the protein readings of the instrument calibrated to the HRS wheat samples. The regression line for HRS wheat has a slope of one and goes through the origin as forced by the multiple regression analysis. The same was true for the other classes of wheat if the regression equation was obtained using the regression analysis of method A (log values). However, this is not true for the regression equation obtained from the Kjeldahl and the NIR protein of the InfraAlyzer calibrated to HRS wheat (method B). For example, the regression equation for the SRW wheat obtained by method A was $Y = 1.00X - 0.01$, whereas for method B the regression equation was $Y = 1.318X - 3.121$. The difference in slope (0.318) between the two equations demonstrates the need to calibrate the InfraAlyzer to the particular class of wheat. The HRW wheat line has nearly the same slope as the HRS wheat line, but it is shifted upward. Therefore, the HRW wheat protein content averaged about 0.7% lower from the InfraAlyzer, with the HRS wheat calibration, than from the Kjeldahl. Durum readings averaged about 0.5% low at 10% protein, and about 1.1% low at 14% protein; *i.e.*, the slope of the regression line was not the same for D and HRS. The difference in slope was greater between SRW and HRS than between D and HRS (Table II). At 10% protein the InfraAlyzer reading was 0.1% low and at 14% protein, 1.4% for SRW. The shift in regression line was greatest with W wheat. At 10% protein the InfraAlyzer readings were 1% low and at 14% protein, 1.9%. The slopes for HRS and HRW are about the same—nearly one; the slope for D is less than one; and the slopes for SRW and W are greater than one. The slopes of the regression line are in ascending order (less than one to greater than one) in the same general order of hardness of each class of wheat (D, hardest to SRW, softest). These comparisons indicate that hardness which influences

TABLE III
Average Particle-Size Distribution of Each Class of Wheat

	Per Cent of Sample in Size Range						
Wheat Class	**>297 μ**	**210–297 μ**	**149–210 μ**	**105–149 μ**	**74–105 μ**	**<74 μ**	**<105 μ**
Hard red spring	6.5	11.5	15.6	15.6	32.6	18.2	50.8
Hard red winter	5.8	10.9	16.2	18.9	42.4	5.8	48.2
Durum	4.4	12.4	22.3	22.2	33.3	5.4	38.7
Soft red winter	5.2	7.0	11.8	18.9	49.2	7.9	57.1
White	6.9	9.1	19.1	39.9	19.5	5.5	25.0

TABLE IV
Means of Log Values for Each Class of Wheat, Wavelength, and Factor Measured

	Means					
Wheat Class	**Log 1**	**Log 2**	**Log 3**	**Log 4**	**Log 5**	**Log 6**
Hard red spring	667	484	157	314	500	109
Hard red winter	656	477	144	324	524	135
Durum	738	537	209	367	538	146
Soft red winter	478	321	−10	144	337	37
White	492	342	6	165	339	55
Wavelength, nm	2310	2230	2180	2100	1940	1680
Factor measured	Oil	Reference	Protein	Starch	Water	Reference

particle size, shape, and the manner of facture of the protein-starch granules affects NIR determinations.

As expected, the standard deviation for the combined data for all classes of wheat was larger than the standard deviations for the individual classes (Table II). This comparison shows that the accuracy of measurements of protein content by NIR is increased by calibrating the instrument for each class of wheat as recommended in the instruction manual.

Particle-size distribution of composite samples made in 1% protein increments within each class of wheat showed no difference due to protein content. Therefore, the data were averaged and are reported in Table III. These data show no consistency or trend of particle-size distribution with NIR data. The greatest variation in distribution between wheat classes occurred at 105–149 μ and below.

Personal communication[4] suggested that there might be some relation between certain log values and wheat hardness, especially the difference between logs 5 and 6. Therefore, the mean log values for each class of wheat are reported in Table IV. These data show distinct differences among mean log values and are related to the general hardness classification of the wheats. The log values were similar for HRS and HRW and for SRW and W, but were higher for D than for any other class. In terms of general hardness, HRS and HRW are about equal; SRW and W are about equal and softer than HRS and HRW; and D is the hardest. All possible additions, differences, and ratios of the mean log values were determined. The combinations that showed the most promise for differentiation of classes of wheat by addition, subtraction, and ratio were logs 1 and 3, logs 1 and 6, logs 2 and 6, logs 3 and 6, and logs 5 and 6. These combinations warrant further investigation.

[4]Personal communication with G. W. Schiller, Dixie-Portland Flour Mills, Inc., Arkansas City, KS 67005.

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Literature Cited

1. ROSENTHAL, R. D. Introducing: The grain quality analyzer. Annual meeting, Kansas Assoc. Wheat Growers, Hutchinson, KS, Dec. 1971.
2. HYMOWITZ, T., DUDLEY, J. W., COLLINS, F. I., and BROWN, C. M. Estimations of protein and oil concentration in corn, soybean, and oat seed by near-infrared light reflectance. Crop Sci. 14: 713 (1974).
3. WILLIAMS, P. C. Application of near infrared reflectance spectroscopy to analysis of cereal grains and oilseeds. Cereal Chem. 52: 561 (1975).
4. WATSON, C. A., CARVILLE, D., DIKEMAN, E., DAIGGER, G., and BOOTH, G. D. Evaluation of two infrared instruments for determining protein content of hard red winter wheat. Cereal Chem. 53: 214 (1976).
5. POMERANZ, Y., and MOORE, R. B. Reliability of several methods for protein determination in wheat. Baker's Dig. 50(1): 44 (1975).
6. RINNE, R. W., GIBBONS, S., BRADLEY, J., SEIF, R., and BRIM, C. A. Soybean protein and oil percentages determined by infrared analysis. Agric. Res. Service, USDA, ARS-NC-26, July 1975.
7. POPINEAU, Y., and GODON, B. The effect of sampling grinding times on results from infrared reflectance analysis. TIS News 2(1): 9 (1976).
8. TECHNICON INDUSTRIAL SYSTEMS, Tarrytown, NY 10591. TIS News 2(1) (1976).
9. AMERICAN ASSOCIATION OF CEREAL CHEMISTS. Approved methods of the AACC. Method 44-15, approved April 1967; and Method 46-10, approved April 1961. The Association: St. Paul, Minn.

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