

## Interlaboratory and Intralaboratory Reproducibility of Protein Determination in Hard Red Winter Wheat by Kjeldahl and Near Infrared Procedures<sup>1</sup>

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### ABSTRACT

Protein content was determined in eight samples (three of which were duplicates) of hard red winter (HRW) wheat (10 to 14% protein) by the Kjeldahl method in 24 laboratories and by the near infrared reflectance (NIR) method in 30 laboratories in which Neotec 31 EL or Technicon InfraAlyzer instruments were used. All samples were ground and tested by the NIR method at all 30 laboratories; however, ground samples were returned from only 24 of the 30 laboratories to the U.S. Grain Marketing Research Laboratory (USGMRL). Ranges in particle size of all returned samples were measured and the variability in protein contents was evaluated by comparisons with data from Kjeldahl and NIR analyses conducted in a single laboratory (USGMRL). Finally, portions of the five different wheat samples were ground in the USGMRL to cover the range of particle size that had been measured in the ground samples returned from the 24 laboratories. The samples were analyzed at the USGMRL for protein by the Kjeldahl and NIR methods and for particle size. The standard deviation for Kjeldahl data from Group A laboratories (four government, four university and four

commercial) was judged *a priori* to be low; it was, in fact, significantly lower than the standard deviation for comparable data from the 12 Group B laboratories where wheat in trading channels was analyzed. The standard deviations did not differ significantly among the data from Kjeldahl analyses in Group B laboratories and comparable data from analyses by either Neotec 31 EL or Technicon InfraAlyzer NIR instruments. Therefore, the use of the NIR instruments would not reduce the reliability of protein determinations in the marketing of HRW wheat. In the ground samples returned from the 24 collaborating NIR laboratories, average particle size ranged from 110 to 168  $\mu\text{m}$ . No significant effect on either particle size or protein content could be attributed to the use of different Udy cyclone grinders in different laboratories. In samples ground at the USGMRL, average particle size ranged from 111 to 162  $\mu\text{m}$  and did not consistently affect protein content. We concluded that the Udy cyclone grinder is suitable for grinding HRW wheat samples to analyze protein content by the NIR method.

### INTRODUCTION

Several workers (1-5) have used near infrared reflectance (NIR) spectroscopy to analyze wheat for protein. Hunt *et al.* (4) studied the effect of grinder type. Hunt *et al.* (5) conducted a collaborative study on two NIR instruments by using samples ground at a central laboratory. Miller and Pomeranz (6) reported that NIR methods are suitable for use in binning wheat

according to protein content, but they questioned the reproducibility of the method for measuring protein content of wheat for trading purposes.

We undertook the present study to compare interlaboratory and intralaboratory reproducibility of protein determination by Kjeldahl and NIR procedures in commercial samples of hard red winter (HRW) wheat. Our procedures differed from those used by Hunt *et al.* (5) in that personnel in each collaborating laboratory ground their own samples and used instruments that were individually calibrated for their own use. Hunt *et al.* (5) sent samples that had been ground in a central laboratory to each collaborator and provided for central calibration of the NIR instruments to a single reference Kjeldahl laboratory.

We also investigated the effect of particle size on the protein determination by two types of commercial NIR instruments. We compared results of this part of the study with those of the study by Hunt *et al.* (5).

### MATERIAL

Five 50-pound samples of commercial HRW wheat ranging from 10 to 14% in protein and from 10.5 to 12.5% in moisture contents were obtained from Ross Industries, Wichita, KS. The five wheat samples were cleaned with a Carter Dockage Tester and Forster Cyclone Laboratory Scourer. Each sample was mixed in a McClellan batch mixer for 30 min. Three of the five samples (low-, medium- and high-protein contents) were

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Mention of specific instruments or trade names is made for identification purposes only and does not imply any endorsement by the U.S. government.

divided with a Boerner divider. Those samples served as internal duplicate samples, designated as A and B in the tables. All eight samples were assigned coded numbers selected at random and were subdivided with a Boerner divider into uniform subsamples; subsamples were sent to all collaborating laboratories.

### Experimental Design

Two groups of 12 laboratories each were selected to do the Kjeldahl analyses. Group A laboratories included research laboratories that conduct precise Kjeldahl analyses and thus could be expected to establish a lower limit of interlaboratory variability. Group A comprised four USDA, four university and four commercial analytical service laboratories. Laboratories in Group B were chosen at random from 37 laboratories at which wheat in trading channels is analyzed for protein; they were paid their normal fee for analysis. The intralaboratory variation of the Group B laboratories is representative of the reproducibility of protein determinations made on a lot of wheat as it moves through marketing channels and is analyzed by different laboratories.

Two other groups of laboratories, one of 14 laboratories and the other of 16 laboratories, were selected to determine protein with NIR instruments. One group used the Neotec 31 EL instrument and the other the Technicon InfraAnalyzer. Protein contents were reported as determined with the existing calibration for each machine.

Group B Kjeldahl laboratories received 250-gram samples (each sealed in a metal paint can)—enough to measure moisture of whole grain with a Motomco electronic moisture tester. All other laboratories received 90-gram samples (each sealed in a plastic bottle)—enough to measure moisture by an oven method.

Each collaborator measured the protein in each of the eight coded samples. All collaborators were asked to measure moisture.

The effect of differences in particle size, resulting from the use of different grinders to prepare samples for NIR protein determinations, was part of the between-laboratory variance measured by the collaborative study. For evaluation of the effect of different grinders, samples were ground with Udy cyclone mills in 24 laboratories and then returned to the USGMRL for analysis. These samples were analyzed for average particle size on a sonic sifter and for protein with both a Neotec 31 EL and an InfraAnalyzer. As a further check, the returned samples were also analyzed at the USGMRL for protein by the Kjeldahl and for moisture by the air-oven methods.

Finally, to encompass the range of particle size that had been determined in ground samples returned by 24 of the 30 NIR laboratories, samples of the five different wheat samples were ground at the USGMRL. Those five samples were analyzed for protein and particle size to verify the effect of particle size, within the range obtained in various laboratories, on protein determined by NIR methods.

### METHODS

Moisture and Kjeldahl protein were determined at the USGMRL by AACC methods (7). NIR determinations were made according to the manufacturers' instructions. Distribution of particle sizes was measured on a sonic sifter. Mean particle size was computed from those results according to Method ASAES 319 of the American Society of Agricultural Engineers (8).

To produce samples with particle sizes within the range of samples submitted by laboratories employing the NIR method, we ground subsamples of the five wheat samples on a modified Udy Weber mill. We used these four methods: A) grinding a sample to pass a screen with 0.040-inch diameter openings; B) sieving (A) through a 100-mesh sieve, regrinding the "overs" to pass through a screen with 0.040-inch diameter openings and mixing those with the "thrus"; C) grinding a sample to pass a

screen with 0.024-inch diameter openings; and D) sieving (C) through a 120-mesh sieve, regrinding the "overs" to pass through a screen with 0.024-inch diameter openings and mixing those with the "thrus."

### RESULTS AND DISCUSSION

Our original intent was to express protein on a constant-moisture basis. However, only 10 of 30 laboratories using NIR devices were able to make a moisture determination. Therefore, all protein contents are given on an "as-is" moisture basis to make the results as comparable as possible. In several foreign countries protein of wheat in marketing channels is expressed on a constant-moisture basis, but that practice is not currently followed in the United States. Therefore, analysis of protein on an as-is moisture basis accurately indicates the reproducibility of protein determination as it is presently conducted during the marketing of wheat in the United States.

In our study the low-moisture contents of the samples, and small moisture variations probably associated with grinding and handling, had little effect on the results for protein. Ideally, however, protein values from both Kjeldahl and NIR methods should be expressed on a fixed-moisture basis. A difference of 2% in moisture could effect a 0.3% difference in protein content for a 15% protein wheat. NIR instruments should be calibrated for moisture by use of samples with known moisture content as determined by the oven method (7).

**Kjeldahl Results**—The Kjeldahl analyses by 24 participating laboratories are summarized in Table I. The between- and within-laboratory variance components were computed for each sample. These variances were then pooled across samples and reported (Table II). Data from the laboratories in Group A were comparable. Any difference in protein determinations among these laboratories apparently was largely due to sampling variation. The between-laboratory component of variance (0.175) for Group B laboratories indicated that they were less well standardized than were Group A laboratories. For both groups (A and B), the within-laboratory variance was mainly attributable to sampling variation. We believe that to be true because the within-laboratory variance for our data was essentially the same as that found by Hunt *et al.* (5) and others involved in sampling-variation studies with which we are familiar.

**Infrared Results**—The NIR analyses by the 30 participating laboratories are summarized in Table III. The between- and within-laboratory variance components were computed and pooled across samples (Table IV) in the same way as were results from the Kjeldahl laboratories (Table II).

For the NIR laboratories and the Kjeldahl laboratories, the within-laboratory variance comprised mainly sampling variation. The between-laboratory component for the NIR instruments tested was comparable with the between-laboratory component for the Group B Kjeldahl laboratories (Table II).

Although all NIR instruments are calibrated initially by their respective manufacturers, each user generally changes the factory calibration on the basis of his own calibration samples as analyzed by his own reference Kjeldahl laboratory. This practice adds an unknown amount to the between-laboratory variance for the instrument. That additional variance is part of the total variation in NIR determinations unless calibration and standardization are based on results from a single Kjeldahl laboratory. The between- and within-laboratory variances for Kjeldahl analyses and the within-laboratory variances for NIR analyses were unaffected by the uncontrolled sources of variation that arose from differences in calibration practices.

**Interlaboratory Agreement**—The data presented in Tables II and IV were summarized (Table V) as total interlaboratory standard deviations for the Kjeldahl and NIR methods. For the Kjeldahl data, the standard deviation was significantly lower (the data were more reproducible) for Group A than it was for Group B laboratories. The standard deviations did not differ significantly for Group B Kjeldahl laboratories and comparable data for laboratories that used either of the two NIR

instruments.

Group B Kjeldahl laboratories now conduct protein determinations on grain moving in marketing channels. Our data indicate, therefore, that NIR instruments could be substituted for Kjeldahl analyses in Group B laboratories without reducing the reliability of the protein determinations used in marketing HRW wheat.

**Grinder Effect**—The protein contents determined with the Neotec and Technicon instruments at the USGMRL for the eight samples ground on Udy cyclone mills at 24 participating laboratories are summarized in Table VI. Between- and within-laboratory variance components are summarized in Table VII. All samples were analyzed on a single NIR instrument of each kind, so the between-laboratory variance component was

essentially the effect of different grinders. Because the wheat samples were divided and sent to the various laboratories to be ground and analyzed, the between-laboratory variance contained variation due to sampling. Within-laboratory variance primarily measured sampling variation.

The between-laboratory variance (grinder effect) for both instruments was only slightly less than the within-laboratory variance. Because the effect due to grinder was about the same as the sampling error, total variation would not be significantly reduced by further standardizing the grinders.

Average particle size of individual samples ground in the NIR laboratories ranged from 110 to 168  $\mu\text{m}$ ; the data for each sample are summarized in Table VIII. The average particle size was remarkably uniform for all samples.

TABLE I. SUMMARY OF PROTEIN CONTENTS OF EIGHT SAMPLES OF GROUND WHEAT AT 24 LABORATORIES

| Sample No. | Grinder   |           |
|------------|-----------|-----------|
|            | Average % | Range %   |
| 1A         | 9.9       | 9.2-10.6  |
| 1B         | 10.0      | 9.3-10.7  |
| 2          | 11.5      | 10.8-12.2 |
| 3A         | 12.6      | 11.9-13.3 |
| 3B         | 12.7      | 12.0-13.4 |
| 4          | 14.0      | 13.3-14.7 |
| 5A         | 14.7      | 14.0-15.4 |
| 5B         | 14.8      | 14.1-15.5 |

Four USDA, four university, and eight government laboratories.  
Twelve grain trade laboratories.

TABLE II. VARIANCE COMPONENTS FOR NIR PROTEIN DATA

| Variance             | Component |
|----------------------|-----------|
| Between laboratories | 0.020     |
| Within laboratories  | 0.035     |
| Total                | 0.055     |

TABLE III. SUMMARY OF NIR ANALYSES AT 30 LABORATORIES

| Sample No. | Instrument 1 |         | Instrument 2 |         |
|------------|--------------|---------|--------------|---------|
|            | Average %    | Range % | Average %    | Range % |
| 1A         | 10.5         | 2.2     | 10.4         | 0.9     |
| 1B         | 10.6         | 2.2     | 10.7         | 1.0     |
| 2          | 12.1         | 1.5     | 11.9         | 1.3     |
| 3A         | 12.6         | 1.5     | 12.6         | 1.4     |
| 3B         | 12.3         | 1.5     | 12.4         | 1.4     |
| 4          | 14.3         | 1.2     | 14.3         | 2.1     |
| 5A         | 14.8         | 1.5     | 14.6         | 2.3     |
| 5B         | 14.7         | 2.1     | 14.6         | 2.0     |

TABLE IV. VARIANCE FOR NIR PROTEIN DATA

| Variance             | Instrument 1 (14 Laboratories) |           | Instrument 2 (16 Laboratories) |           |
|----------------------|--------------------------------|-----------|--------------------------------|-----------|
|                      | Component                      | Component | Component                      | Component |
| Between laboratories | 0.020                          | 0.015     | 0.020                          | 0.015     |
| Within laboratories  | 0.035                          | 0.029     | 0.035                          | 0.029     |
| Total                | 0.055                          | 0.044     | 0.055                          | 0.044     |

TABLE V. INTERLABORATORY AGREEMENT FOR PROTEIN DATA

| Sample | Standard Deviation |
|--------|--------------------|
| 1A     | 0.24               |
| 1B     | 0.44               |
| 2      | 0.45               |
| 3A     | 0.38               |

TABLE VI. SUMMARY OF NIR ANALYSES AT 24 LABORATORIES OF GROUND WHEAT RETURNED BY 24 OF THE 30 LABORATORIES THAT CONDUCTED THE NIR ANALYSES

| Sample | Grinder | Instrument 2 |         |         |
|--------|---------|--------------|---------|---------|
|        |         | Average %    | Range % | Range % |
| 1A     | 0.5     | 9.8          | 1.0     | 1.0     |
| 1B     | 1.0     | 9.8          | 1.1     | 1.1     |
| 2      | 0.6     | 11.9         | 0.7     | 0.7     |
| 3A     | 0.7     | 12.7         | 0.4     | 0.4     |
| 3B     | 0.9     | 12.8         | 0.6     | 0.6     |
| 4      | 0.5     | 14.2         | 0.6     | 0.6     |
| 5A     | 0.9     | 14.8         | 0.6     | 0.6     |
| 5B     | 1.0     | 14.9         | 0.5     | 0.5     |

TABLE VII. VARIANCE FOR NIR DATA RESULTING FROM GRINDER DIFFERENCES AMONG 14 OF THE 30 LABORATORIES THAT CONDUCTED THE ANALYSES

| Variance             | NIR Instrument 1 | NIR Instrument 2 |
|----------------------|------------------|------------------|
| Between laboratories | 0.020            | 0.015            |
| Within laboratories  | 0.035            | 0.019            |
| Total                | 0.055            | 0.035            |

TABLE VIII. SUMMARY OF MEAN PARTICLE SIZES OF GROUND WHEAT RETURNED BY 24 OF THE 30 LABORATORIES THAT CONDUCTED THE NIR ANALYSES

| Sample | Average ( $\mu\text{m}$ ) | Range ( $\mu\text{m}$ ) |
|--------|---------------------------|-------------------------|
| 1A     | 131                       | 54                      |
| 1B     | 138                       | 50                      |
| 2      | 134                       | 50                      |
| 3A     | 135                       | 43                      |
| 3B     | 135                       | 44                      |
| 4      | 131                       | 47                      |
| 5A     | 129                       | 46                      |
| 5B     | 131                       | 48                      |

As a further check on the effect of particle size, protein was determined with the Kjeldahl procedure and with Neotec 31 EL and Technicon instruments for the five samples ground at the USGMRL to four degrees of fineness. Average particle size of those samples ranged from 111 to 162  $\mu\text{m}$ . As with the samples ground at 24 NIR laboratories, particle size—within the range employed—did not consistently affect protein determination by the Kjeldahl and NIR methods. We concluded that the Udy cyclone grinder is suitable for grinding HRW wheat samples for analysis of protein by NIR procedures.

*Present Results Compared with Those from a Previous Collaborative Study*—In 1975, through a joint effort of the Agricultural Marketing Service, Agricultural Research Service and the National Bureau of Standards, a collaborative study was conducted to evaluate the performance of infrared instruments for determining protein in HRW wheat (5). Eighty samples of HRW wheat were ground on a Mikro mill. Fifty-five of the samples were used to calibrate individually twelve Neotec and six Technicon instruments on the basis of protein analyses supplied by the USDA Kjeldahl laboratory in Beltsville, MD. The remaining 25 samples were used to estimate the between- and within-laboratory variance components. Six Kjeldahl laboratories participated in the study.

The results of the current study corroborated the findings of Hunt *et al.* (5). The pooled within-laboratory variance component for protein determined by NIR was 0.029 for that study (5) and 0.028 for our study (Table IV). The between-laboratory variance component for the NIR method, however, was less in the previous study (0.102) than in our study (0.148). However, we expected an increase in variation because the instruments used in our study were not calibrated against results from a single Kjeldahl laboratory.

The grinder effect was small in the present study, so the use of a single grinder in the previous study should not cause the between-laboratory component to be lower than that encountered in practice. Thus, the published variance components (5) are reasonable estimates of the between- and within-laboratory variation in protein contents of HRW wheat as determined with NIR instruments that have been properly standardized.

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