# INFLUENCE OF WHOLE MEAL GRANULARITY ON ANALYSIS OF HRS WHEAT FOR PROTEIN AND MOISTURE BY NEAR INFRARED REFLECTANCE SPECTROSCOPY (NRS)[1]

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**ABSTRACT**

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Granulation of a wheat whole meal embodies mean particle size and particle size distribution. Granulation affects the accuracy and precision of testing of wheat and other grains for protein and moisture by means of near-infrared reflectance spectroscopy (NIRS). The grinding procedure and the moisture and protein levels of the wheat before grinding principally affect the granulation itself. Some of these factors are nonlinear in influence. This article describes the manner in which granularity, moisture, and protein levels influence the accuracy and precision of NIRS protein and moisture testing of hard red spring wheat. Calibration practices minimize errors caused by these factors.

Wheat whole meal is the pulverized material that results from subjecting wheat to a grinding process. The granularity of the whole meal means the state of division, which encompasses both the mean particle size (MPS) and particle size distribution (PSD). Several factors (Table I) affect granularity. These may be subdivided into factors arising from the nature of the wheat itself, and those introduced through the grinding process. In an earlier study (1), grinding wheat before near-infrared reflectance spectroscopy (NIRS) analysis was noted to exert a marked effect on the accuracy of the results. This article describes in more detail investigations of the influence of grinding processes on the granularity and reflectance characteristics of wheat whole meal.

## MATERIALS AND METHODS

Samples of wheat of different types and grades were drawn from the wheat "bank," which has been established at the Canadian Grain Research Laboratory over the past seven years. Only varieties licensed for commercial production were used in the study. Granularity was determined by a modification of the Symes particle size index (PSI) test (2). Samples (25 × 25 g) of hard red spring (HRS) wheat were ground on all of the grinders studied. The Canadian Grain Commission has found a sample size of 20–25 g to be optimum in terms of precision and throughput for routine determination of protein in HRS wheat. Duplicate samples of well-mixed whole meal were weighed and sieved for 10 min through U.S. standard 200-mesh stainless steel wire screen using a Ro-tap sieve shaker.

To compare the granularity of different grinds from the aspects of MPS and PSD, an arbitrary method of determining weighted mean particle size was developed. Preliminary experiments using different screens, sample weights ranging from 5 to 50 g, and sieving times from 5 to 30 min gave optimum conditions as follows: 10-g samples were weighed and transferred to the

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uppermost of a nest of five sieves. The lid was placed in position, and the sieves shaken for 15 min on a Ro-tap sieve shaker. The individual fractions were weighed carefully. Fine material adhering to the underside of individual screens was transferred to the contents of the screen immediately below with a fine brush. The weighted mean particle size was calculated using the formula:

$$MPS = (W_1d_1 + W_2d_2 + \ldots\ldots W_6d_6) \div R$$

where MPS is weighted mean particle size; $W_1$, weight of overs on screen 1 in grams; $W_{2-6}$, weight of throughs of respective screens; $d_1$, 1,000 $\mu$; $d_{2-6}$, diameter of aperture of the respective screens in microns; and R, total recovery of whole

**TABLE I**
**Factors Affecting Granularity of Wheat Whole Meal**

A. Factors introduced by the wheat itself
  1. Genetic constitution
    a. Chromosome number
    b. Wheat type
    c. Wheat variety
  2. Growing location
    a. Soil factors
    b. Climate
  3. Moisture content
  4. Protein content
  5. Damage incurred by grain as a result of weather
    a. Bleaching (intermittent wetting and drying during late stages of maturation)
    b. Frost
    c. Disease
    d. Drought

B. Factors introduced by grinding process
  1. Type of grinder, including grinding action
    a. Hammer mill
    b. Burr mill
    c. Impeller mill
    d. Cutting-type mill
    e. Ball mill
    f. Pin mill
  2. Size of screen used in grinder
  3. Grinder rpm
  4. Grinder feed rate
  5. Sample size

C. Factors introduced by granularity testing itself
  1. Test procedure
    a. Penetrometer
    b. Compression
    c. Pearling
    d. Torque measurement
    e. Grinding and sifting
      i. Number and mesh size of sieves
      ii. Size of sieves
      iii. Time of sifting
    f. General carelessness

meal. The value of 1,000 $\mu$ was assigned arbitrarily to the mean diameter of the overs on the uppermost screen. U.S. standard stainless steel gauze screens were used. These are listed in Table II.

The values for the MPS attained in this manner were verified by microscopic examination of the whole meal of HRS wheat ground on the Hobart, Krups 75, and Cyclotec (1.0-mm screen) grinders using a Zeiss photomicroscope in conjunction with a hemacytometer grid. Duplicate counts of 1,000 particles were made for each grind. The application of the more sophisticated sedimentation methods for MPS determination to the estimation of MPS in coarse whole meals presents problems due to the settling velocity of coarse particles, while the Coulter Counter technique requires preliminary removal of the coarsest particles to achieve reproducible results. The weighted MPS approach was somewhat more arbitrary than those methods, but it afforded a simple, precise procedure for comparing the granularity of the whole meals and also gave a useful estimate of the PSD. The precision of the technique was verified by interposing check samples of HRS wheat ground on the Hobart 2040 burr mill, the Tecatur/Udy Cyclotec grinder, and the Krups 75 impeller mill. Protein was determined by the Kjeldahl procedure (3) and moisture by the single-stage air oven method (4). A Neotec Grain Quality Analyzer (GQA) Model 31, was used for all NIRS testing.

Seven grinders were used, including three burr mills, the LabConco Model 900, the Buhler Model MLI 204, and the Hobart Model 2040; two impeller mills, the Krups 75 and the Janke/Kunkel (Culatti) Model DHF48; and the Tecatur/Udy Cyclotec grinder, with 0.5- and 1.0-mm screens. This gave a total of seven grinding procedures. In a subsequent study to verify the effect of varying MPS without changing grinding action, a hammer mill, the Christie/Norris 8-in. grinder, was used with five screen sizes varying from 0.5 to 2.5 mm.

To assess the effect of grinding procedure on the accuracy of subsequent NIRS analysis, the protein content of each series of wheats was calculated from the respective K values and C values for each series. The results of all protein tests were converted to constant moisture basis (13.5%) for purposes of comparison unless otherwise stated. Next, the discrepancies between the NIRS and Kjeldahl protein values for each sample in each series were compared with the Kjeldahl values. A significant correlation between discrepancies and protein content indicated a slope change. This meant that, as distinct from a linear offset, the discrepancies, eg, those of grinder A NIRS protein from Kjeldahl protein using grinder B NIRS K values, could not be corrected by the adjustment of the intercept $K_o$, since the magnitude of the discrepancies would not be linear over a

**TABLE II**
**Sieves Used in Granularity Testing**

| U.S. Standard Mesh | Aperture ($\mu$) |
|---|---|
| 35 | 500 |
| 45 | 354 |
| 75 | 212 |
| 100 | 149 |
| 200 | 74 |

protein range. When this occurred, separate calibrations were deemed necessary for individual grinders. Figure 1 illustrates some typical slope changes. A hi-lo slope change, for example, indicates that at high Kjeldahl protein levels, all NIRS results will read higher than standard, while the reverse holds at low Kjeldahl protein levels.

K. H. Norris, of the Instrument Research Laboratory, USDA, ARC, Beltsville, MD, measured reflectance traces of various types of wheat. A tilting filter system involving three filters was used to simulate the scanning system used in the GQA. The first filter (moisture) scanned from 1.95 to 2.08 $\mu$, the "protein" filter from 2.01 to 2.18 $\mu$, and the "oil" filter from 2.15 to 2.32 $\mu$. The reflectance values were recorded as percent of reflectance from the sample surface relative to dry cornstarch. Since the various influences of grade, wheat type, moisture, and grinder caused pronounced scatter, the scales were adjusted somewhat for plotting, to accommodate the most widely divergent traces.

## RESULTS

### Precision of Granularity Measurements

An HRS wheat sample was mechanically divided into subsamples of 25 g. The subsamples were interpolated periodically into the MPS testing runs for three of

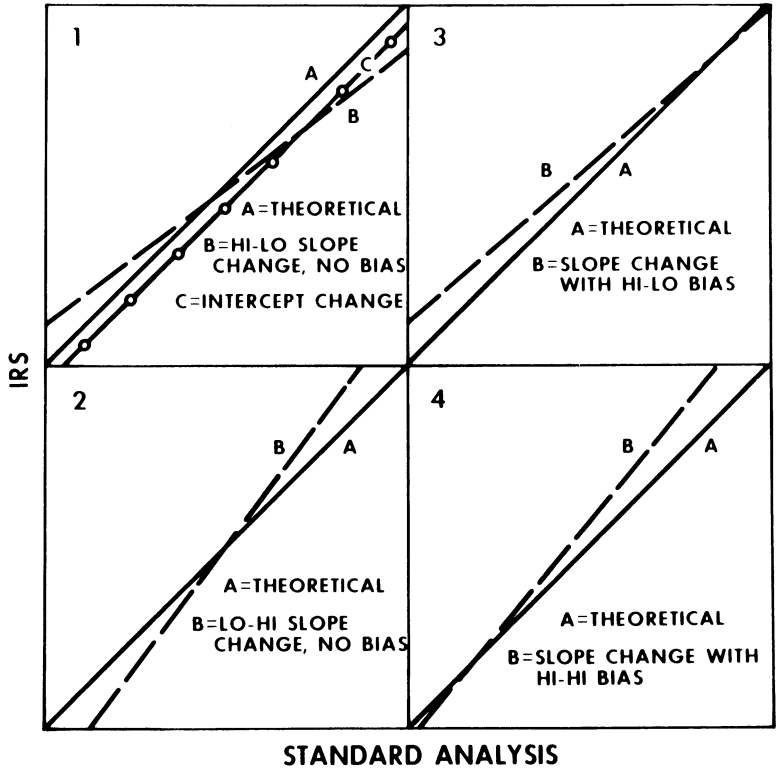


Fig. 1. Types of slope change. IRS = infrared reflectance spectroscopy.

**TABLE III**
**Precision of MPS[a] Measurement for Three Grinders**

| Grinder | | >500 μ | 500–355 μ | 355–212 μ | 212–149 μ | 149–74 μ | <74 μ | MPS | Recovery (%) |
|---|---|---|---|---|---|---|---|---|---|
| Cyclotec 1.0-mm screen | Mean % distribution | 1.86 | 5.74 | 15.89 | 17.58 | 21.71 | 36.62 | 201.7 | 99.4 |
| | SD[b] | 0.29 | 0.51 | 1.01 | 0.41 | 0.86 | 1.39 | 5.41 | 0.39 |
| | CV[c] | 15.6 | 8.9 | 6.4 | 2.4 | 4.0 | 3.8 | 2.7 | 0.4 |
| Hobart 2040 | Mean % distribution | 3.61 | 8.62 | 24.58 | 16.63 | 21.57 | 24.15 | 253.0 | 98.5 |
| | SD | 0.35 | 0.32 | 1.25 | 0.59 | 0.92 | 1.11 | 6.87 | 1.04 |
| | CV | 9.7 | 3.7 | 5.1 | 3.6 | 4.3 | 4.6 | 2.7 | 1.1 |
| Krups 75 | Mean % distribution | 4.10 | 11.71 | 22.97 | 11.74 | 15.80 | 31.30 | 258.4 | 97.7 |
| | SD | 1.07 | 1.96 | 0.86 | 0.62 | 1.22 | 2.83 | 19.06 | 1.24 |
| | CV | 26.1 | 16.8 | 3.7 | 5.3 | 7.8 | 9.1 | 7.4 | 1.3 |

[a]MPS = mean particle size.
[b]SD = standard deviation.
[c]CV = coefficient of variability.

the grinders to establish the reproducibility of the technique. The results are summarized in Table III for each of the six fractions together with the MPS and total recovery.

The Cyclotec grinder showed a slightly better precision than did the Hobart in terms of MPS and recovery. Both of these grinders showed satisfactory levels of precision at the six intervals estimated, which verified the precision of the technique. The Cyclotec grinder with 0.5-mm screen showed the highest degree of precision, followed closely by the Cyclotec with a 1.0-mm screen. At moisture levels up to 13%, the Hobart 2040 burr mill gave excellent precision. The Krups showed rather poor reproducibility at four of the intervals and for the overall MPS.

At higher moistures, measurements of the MPS and PSD of whole meals from the Hobart, LabConco, and Buhler mills were practically meaningless, because the distance between the burrs had to be opened for the wheat to pass. In general, the Krups and Culatti mills were the least precise grinders in terms of MPS and PSD, and the Christie/Norris mill (1.0-mm screen) was equivalent to the Hobart grinder (at "normal" moisture levels).

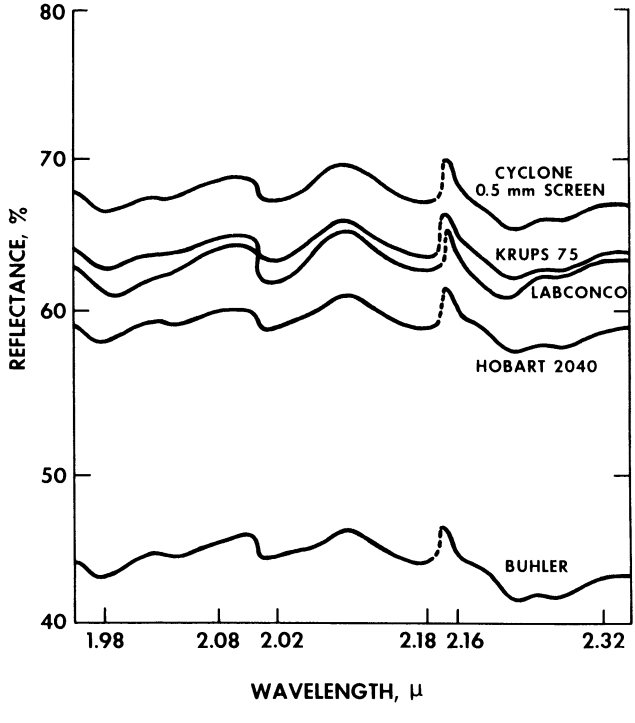


Fig. 2. Influence of grinding on near-infrared reflectance spectrum of hard red spring (HRS) wheat.

**Influence of Grinding Procedure on Granularity of HRS Whole Meal**

Grinding wheat in different grinders, or using different screens, caused major differences in the granularity of the resultant whole meals, which in turn affected the reflectance characteristics. Figure 2 illustrates the gross differences in reflectance of a sample of HRS wheat of 13.8% protein when ground by five different grinding procedures. In general, the finer the whole meal, the higher the reflectance. The Cyclotec grinder with a 0.5-mm screen gave the finest whole meal and the Buhler the coarsest. The reflectance spectra follow this pattern, with the Buhler whole meals giving low reflectance. The LabConco whole meal was anomalous in occupying an intermediate position with regard to reflectance. MPS determinations indicated that this whole meal contained a higher proportion of fine particles (<74 $\mu$) than did the Buhler whole meal (21.0 compared with 10.8%). This was more comparable with the Hobart (24.1%) and resulted in a much brighter sample.

The MPS of four samples of HRS wheat was determined in quadruplicate after grinding by all seven procedures enumerated above. The wheats were selected to give a range in protein content, and were all at an equilibrium moisture level of 11.5–12% before grinding. The complete results are given in Table IV; the analysis of variance is summarized in Table V. An interaction between samples and grinders was revealed. All of the wheats were HRS type; since the principal difference between them was the protein content, this interaction was attributed to protein content. Figures 3–7 illustrate differences in MPS of whole meals ground by four of the grinding procedures. The other three were intermediate. Variation in protein of 5% led to significant differences in both the MPS and PSD of the Krups whole meals. The fines increased from 31 to 42.5% coincident with the increase in protein content. The MPS of LabConco whole meals did not show a consistent trend with variation in protein content, but in general the MPS varied in the reverse direction to that of all other grinders. The Cyclotec and Hobart grinders showed least sensitivity to variation in protein content.

Figure 8 illustrates the effects of protein level on the reflectance characteristics of Hobart-ground whole meals. In general, the pattern follows that of MPS. Since the factors that affect MPS and PSD are not necessarily the same type as those affecting reflectance, numerous exceptions would be expected to occur with this relationship.

Due to the difficulty of grinding high-moisture wheat in the three burr mills, investigating the influence of moisture level on the MPS and PSD of the wheats was not possible. The general trend observed in the reflectance traces of whole meals from wheats with moisture contents ranging from 9 to 13% was that reflectance was related inversely to moisture level. Other workers (5) have commented on the decreased brightness of samples at higher moisture levels. Differences in moisture level were observed to affect the scatter of light from the sample surface.

**Influence of Grinding Procedure on Accuracy of Protein and Moisture Testing by NIRS**

*Precision.* The precision or reproducibility of testing different whole meals was assessed by dividing four bulk wheat samples ranging in protein into seven sets of 25-g samples. The samples were all ground on each grinder and analyzed in duplicate by NIRS to assess the respective standard errors of duplicate testing

**TABLE IV**
**Average Mean Particle Size ($\mu$) of Four Samples of Whole Meals From HRS Wheat of Different Protein Content After Grinding by Seven Different Procedures**

| Sample | Protein (%)[a] | Grinders | | | | | | | Average All Grinders |
|---|---|---|---|---|---|---|---|---|---|
| | | Cyclotec 0.5-mm screen | Cyclotec 1.0-mm screen | Krups 75 | Culatti DHF 48 | Hobart 2040 | LabConco 900 | Buhler | |
| PR 54 | 12.4 | 165 | 199 | 248 | 276 | 284 | 439 | 411 | 288.8a |
| PC 101 | 13.7 | 165 | 209 | 245 | 263 | 266 | 364 | 435 | 282.4ab |
| PR 7 | 15.1 | 161 | 207 | 219 | 254 | 254 | 398 | 437 | 275.6b |
| PR 72 | 17.4 | 148 | 176 | 189 | 216 | 264 | 465 | 408 | 266.5c |
| Average[b] all wheats | ... | 160a | 198b | 225c | 252d | 267e | 417f | 423f | |

[a]13.5% mb.
[b]Means in same row with different letters are significantly different (p = 0.05).

and duplicate grinding. The results are summarized in Table VI for protein on a constant moisture basis. When the grinding error was isolated from the overall testing error (standard deviation between duplicates), the error induced by grinding per se increased linearly with increasing whole meal MPS (r, 0.87). For every increment of 25 $\mu$ in MPS, the grinding error increased by about 0.02%. The Cyclotec with 1.0-mm screen indicated the highest overall degree of precision in both grinding and testing.

Differences between duplicate tests of the same grind reflect differences in the efficiency of mixing that are reflected in the significantly higher standard errors

TABLE V
Analysis of Variance of Grinder (Wheat MPS[a] Study)

| Source | df | MSS | F | P |
|---|---|---|---|---|
| Wheat | 3 | 2,555.546 | 24.7 | 0.01 |
| Grinders | 6 | 168,214.936 | 1,677.4 | 0.01 |
| W × G | 18 | 2,685.149 | 26.0 | 0.01 |
| Error | 84 | 103.366 | ... | |
| Total | 111 | ... | | |

[a]MPS = mean particle size.

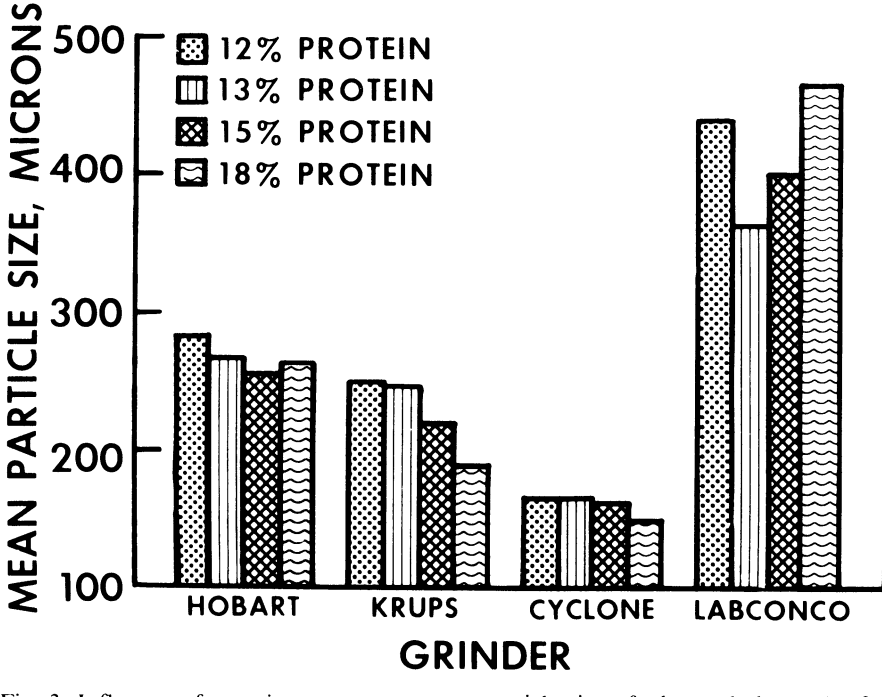


Fig. 3. Influence of protein content on mean particle size of wheat whole meals after grinding by four different procedures.

of duplicate testing. The protein content of all six subfractions resulting from the grinding of four samples of different protein content by the seven grinding procedures was determined by the Kjeldahl method on the bulked material from the testing of the four replicate grinds. The results are summarized in Table VII. The range of up to 5.6% protein in the fractions indicate the variability that can

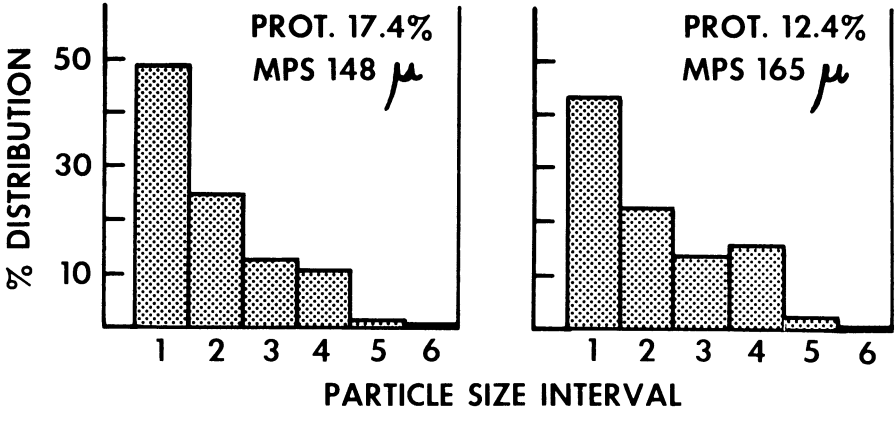


Fig. 4. Influence of protein content on mean particle size distribution (MPS) in wheat whole meals after grinding on Cyclotec grinder (0.5-mm screen).

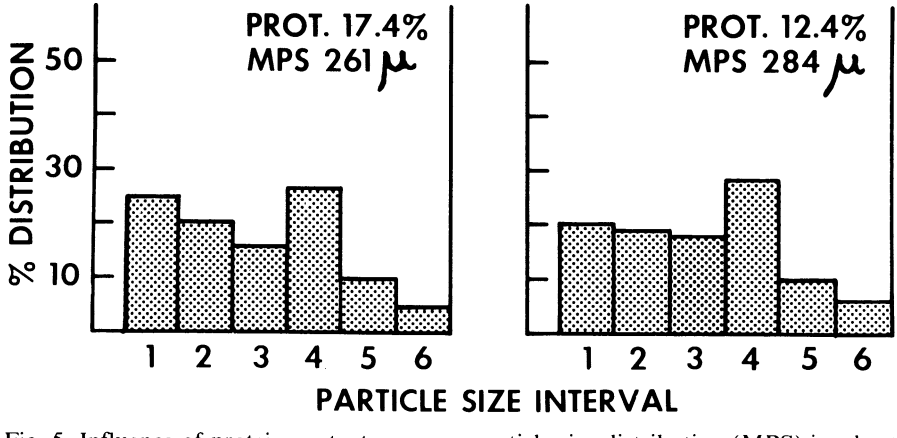


Fig. 5. Influence of protein content on mean particle size distribution (MPS) in wheat whole meals after grinding on Hobart 2040 burr mill.

exist in a sample of whole meal. The variability of the protein contents of the fractions increased significantly at higher protein levels. In general,the fines separate more easily from coarse whole meals than from finer material, and are more difficult to incorporate uniformly. This factor coupled with the higher protein content of the fine fraction of the two coarsest whole meals (Buhler and LabConco) affected the precision of NIRS testing in the above exercise. This

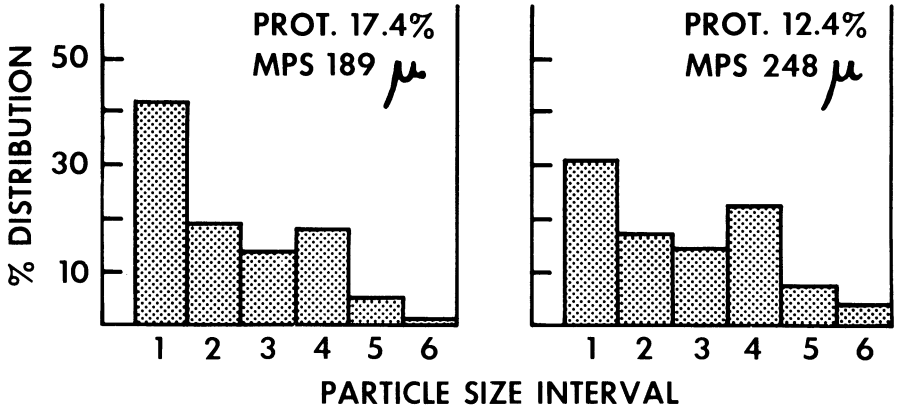


Fig. 6. Influence of protein content on mean particle size distribution (MPS) in wheat whole meals after grinding on Krups 75 impeller mill.

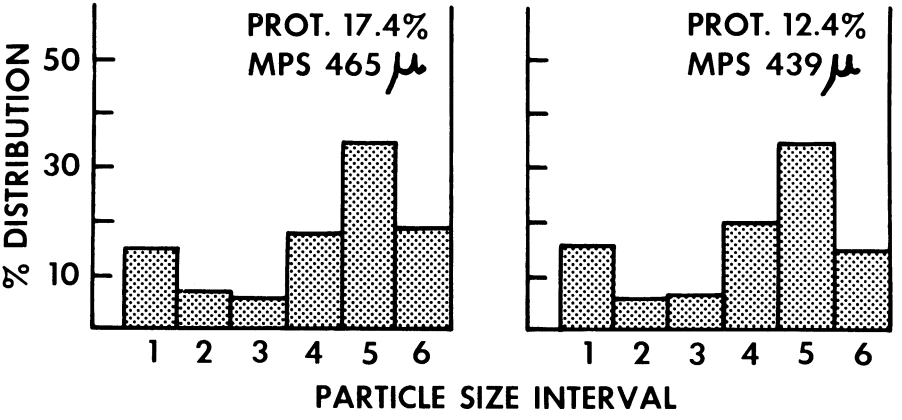


Fig. 7. Influence of protein content on mean particle size distribution (MPS) in wheat whole meals after grinding on LabConco Model 900 burr mill.

phenomenon is not limited to NIRS testing. Adequate mixing is essential to all analysis. Accumulation of fines inside grinders may become dislodged periodically and fall into a sample if the grinders are not of the self-cleaning type. The figures in Table VII indicate the extent of contamination that can occur by the unwitting incorporation of high protein fines into a sample. The converse is true for the loss of fines for any reason.

*Accuracy.* In assessing the accuracy of an NIRS calibration, the usual practice is to analyze a number of samples of known protein and moisture on the instrument. The standard error of difference (SED) between standard and NIRS results is calculated together with the respective means. If the SED multiplied by 100 exceeds 2.5 times the mean result, the calibration should be repeated. If the SED is satisfactory, the difference between the two means (NIRS and standard) can be corrected by adjusting the intercept on the instrument. The SED does not reveal the existence of a bias, nor the type of bias if one exists. For example, a symmetric hi-lo bias may give an accurate result for the overall mean, but all samples occurring below the mean are biased upward by an amount that depends on the deviation from the mean; values higher than the mean are biased downward. Figure 1 illustrates some typical patterns of bias.

If individual deviations are regressed against the original standard results, the

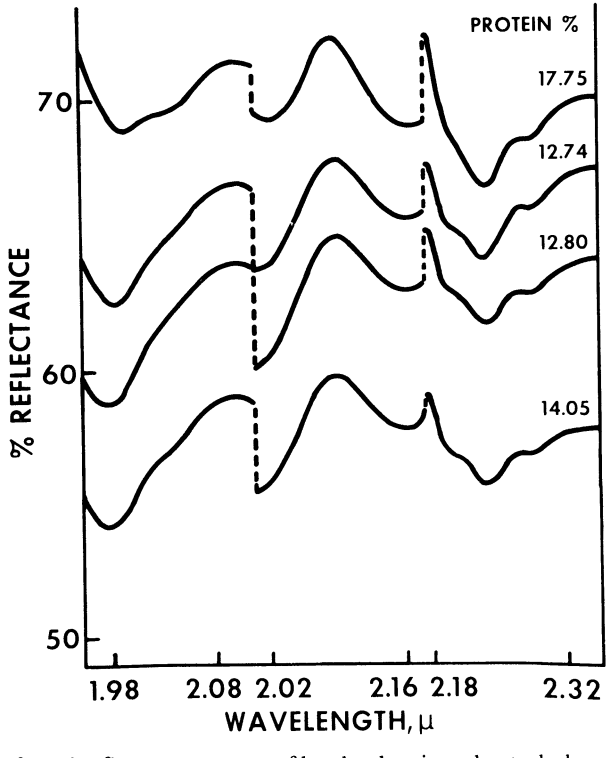


Fig. 8. Near-infrared reflectance spectra of hard red spring wheat whole meals of different protein contents.

regression equation provides the means of calculating the deviations that occur at various levels of, eg, protein, while the correlation coefficient indicates the closeness or validity of the relationship. If a factor does not cause a bias to the extent of affecting the accuracy of a calibration, the correlation coefficient will be low, which means that deviations between NIRS and standard results are distributed randomly, and no bias exists. When a factor exerts a significant influence on the accuracy of a calibration, there is a significant correlation between deviations of NIRS from standard results. For the purpose of this discussion, a statistically significant correlation coefficient was considered to affect the accuracy of a calibration to the extent that inaccuracies cannot be rectified by intercept control.

A set of 40 samples of HRS wheat was subdivided into 7 × 25-g subsamples. Each subseries of 40 samples was ground by a different grinding procedure and the resulting whole meals used to calibrate the GQA. The protein and moisture contents of the wheat were then calculated for all seven series of C values using the seven series of K values, and the mean deviation and root mean square deviation from standard analysis were calculated. The results are summarized in Tables VIII and IX. Some large discrepancies were apparent, as may be expected from the variation in granularity noted above. The boxes in the tables denote the mean results for the analysis of the material of each individual grind on the calibration based on that grind. This procedure of comparing the efficiency of individual calibrations eliminates the errors incurred by remixing and rereading the samples.

Next, the discrepancies for each individual calibration were regressed against the original standard Kjeldahl protein and oven moisture results for all series and the results scrutinized for slope changes. These results are summarized in Tables X and XI. The many instances in which slope changes occurred are additional verification that individual calibrations were necessary for different grinding procedures. In many instances, the discrepancies in moisture figures were correlated to both moisture and protein levels of the original samples. In general, protein discrepancies were not correlated to the moisture level of the samples. The range in moisture in Cyclotec-ground wheat, however, usually is rather restricted; studies were set up to investigate the influence of both protein and moisture status of calibration samples on the accuracy of subsequent testing. These experiments are described below.

**TABLE VI**
**Precision of Duplicate Grinding and Testing of HRS Wheat by Seven Different Grinding Procedures**

| Grinder | SD Duplicate Grinds | SD Duplicate Tests[a] | Grinding Error |
|---|---|---|---|
| Cyclotec (0.5 mm) | 0.216 | 0.190 | 0.103 |
| Cyclotec (1.0 mm) | 0.186 | 0.134 | 0.129 |
| Krups | 0.293 | 0.192 | 0.221 |
| Culatti | 0.284 | 0.178 | 0.221 |
| Hobart | 0.304 | 0.190 | 0.237 |
| LabConco | 0.355 | 0.247 | 0.255 |
| Buhler | 0.434 | 0.236 | 0.364 |

[a]Duplicate tests within a grind.

**TABLE VII**
**Mean Protein Content of Whole Meal Sieved Subfractions From Two Samples of HRS Wheat After Grinding by Seven Different Procedures (13.5% mb)**

| Grinder | Interval | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 500 $\mu$ | 500–354 $\mu$ | 354–212 $\mu$ | 212–149 $\mu$ | 149–74 $\mu$ | 74 $\mu$ | Range (%) | SD |
| | | | Sample A. Unsieved whole meal protein = 12.4% | | | | | |
| Cyclotec | | | | | | | | |
| 0.5-mm screen | ... | 12.0 | 10.7 | 11.1 | 11.9 | 13.7 | 3.0 | 1.15 |
| 1.0-mm screen | 12.8 | 11.3 | 10.6 | 10.9 | 11.7 | 14.2 | 3.6 | 1.36 |
| Krups | 13.6 | 11.1 | 10.5 | 11.6 | 13.1 | 14.0 | 3.5 | 1.44 |
| Culatti | 13.9 | 10.8 | 10.6 | 11.8 | 12.9 | 15.4 | 4.8 | 1.60 |
| Hobart | 12.9 | 12.4 | 11.6 | 11.8 | 12.4 | 14.8 | 3.2 | 1.15 |
| LabConco | 12.1 | 11.3 | 11.8 | 12.3 | 13.6 | 16.1 | 4.8 | 1.76 |
| Buhler | 13.3 | 11.9 | 11.7 | 12.3 | 13.1 | 15.4 | 3.7 | 1.36 |
| | | | Sample B. Unsieved whole meal protein = 17.4% | | | | | |
| Cyclotec | | | | | | | | |
| 0.5-mm screen | ... | 17.1 | 14.5 | 14.2 | 17.1 | 19.5 | 5.3 | 2.18 |
| 1.0-mm screen | 18.2 | 17.3 | 15.0 | 14.6 | 15.5 | 19.8 | 5.2 | 2.05 |
| Krups | 19.6 | 18.8 | 15.3 | 15.0 | 16.3 | 20.3 | 5.3 | 2.30 |
| Culatti | 19.5 | 18.2 | 14.8 | 14.8 | 16.1 | 20.4 | 5.6 | 2.42 |
| Hobart | 18.9 | 18.2 | 15.9 | 15.5 | 16.6 | 20.8 | 5.3 | 2.03 |
| LabConco | 17.2 | 16.0 | 15.8 | 15.1 | 16.1 | 21.9 | 6.8 | 2.49 |
| Buhler | 19.8 | 17.0 | 16.1 | 16.4 | 17.7 | 21.6 | 5.5 | 2.16 |

**TABLE VIII**
**Mean Deviations From Kjeldahl of NIRS Protein Results for HRS Wheat Ground by Seven Different Grinding Procedures and Calculated From Individual Series of C Values and K values**

| | C Values | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Cyclotec 0.5 mm | | Cyclotec 1.0 mm | | Krups | | Culatti | | Hobart | | LabConco | | Buhler | |
| K Values | $\bar{d}$ | SED[a,b] | $\bar{d}$ | SED | $\bar{d}$ | SED | $\bar{d}$ | SED | $\bar{d}$ | SED | $\bar{d}$ | SED | $\bar{d}$ | SED |
| Cyclotec (0.5 mm) | .002 | .25 | .34 | .27 | −.26 | .30 | −.45 | .31 | .43 | .50 | −.98 | .31 | .66 | .46 |
| Cyclotec (1.0 mm) | −.41 | .26 | .004 | .24 | −.69 | .22 | −.75 | .23 | .16 | .27 | −1.34 | .23 | .28 | .35 |
| Krups | .23 | .30 | .44 | .30 | −0.5 | .29 | −.30 | .26 | .61 | .35 | −.92 | .22 | .85 | .53 |
| Culatti | .58 | .33 | .78 | .25 | .30 | .22 | .004 | .19 | .6 | .21 | −.54 | .21 | 1.11 | .25 |
| Hobart | −.45 | .46 | .09 | .35 | −84 | .32 | −.39 | .34 | .01 | .25 | −1.36 | .29 | .11 | .25 |
| LabConco | 1.17 | .68 | 1.37 | .31 | 1.03 | .29 | .52 | .32 | 1.57 | .34 | .06 | .30 | 1.75 | .40 |
| Buhler | −.59 | .52 | .06 | .44 | −1.07 | .38 | −.27 | .42 | −.19 | .33 | −1.48 | .44 | −.11 | .33 |
| MPS ($\mu$) | 160 | | 198 | | 225 | | 252 | | 267 | | 417 | | 423 | |

[a]SED = standard error of difference.
[b]Values in boxes were obtained from individual grinder calibration on its own material. In these cases, SED values are standard errors of estimate.

**TABLE IX**
**Mean Deviations From Oven Moisture of NIRS Moisture Results for HRS Wheat Ground by Seven Different Grinding Procedures and Calculated From Individual Series of C Values and K Values**

| | C Values | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Cyclotec 0.5 mm | | Cyclotec 1.0 mm | | Krups | | Culatti | | Hobart | | LabConco | | Buhler | |
| K Values | $\bar{d}$ | SED[a,b] | $\bar{d}$ | SED | $\bar{d}$ | SED | $\bar{d}$ | SED | $\bar{d}$ | SED | $\bar{d}$ | SED | $\bar{d}$ | SED |
| Cyclotec (0.5 mm) | −.06 | .11 | −.18 | .27 | .64 | .58 | .47 | .34 | .80 | .61 | .65 | .32 | 1.37 | .81 |
| Cyclotec (1.0 mm) | .06 | .35 | −0.1 | .14 | .74 | .80 | .56 | .41 | .88 | 1.21 | .57 | .36 | 1.73 | .75 |
| Krups | −.54 | .71 | −.72 | .61 | .02 | .17 | −.13 | .42 | .36 | .28 | −.03 | .59 | .68 | .51 |
| Culatti | −.60 | .32 | −.72 | .35 | .08 | .61 | −.07 | .12 | .56 | .67 | −.03 | .3 | 1.05 | .54 |
| Hobart | −.87 | .44 | −.94 | .54 | −.12 | .34 | −.30 | .53 | −.12 | .40 | −.15 | .66 | .37 | .51 |
| LabConco | −1.23 | .41 | −1.52 | .22 | −.30 | .71 | −.45 | .29 | −.65 | .82 | −.02 | .23 | −.66 | .80 |
| Buhler | −1.16 | .42 | −1.25 | .56 | −.63 | .52 | −.68 | .38 | −.24 | .41 | −.26 | 1.11 | .08 | .16 |

[a]SED = standard error of difference.
[b]Values in boxes were obtained from individual grinder calibration on its own material. In these cases, SED values are standard errors of estimate.

**Influence of MPS on Accuracy of NIRS Testing of HRS Wheat for Protein and Moisture Under Conditions of Constant Grinding Action but Different Screen Size**

The foregoing details with regard to the influence of granularity on NIRS testing were based on data gathered from whole meals using seven different grinding procedures and six different grinders. The next investigation dealt with the influence of MPS when the same grinder but different screen apertures were used. The Christie/Norris 8-in. hammer mill was used. This mill operated at 8,500 rpm and was fitted with five screens ranging in aperture from 0.5 to 2.5 mm. MPS ranged from 231 to 564 $\mu$. Five series of whole meals were prepared, each using 40 samples, and the GQA was calibrated with each series. The protein and moisture values were calculated using the respective C and K values. Discrepancies in protein and moisture from standard values were regressed against standard values as described above. The results are summarized in Tables XII–XIV. In general for each set of C values, both protein and moisture were correlated inversely with MPS, so that using K values derived from material of increasing MPS, the results for both protein and moisture were progressively lower.

Using constant K values, and C values that were MPS dependent, we found that deviations of GQA protein from Kjeldahl were generally insignificant up to K values derived from material with MPS of 420 $\mu$; the K values derived from coarser material gave progressively larger negative deviations. Deviations changed little when moving from "fine" to "coarse" C values at coarser K values, although in analyzing progressively coarser material using fine K values, the finest series consistently gave the largest discrepancies from Kjeldahl. Deviations in GQA moisture became more positive with C values of increasing coarseness at all sets of K values. For K values derived from finer material, moisture deviations were positive, and increased to nearly +1% for the coarsest C values at the finest K values. As the K values were derived from coarser material, the moisture deviations become progressively more negative, and at the coarsest set of K values, the finest C values gave negative discrepancies of nearly −0.8%.

With individual calibrations, evidence of slope changes appeared in all series

**TABLE X**
**Correlation Coefficients Between Discrepancies of NIRS From Kjeldahl Protein Against Kjeldahl Protein for HRS Wheat Ground by Seven Procedures and Calculated Using Individual K Values and C Values[a]**

| | C Values | | | | | | |
|---|---|---|---|---|---|---|---|
| K Values | Cyclotec 0.5 mm | Cyclotec 1.0 mm | Krups | Culatti | Hobart | LabConco | Buhler |
| Cyclotec (0.5 mm) | +.02 | +.40 | +.60 | +.61 | +.58 | +.61 | +.87 |
| Cyclotec (1.0 mm) | −.47 | −.19 | −.26 | +.20 | +.59 | +.21 | +.80 |
| Krups | −.20 | +.17 | +.40 | +.37 | +.71 | +.49 | +.91 |
| Culatti | −.77 | −.58 | −.34 | −.15 | +.21 | −.28 | +.62 |
| Hobart | −.77 | −.62 | −.55 | −.43 | −.04 | −.44 | +.36 |
| LabConco | −.40 | +.03 | +.23 | +.34 | +.59 | +.31 | +.73 |
| Buhler | −.64 | −.46 | −.40 | −.27 | −.03 | −.20 | −.28 |

[a]Correlation coefficients greater than 0.39 were statistically significant in this study.

**TABLE XI**
**Correlation Coefficients Between Discrepancies of NIRS From Oven Moisture Against Oven Moisture and Kjeldahl Protein for HRS Wheat Ground by Seven Procedures and Calculated From Individual K Values and C Values[a,b]**

| | C Values | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Cyclotec 0.5 mm | | Cyclotec 1.0 mm | | Krups | | Culatti | | Hobart | | LabConco | | Buhler | |
| K Values | r $H_2O$ | r Kj.[a] | r $H_2O$ | r Kj. | r $H_2O$ | r Kj. | r $H_2O$ | r Kj. | r $H_2O$ | r Kj. | r $H_2O$ | r Kj. | r $H_2O$ | r Kj. |
| Cyclotec (0.5 mm) | −.11 | +.14 | −.51 | +.67 | +.41 | +.03 | −.23 | +.55 | +.44 | −.38 | −.63 | +.63 | −.39 | +.03 |
| Cyclotec (1.0 mm) | +.64 | −.44 | +.23 | +.01 | +.70 | −.17 | +.40 | +.08 | +.27 | −.11 | +.28 | −.08 | +.80 | −.33 |
| Krups | +.49 | +.07 | +.52 | +.19 | +.23 | −.01 | +.37 | −.33 | +.27 | −.85 | +.42 | +.23 | +.57 | −.08 |
| Culatti | +.58 | −.46 | +.28 | −.09 | +.52 | −.27 | +.23 | −.15 | +.54 | −.65 | −.03 | −.05 | +.74 | −.41 |
| Hobart | +.37 | +.45 | +.26 | +.60 | −.03 | +.67 | +.11 | +.74 | +.22 | −.31 | +.30 | +.60 | +.64 | +.30 |
| LabConco | +.76 | −.70 | +.45 | −.29 | +.69 | −.42 | +.44 | −.34 | +.63 | −.60 | +.01 | −.52 | +.83 | −.44 |
| Buhler | +.38 | +.04 | +.24 | +.24 | +.35 | −.29 | −.13 | +.38 | +.22 | −.56 | −.22 | +.37 | +.20 | −.13 |

[a]r $H_2O$ = coefficient of correlation between discrepancies in NIRS and oven moisture values and oven moisture value itself.
r Kj. = coefficient of correlation between moisture discrepanices and Kjeldahl protein.
[b]Correlation coefficients of greater than 0.39 were statistically significant in this study.

**TABLE XII**
**Mean Discrepancies of NIRS Protein and Moisture Values From Standard Oven Moisture Values and Kjeldahl Protein Contents of Original Samples[a]**

| | C Values | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | I | | II | | III | | IV | | V | | Overall | |
| K Values | $\bar{d}$ $H_2O$ | $\bar{d}$ Protein | $\bar{d}$ $H_2O$ | $\bar{d}$ Protein | $\bar{d}$ $H_2O$ | $\bar{d}$ Protein | $\bar{d}$ $H_2O$ | $\bar{d}$ Protein | $\bar{d}$ $H_2O$ | $\bar{d}$ Protein | $\bar{d}$ $H_2O$ | $\bar{d}$ Protein |
| I[b] | −.02 | −.21 | .23 | .04 | .70 | .16 | .92 | −.08 | .92 | −.03 | .54 | −.02 |
| II | −.12 | −.58 | .04 | −.17 | .41 | .22 | .60 | .20 | .57 | .28 | .30 | −0.1 |
| III | −.52 | −.60 | −.36 | −.36 | −.02 | −.12 | .14 | −.22 | .15 | −.20 | −.12 | −.30 |
| IV | −.77 | −1.46 | −.58 | −1.38 | −.19 | −1.26 | .03 | −1.44 | .02 | −1.48 | −.30 | −1.40 |
| V | −.79 | −2.04 | −.64 | −1.90 | −.27 | −1.74 | −.10 | −1.90 | −.14 | −1.92 | −.39 | −1.90 |
| Overall | −.44 | −.98 | −.26 | −.75 | .13 | −.55 | .32 | −.69 | .30 | −0.66 | .006 | −.77 |

[a]Negative figures indicate that NIRS figures were lower than standard.
[b]MPS for respective screens were I = 231 $\mu$, II = 289 $\mu$, III = 421 $\mu$, IV = 519 $\mu$, V = 564 $\mu$.

above 290 $\mu$ for both protein and moisture testing when individual deviations were regressed against the standard analytic results for each sample. This verified that in the case of coarser whole meals, the accuracy of NIRS testing is affected by both the protein and moisture levels in addition of MPS. In view of the fact that whole meals obtained from burr mills are usually well above 300 $\mu$ in MPS, these grinders generally are not to be recommended for use in any other than screening operations.

In the case of such screening operations as breeding programs in which large populations are to be classified roughly into sublevels of widely different protein or moisture levels or both, burr mills are adequate.

**Influence of Protein Level of Calibration Samples on Subsequent Analysis**

The interactions between protein and MPS appeared to be particularly important in the case of coarser material. A separate study has shown that the correlation of MPS to protein was 0.024 in the case of Cyclotec-ground HRS wheat (overall MPS, 196 $\mu$) and −0.491 for Hobart-ground whole meals (MPS, 321 $\mu$). In the case of the Hobart whole meals, the PSI and protein were only weakly correlated (r, +0.168).

To investigate the extent to which this influence affected the accuracy of NIRS protein measurement, six separate calibrations were set up, on a GQA Model 31. Each calibration used 40 samples; the mean protein content of the six individual series ranged from 10.5 to 15.5% (13.5% mb). After the analysis of 12 "unknowns" of each level, 6 new series of 12 unknowns were reanalyzed on each calibration to verify the accuracy of individual calibrations. The results are summarized in Table XV. In general, the following results were found:

1. The analysis of higher-protein samples on calibrations based on low-protein material resulted in a downward bias to the results of up to 0.7%.
2. The analysis of low-protein samples on "high-protein" calibrations also led to a significant downward bias. In other words, low-protein calibrations were only suitable for the analysis of low-protein samples, and vice versa.
3. With the exception of the high-protein samples/low-protein calibration combination, the deviations were not generally large, and the recommended calibration practice of including a number of samples at different protein levels over a range of about 6% protein was found for all practical purposes to eliminate possible errors introduced by protein content itself.

**TABLE XIII**
**Correlations Between Discrepancies in NIRS and Kjeldahl Protein Values and Original Kjeldahl Protein Contents[a]**

| | C Values | | | | | |
|---|---|---|---|---|---|---|
| K Values | I | II | III | IV | V | Overall |
| I | +.21 | +.31 | +.34 | +.02 | +.13 | +.20 |
| II | +.14 | −.20 | +.22 | +.20 | −.09 | +.05 |
| III | +.77 | +.80 | +.67 | +.66 | +.56 | +.69 |
| IV | +.70 | +.82 | +.65 | +.68 | +.57 | +.68 |
| V | +.81 | +.87 | +.69 | +.71 | +.71 | +.76 |

[a]Correlation coefficients greater than 0.39 were statistically significant in this study.

**TABLE XIV**
**Correlations Between Discrepancies in NIRS Moisture Values and Oven Moisture and Kjeldahl Protein Values[a]**

| | C Values | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | I | | II | | III | | IV | | V | |
| K Values | r $H_2O$ | r Protein | r $H_2O$ | r Protein | r $H_2O$ | r Protein | r $H_2O$ | r Protein | r $H_2O$ | r Protein |
| I | −.73 | .61 | −.85 | .60 | −.73 | .46 | −.20 | −.14 | −.54 | .22 |
| II | .08 | −.52 | −.34 | −.29 | −.22 | −.38 | −.43 | −.16 | .06 | −.53 |
| III | −.28 | .40 | −.65 | −.04 | −.41 | −.26 | −.62 | −.11 | −.28 | −.39 |
| IV | −.24 | −.14 | −.62 | .16 | −.38 | −.06 | −.54 | −.1 | −.15 | −.26 |
| V | −.68 | .40 | −.87 | .58 | −.82 | .52 | −.83 | .42 | −.74 | .52 |

[a]Correlation coefficients of greater than 0.39 were statistically significant in this study.

**Influence of Moisture Status of Samples Used in Calibration on Accuracy of Subsequent NIRS analyses for Protein and Moisture**

Calibrations are sometimes set up using samples of grain that have been on hand for several days or weeks, usually due to the time involved in accumulating sufficient samples of different protein content. Unless the samples are carefully stored to prevent moisture loss, they may lose several percentage points of moisture under normal laboratory conditions. On the other hand, tempering grain to obtain calibration samples with sufficient range of moisture is sometimes necessary. To investigate the influence of moisture status on the accuracy of subsequent NIRS analysis, a series of 50 samples were compiled with a range of more than 6% protein, and an average moisture content of 12% before grinding.

**TABLE XV**
**Influence of Protein Level of Calibration Samples on Deviations of IRS From Kjeldahl Protein in HRS Wheat**

| Calibration Protein Level | Wheat Protein | | |
|---|---|---|---|
| | 10.5 | 13.5 | 15.5 |
| 10.5 | −.02 | −.22 | −.67 |
| 11.5 | +.03 | −.08 | −.38 |
| 13.5 | −.27 | −.06 | −.06 |
| 15.5 | −.20 | +.05 | ± 0 |

**TABLE XVI**
**Influence of Moisture Level of Calibration Samples on Deviations of IRS From Kjeldahl Protein in HRS Wheat**

| Calibration $H_2O$ Status | | Wheat $H_2O$ Status | | |
|---|---|---|---|---|
| | | Low | As Is | Tempered |
| Low | ( 9.2)[a] | +.04 | +.16 | +.27 |
| As is | (10.5) | +.07 | ± 0 | −.11 |
| Tempered | (12.3) | −.38 | −.14 | +.05 |

[a]Mean oven moisture level of series.

**XVII**
**Influence of Moisture Level of Calibration Samples on Deviations of IRS From Standard Oven $H_2o$ in HRS Wheat**

| Calibration $H_2O$ Status | | Wheat $H_2O$ Status | | |
|---|---|---|---|---|
| | | Low | As Is | Tempered |
| Low | ( 9.2)[a] | ± 0 | −.15 | −.67 |
| As is | (10.5) | −.12 | +.01 | +.11 |
| Tempered | (12.3) | −.08 | −.04 | −.05 |

[a]Mean oven moisture level of series.

The samples were divided into three subseries, the first, or "low moisture," series was allowed to dry in open dishes for several days under low humidity conditions. The second series was left as is, while the third, or "high moisture," series was tempered to 14–18% moisture by adding distilled water, and stored in sealed cans for two weeks. All three series were ground on a Cyclotec grinder, and 38 samples of each series used to set up calibrations on the GQA. The remaining 12 samples were analyzed as unknowns to verify the accuracy of individual calibrations, and the intercepts adjusted where necessary.

Next, all three series of unknowns were analyzed for protein and moisture on the three calibrations. The results, summarized in the form of deviations from standard analysis are presented in Tables XVI and XVII. The following observations were apparent:

1. The NIRS analysis for protein of samples of as-is (or "normal") and high-moisture status on calibrations based on low-moisture samples gave results that were biased upward. As-is samples were 0.16% high on an average, with individual deviations of up to +1%. High-moisture samples were 0.27% high on an average, with individual deviations of up to +1.6%.
2. The NIRS analysis for protein of low-moisture samples on calibrations based on high-moisture material gave results with a downward bias of nearly 0.4%, and individual deviations of up to −1.4%.
3. In NIRS moisture analysis, the analysis of high-moisture samples on low-moisture calibrations led to an overall downward bias of nearly 0.7%, with individual errors of 1.5%. A significant correlation existed between the deviations of low-moisture samples from standard results when analyzed on high-moisture calibrations, but since the maximum moisture content of samples classified as low was 10.2%, the average deviation was only about 0.1%.
4. Ideally for North American conditions, the mean moisture level for samples of cereals used in NIRS calibration should be about 10.5–12.5% after grinding.

## Summary

The foregoing commentary has indicated some of the reasons underlying the influence of whole-meal granularity and related factors on the analysis of HRS wheat for protein and moisture by NIRS instrumentation. Certain situations

**TABLE XVIII**
**Summary of Situations Causing Slope Changes in HRS Wheat Calibrations**

| Calibration Samples | Samples to Be Analyzed | |
|---|---|---|
| | Protein | Moisture |
| 500 μ MPS | All | All |
| 400 μ MPS | All | 400 μ + |
| 300 μ MPS | ... | 500 μ + |
| 200 μ MPS | ... | 400 μ + |
| Low $H_2O$ | High $H_2O$ | Medium High $H_2O$ |
| Medium $H_2O$ | ... | Low $H_2O$ |
| High $H_2O$ | ... | Low $H_2O$ |
| Low Protein (10.5) | Low and High Protein | ... |
| High Protein (15.5) | Low Protein | ... |

have been shown in which simple intercept changes were inadequate to correct for the possibility of discrepancies in the results. Situations in which such slope changes occur are summarized in Table XVIII. In brief, the steps necessary to achieve the most accurate test results can be embodied in an efficient calibration procedure. These include:

1. Avoid the use of burr mills or impeller mills for sample preparation; use a high-speed hammer mill, or similar mill fitted with a screen to regulate particle size. Regulate the amount ground by means of a sampling cup. Grind as much sample as possible, but for rapid throughput, about 20–25 g is optimum.
2. Optimum MPS for NIRS analysis of HRS wheat whole meals lies between 180 and 220 $\mu$.
3. Use at least 50 samples.
4. Select the samples to include at least six in all protein percentage levels likely to be encountered, eg, with a range from 10–16% protein, provide at least six samples between 10 and 11% protein. This sample distribution is non-Gaussian, but leads to improved accuracy, since the variance incorporated into the calibration enables the instrument to accept a wider range of protein.
5. The same considerations apply to moisture levels. If necessary, adjust the moisture levels of samples by tempering or air drying, so that at least five levels of moisture (eg, 10–15%) occur *within each protein percentage subgroup.*
6. Proceed according to the manufacturer's manual after assembling and preparing calibration samples as described in steps 1–5. This calibration procedure should minimize the possibility of errors incurred by slope factors referred to above.

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