Screening Wheat for Protein and Hardness by Near Infrared Reflectance Spectroscopy

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ABSTRACT

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Wheat was screened for protein and hardness by near infrared reflectance spectroscopy. Two series of wheats, I and II, were assembled, both of which varied widely in Kjeldahl protein and hardness, as assessed by the particle size index (PSI) test. All wheats were ground on both a burr mill and an impeller-type mill that was fitted with a 1.0-mm screen. A Neotec Model 31 Grain Quality Analyzer was calibrated against protein and PSI for the burr milled series I samples and for protein only on the impeller milled series I samples. In series II of burr milled samples, protein was predictable to within 0.7% and PSI to within less than two units. Both parameters are satisfactory for screening early generations of wheat for protein and hardness in breeding programs. Protein was predictable to within 0.31% in series II of impeller-ground wheat, which contained wheat ranging from durum to soft white and club wheats. Correlations between PSI and mean particle size (MPS) were high for burr mill-ground wheats but much lower for impeller-ground wheats. PSI figures for wheats ground by burr and impeller mills were closely correlated. Correlations between protein and MPS were −0.49 for burr mill-ground wheat and +0.02 for impeller mill-ground wheat.

In breeding wheat for processing quality and nutritional value, two main factors must be considered, namely those that are genetically controlled and those that are affected mainly by environmental and cultural agents. The protein content of wheat is well documented as the most important criterion for most aspects of processing capability and nutritional value. Also, the physical structure, texture, or hardness of the wheat kernel affect both the manner of its breakdown to a meal or a flour and, in particular, the behavior of flours during their subsequent use. Wheat flour is usually processed into products that require some form of fermentation or other method of gas production, and the condition of the starch has the most effect on this aspect of wheat processing. Mechanical damage to starch granules always occurs to varying degrees during the grinding or milling of wheat, and the hardness or texture of the wheat kernel largely controls this characteristic. An earlier publication summarized the influence of wheat kernel texture on the damaged starch level and related properties of flour milled from the wheat (Williams 1967).

The amount of protein incorporated in the wheat kernel is controlled to a great extent by environmental factors. Weather conditions during maturation, soil nitrogen status, cultivation practice in general, and use of fertilizers account for about 95% of the reasons underlying variance in the protein content of wheat. On the other hand, the primary factor responsible for variance in wheat texture is the variety or genetic background of the wheat (Symes 1965). To a lesser extent, protein content and weather conditions, particularly during maturation, also exert an effect on the texture or hardness of the grain.

The hardness of wheat is conveniently assessed by the particle size index (PSI) test (Symes 1961). The Kjeldahl test remains the standard method for determining protein in wheat (AACC Method 44-16). One PSI test requires about 12–15 min. Using a batch system one worker can process up to 80 samples per day. A worker using Kjeldahl equipment can process about the same number of samples, including sample preparation. In wheat breeding programs involving hard and soft cultivars at different protein levels, screening large populations of early generation (to F_4) material presents such a large task in terms of time and expense that screening for protein and texture is often delayed to later generations.

Analysis by near infrared reflectance spectroscopy (NIRS) is markedly affected by the mean particle size (MPS) of the ground material (Williams 1975, Williams and Thompson 1978). We assumed that if a satisfactory relationship existed between the MPS of pulverized grain and the hardness of the original wheat grain, the NIRS system should be able to screen early and subsequent filial generations simultaneously for protein and hardness.

EXPERIMENTAL

Common wheats can be subdivided into eight classes, based on color, habit, and texture, ie, red or white, spring or winter, and hard or soft. Two series of wheats were assembled that contained examples of all these types. Wheat varieties from Canada, the United States, South America, South Africa, Europe, India, and Australia and also some varieties of *Triticum durum* and *T. compactum* were included. Series I consisted of 88 samples and series II of 66 samples. The large number of samples was necessary so that varieties of all of the eight classes of common wheat could be included. Calibrations are normally done on 40–50 samples. Both series were divided into two equal subgroups, one of which was ground on the Udy Cyclotec sample mill (1.00-mm screen), which is the grinder normally used by the Canadian Grain Commission in conjunction with NIRS testing for protein. The second set was ground on the Hobart Model 2040 grinder, using pulverizing burrs. Hardness was assessed by a modification of the PSI test. Samples (25g) of wheat were ground, well mixed, and 10-g subsamples sieved for 10 min on a Rotap sieve shaker, using 200-mesh stainless-steel screens with an aperture of 74μ. Usually about 25 g is ground for testing by NIRS and Kjeldahl at the Canadian Grain Commission, but since the MPS of wheat is affected by the volume of grain ground, and by its hardness, PSI and MPS had to be assessed on the basis of 25-g grinds instead of the standard 10 g. To establish the integrity of the modified PSI test, a set of check tests was done using the LabConco burr mill with the standard PSI test procedure as modified by Symes (1961). The Hobart Model 2040 coffee grinder is much faster than the LabConco and is self-cleaning. A control sample was tested every tenth sample to assess the precision of the standard and the modified PSI procedures. The MPS of the ground wholemeals was assessed by an arbitrary system that involved sieving for 15 min through a nest of five stainless-steel sieves (Williams and Thompson 1978). The weights of the throughs and the overs on the top sieve, were multiplied by the apertures of the sieves to give a standard of comparision between the MPS of the wholemeals of the various types of wheat. The sieves and the respective apertures are listed in Table I. The overs on the top sieve were assigned a value of 1,000 μ after microscopic evaluation (Williams and Thompson 1978). Protein was determined by the Kjeldahl test (AACC Method 44-16). A Neotec Model 31 Grain Quality Analyzer (GQA)[2] was used for all NIRS testing.

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[2]Citation of equipment does not constitute endorsement by the Canadian Grain Commission.

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RESULTS

Assessment of Hardness by PSI Test

Table II summarizes the PSI figures for some typical wheats after grinding on three grinders: the LabConco (using the standard 10-g sample of whole grain) and the Hobart and Cyclotec using about 25 g of whole grain, with 10-g subsamples used for PSI testing. The PSI figures obtained by the modified Hobart procedure were closely related to the standard LabConco PSI figures but not the Cyclotec figures. The 1.0-mm screen in the Cyclotec grinder reduced the variance in PSI caused by the natural texture of the grain. The PSI figures yielded by Hobart and LabConco grinds established durum wheats as the hardest, followed by Australian varieties of hard white spring (HWhS), hard red spring (HRS), hard red winter (HRW), hard white winter (HWhW), soft red winter (SRW), soft white winter (SWhW), and soft white spring (SWhS) wheats, in that sequence.

Relation of Hardness to MPS of Wheat Wholemeals

The MPS as assessed by the sieving technique (Williams and Thompson 1978) affords a satisfactory comparison of wheats and a method for monitoring the reproducibility of a grinding technique. Table III illustrates the statistical relationships of some typical wheats of different PSI to the MPS of wholemeals from Cyclotec and Hobart grinds. As noted earlier (Williams and Thompson 1978), the MPS of LabConco-ground wholemeals is much higher than those of Hobart or Cyclotec-ground wheats and is more susceptible to fluctuations in protein content. As a result, LabConco wholemeals generally are not recommended for NIRS. The MPS of the Hobart wholemeals was highly correlated with the PSI. Precision of the MPS test (±7.9 μ), together with the root mean square difference (RMSD) of 5.6 between observed and calculated values for the MPS of "unknowns," enabled a satisfactory prediction of the MPS from the PSI figure. The reverse relationship gave a RMSD of 3.3 PSI units and a standard error per test of 1.2 PSI units.

The similarity of the Hobart and LabConco PSI figures, the close relationship between Hobart wholemeal MPS and PSI values, and the sensitivity of NIRS instrumentation to variations in MPS indicated that hardness, as assessed by PSI, could be estimated in wheat by calibrating a NIRS instrument to PSI values for Hobart-ground wheat. The 88 wheat samples were ground on the Hobart grinder and the GQA calibrated, the three C values being regressed directly against the PSI figures. Although the standard error of estimate and other statistical data were satisfactory, the regression coefficients were too high for programming the cue-card because of the high variance in the PSI values; the standard deviation was ±7.9 PSI units. The regressions were rerun, using the PSI values ÷ 10, which gave satisfactory regression coefficients. The second series of wheats (66 samples) were then analyzed for PSI as "unknowns"; a reground check sample of HRS wheat was included every five samples to establish the precision of the GQA hardness test. Results are summarized in Table IV. The Hobart-ground material gave excellent discrimination among varieties on the basis of PSI hardness. PSI testing by Hobart/GQA was more precise than conventional PSI testing, because the errors induced by the GQA instrumentation were less than those induced by the more cumbersome sieving process. With grinding by Hobart and testing by GQA, one operator could perform more than 200 PSI tests per day.

NIRS Measurement of Protein in Wheats of Different Type and Hardness

A common criticism of NIRS analysis of wheat is that different wheat types require separate calibrations because of variations in the texture and hence the MPS of the ground wheat (Hunt et al 1978). Our study used two sets of 88 Hobart and Cyclotec-ground wheats to calibrate the GQA 31 for protein, with the Kjeldahl results as standards. The two sets of 66 unknowns, consisting of

TABLE I
Sieves Used in MPS Estimation

Mesh (U.S.)	Aperture (μ)
35	500
45	354
70	210
100	149
200	74

TABLE II
Particle Size Index (PSI) Values for Wheats of Different Types after Grinding on Three Grinders[a]

Wheat Type[b]	PSI (A) LabConco	PSI (B) Hobart	PSI (C) Cyclotec
HRSp I	19.5	19.0	50.5
HRSp II	18.2	20.0	51.1
HRW I	19.0	21.9	55.0
HRW II	28.1	25.3	54.3
SRW I	32.0	34.6	69.6
SRW II	35.3	37.8	56.8
HWhSp I	19.4	17.1	47.0
HWhSp II	18.8	16.1	50.2
HWhW I	25.1	27.2	63.8
SWhSp I	29.3	31.1	63.5
SWhSp II	30.0	35.4	70.8
SWhW I	33.4	40.6	66.8
SWhW II	31.3	31.3	54.5
Durum I	9.0	12.0	36.7
Durum II	8.3	10.0	40.7
Standard error per test	0.7	1.2	2.6

Statistical Details (N = 52)

Relationship	r[c]	P	b	a	SEE
A:B	0.98	0.01	0.85	2.9	3.2
A:C	0.89	0.01	0.72	−14.6	5.1
B:C	0.91	0.01	0.84	33.3	5.2

[a] Abbreviated from N = 52.
[b] H = hard; S = soft; Wh = white; R = red; Sp = spring; W = winter.
[c] r = correlation coefficient; P = probability; b = regression coefficient; a = intercept; SEE = standard error of estimate.

TABLE III
Mean Particle Size (MPS) and Particle Size Index (PSI) for Two Series of Wholemeals (N = 66)

Parameter	Cyclotec (1.0-mm Screen) MPS (A) (μ)	Cyclotec (1.0-mm Screen) PSI (B) (%)	Hobart MPS (C) (μ)	Hobart PSI (D) (%)
$\bar{x}$	207	54.7	302	25.5
Standard deviation	16.2	9.2	29.7	9.0
Standard error per test	5.9	2.6	7.9	1.2

Statistical Details (N = 66)

Relationship	r[a]	P	b	a	SEE	RMSD[b]
B:A	0.133	Not Significant	...	...	...	...
D:C	−0.958	0.01	−2.37	363.0	8.7	9.1
B:D	0.972	0.01	0.93	−22.9	2.88	...
A:C	−0.201	Not significant	...	...	...	...

[a] r = correlation coefficient; P = probability; b = regression coefficient; a = intercept; SEE = standard error of estimate.
[b] RMSD = Root mean square difference = $\sqrt{d^2/(N-1)}$, where d = difference between value of actual MPS and MPS as predicted from regression.

wheats of widely different types, were then analyzed for protein by NIRS. Results are given in Table V.

The Hobart-ground series showed the larger deviations from the Kjeldahl values, with an overall RMSD of ±0.69% protein. Segregation of the wheats was reasonably good, however, on a protein basis by Hobart grinding. The superiority of the burr-mill grinder for hardness screening was demonstrated. One objective was to establish the degree to which the near infrared reflectance technique could be used to screen early generation genetic material of widely differing texture into approximate levels of protein. The Hobart grinder afforded a method for screening early filial generations of wheat (to F_5) simultaneously for both hardness and protein, so that one operator could do more than 200 tests for the two parameters, including grinding the samples, in a 7.5-hr day. Two operators could process more than 500 per day.

In the Cyclotec-ground series, the overall RMSD of ±0.31 was remarkable in view of the wide variation of wheats in the analysis. These results demonstrate that if wheat is ground in a grinder such as the Cyclotec, which minimizes variations in wholemeal MPS, and provided that wheat types conveying all of the variance likely to be encountered in future analyses are incorporated in the calibration, then a fairly accurate segregation of wheats by protein can be achieved by using one calibration for all types of wheat. The reason the Cyclotec grinder performs better than the Hobart for protein screening in highly variable populations lies partly in the increased uniformity of the MPS of the Cyclotec wholemeal and partly in the fact that the MPS of Cyclotec wholemeal is less sensitive to variations in protein content than is that of Hobart wholemeal. In a series of HRS wheats with 9.8–17.7% protein (13.5% moisture basis), the correlation between MPS and protein was +0.024 for the Cyclotec series and −0.491 for the Hobart series, which implied that nearly 25% of the variance in Hobart MPS was associated with protein.

As a final experiment, the 88 Cyclotec-ground series I wheats were divided into hard and soft wheat types. Individual calibrations were then run. The first was designated the "hard wheat" calibration and included the HRS, HRW, HWhS, and HWhW types. The "soft wheat" calibration consisted of the SRS, SRW, SWhS, and SWhW varieties. Series II, the 66 unknowns, were similarly grouped and analyzed for protein by the GQA. The results in Table VI indicated the expected improvement in the Hobart-ground series and slight improvement in the prediction of protein in the Cyclone-ground hard wheat series. A standard deviation from Kjeldahl of 0.27% would be satisfactory for most screening and segregation purposes.

TABLE IV
Grain Quality Analyzer (GQA) Predictions of Wheat Particle Size Index Compared with Sieved (LabConco) Abbreviated Technique

Wheat	GQA	LabConco
HRS I	21	19.5
HRS II	18	18.2
HRW I	27	19.0
HRW II	25	28.1
SRW I	31	32.0
SRW II	36	35.3
HWhSp I	22	19.4
HWhSp II	19	18.8
HWhW I	24	25.1
SWhSp I	33	29.3
SWhSp II	31	30.0
SWhW I	37	33.4
SWhW II	32	31.3
Durum I	10	9.0
Durum II	11	8.3
Standard error per test	0.8	0.7
Correlation coefficient = +0.949		
Root mean square difference = 3.7		

TABLE V
Statistical Summary of Grain Quality Analyzer (GQA) Predictions of Protein and Moisture in All-Type Series of Wheat Samples (N = 66)

	Hobart		Cyclotec	
Parameter[a]	Protein	H_2O	Protein	H_2O
r[b]	0.84	0.93	0.98	0.94
RMSD	0.69	0.43	0.31	0.32

[a] r = correlation coefficient; RMSD = root mean square deviation of GQA protein and moisture from Kjeldahl protein and single-stage air oven moisture.
[b] $P = 0.01$ for all correlations.

TABLE VI
Statistical Summary of Grain Quality Analyzer Predictions of Protein and Moisture in Hard and Soft Series of Wheat Samples (N = 27 hard; 39 soft)

	Hobart				Cyclotec			
	Hard		Soft		Hard		Soft	
Parameter	Protein	H_2O	Protein	H_2O	Protein	H_2O	Protein	H_2O
r[a]	0.91	0.96	0.81	0.89	0.99	0.96	0.94	0.91
RMSD[b]	0.41	0.31	0.56	0.45	0.27	0.30	0.32	0.33

[a] r = correlation coefficient. $P = 0.01$ for all correlations.
[b] RMSD = Root mean square difference.

GENERAL DISCUSSION

Protein content and kernel hardness are two of the most important factors in wheat quality. Kernel hardness is the most important factor governing variations in the MPS of wheat samples ground in various types of grinders. In turn, MPS exerts considerable influence on the accuracy of measurements of protein and other constituents by NIRS. These studies illustrate a method of using these relationships to effect a means of simultaneous screening wheat at high throughput for protein and kernel hardness. The Hobart Model 2040 burr mill was suitable for NIRS hardness testing. More recently the Falling Number KT-30 burr mill was found to discriminate clearly between wheats of different hardness.

High-speed hammer mills, or impeller type grinders, minimize MPS variations due to kernel texture and have been recommended for use with NIRS protein testing in wheat (Hunt et al 1978). Use of this type of grinder minimizes the effects of one of the most important variables in applying NIRS to the protein analysis of wheat. Variations in MPS due to season, location, and protein content are reduced to a level at which wheats of all types can be analyzed with reasonable accuracy on one calibration. For best accuracy, it is advisable to calibrate NIRS equipment separately for hard and soft wheats.

Watson and co-workers, (1977), using the Technicon InfraAlyzer, found that separate calibrations were necessary for different classes of wheat. The InfraAlyzer used a 1.68-μ NIR filter as one of the functional wavelengths in the protein measurement. This would be expected to render the InfraAlyzer more sensitive to variations in MPS and, therefore, to distinguish between classes of wheat even more clearly than the Neotec instrument. It is anticipated that this study will be extended, in the near future, to the InfraAlyzer Model 400, the Dickey-john GAC III, and the Neotec GQA 31EL to study algorithm sensitivity to variations in MPS using commercial instruments.

The present study described methods for distinguishing wheat samples of different hardness (as measured by the PSI test), and also for rough screening of large populations of wheat of all classes by protein content. The Hobart 2040 or Falling Number KT-30 burr mills are suitable. Samples were prepared using the Cyclotec sample mill, and wheats of all classes were tested for protein on a single calibration, with an accuracy of ±0.31%. For best accuracy, NIRS instruments should be calibrated separately for hard and soft wheats. However, the above technique allows analysis of HRS, HRW, HWhS, HWhW, and durum wheats on one calibration, whereas the soft wheat calibration allows analysis of SRS, SRW, SWhS, SWhW, and club wheats with an adequate accuracy for most practical on-line applications. In general, analysis of hard wheats was more accurate than that of soft wheats. The high starch content of soft wheats and their flours may interfere with protein measurement more than do variations in MPS. This aspect of the application of NIRS to the analysis of wheat and flour requires further investigation.

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