Hardness Measurement of Bulk Wheat by Single-Kernel Visible and Near-Infrared Reflectance Spectroscopy

Elizabeth B. Maghirang[1] and Floyd E. Dowell[1,2]

ABSTRACT

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Reflectance spectra (400 to 1700 nm) of single wheat kernels collected using the Single Kernel Characterization System (SKCS) 4170 were analyzed for wheat grain hardness using partial least squares (PLS) regression. The wavelengths (650 to 700, 1100, 1200, 1380, 1450, and 1670 nm) that contributed most to the ability of the model to predict hardness were related to protein, starch, and color differences. Slightly better prediction results were observed when the 550–1690 nm region was used compared with 950–1690 nm region across all sample sizes. For the 30-kernel mass-averaged model, the hardness prediction for 550–1690 nm spectra resulted in a coefficient of determination (R^2) = 0.91, standard error of cross validation (SECV) = 7.70, and relative predictive determinant (RPD) = 3.3, while the 950–1690 nm had R^2 = 0.88, SECV = 8.67, and RPD = 2.9. Average hardness of hard and soft wheat validation samples based on mass-averaged spectra of 30 kernels was predicted and compared with the SKCS 4100 reference method (R^2 = 0.88). Compared with the reference SKCS hardness classification, the 30-kernel (550–1690 nm) prediction model correctly differentiated (97%) between hard and soft wheat. Monte Carlo simulation technique coupled with the SKCS 4100 hardness classification logic was used for classifying mixed wheat samples. Compared with the reference, the prediction model correctly classified mixed samples with 72–100% accuracy. Results confirmed the potential of using visible and near-infrared reflectance spectroscopy of whole single kernels of wheat as a rapid and nondestructive measurement of bulk wheat grain hardness.

Wheat (*Triticum aestivum* L.) grain hardness is a primary quality trait relating wheat to milling properties and end-use (Pomeranz et al 1984; Slaughter et al 1992; Ohm et al 1998; Morris et al 1999). An excellent indication of its importance is the manner by which wheat has been generally classified in the United States into three major hardness classes: soft, hard hexaploid, and durum.

Although extensively studied, no direct causal relationship between the genetic and physicochemical basis of endosperm texture has been established (Greenblatt et al 1995). Hong et al (1989) reported a slight correlation (r = 0.58) between the amount of water-soluble pentosans and endosperm texture. Bettge and Morris (2000) noted that among hard wheat samples, pentosans had a minimal role in modifying grain hardness; however, for soft wheat, pentosans appear to have a significant hardness-modifying effect that carries over into end-use quality. Grain hardness is affected by the degree of adhesion between starch granules and endosperm protein matrix (Barlow et al 1973; Simmonds et al 1973). Additionally, Barlow et al (1973) noted that the interaction between starch granules and amyloplast membranes is different between hard and soft wheats. Results from scanning electron microscopy and freeze-etching work showed that fractures during milling of hard wheat tend to pass along endosperm cell walls to yield clean, well-defined particles. Fracture through cell contents in these wheats, when it occurs, involves both starch granules and storage protein resulting in high proportion of damaged and broken starch granules. Soft wheats, on the other hand, had lower adhesion between starch and protein, thus tending to release starch granules more freely during milling. Glenn and Saunders (1990) demonstrated that intracellular space exists around the starch granules of soft, but not hard wheat, forming a discontinuity in the starch-protein matrix. This physical discontinuity provides a natural path for shearing forces during kernel disruption, leading to softer material that is easily reduced in particle size. Turnbull and Rahman (2002) provided a review of the structure of hard and soft wheat endosperm with particular emphasis on when differences in endosperm texture can be detected in the developing seed.

[1] Engineering Research Unit, Grain Marketing and Production Research Center, ARS-USDA, 1515 College Avenue, Manhattan, KS 66502.
[2] Corresponding author. Phone: 785-776-2753. Fax: 785-776-2792. E-mail: fdowell@gmprc.ksu.edu.

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Numerous techniques have been studied to quantify wheat hardness. Some of these techniques include near-infrared reflectance spectroscopy (NIRS) of ground meal (Williams 1979; Norris et al 1989), NIRS of whole wheat grains (Manley et al 1996), near-infrared transmittance spectroscopy (Delwiche 1993), laser light scattering (Plantz 1983), acoustical properties of a kernel during grinding (Massie et al 1993), measuring the force required to crush kernels (Martin et al 1993; Psotka 1997; Morris et al 1999), particle size index (PSI) (Cutler and Brinson 1935;Yamazaki 1972), soft metal hardness testers (Katz et al 1959), pearling index (McCluggage 1943), and visual inspection of crushed endosperm (Mattern 1988). The NIRS of ground meal (Approved Method 39-70, AACC 2000), which was first reported by Norris et al (1989), is the method adopted by the National Institute of Standards and Technology (NIST) for kernel hardness determination. Two other AACC approved physical tests for grain hardness measurements are the particle size index (Approved Method 55-30) and the Single Kernel Characterization System (SKCS) 4100 (Approved Method 55-31).

Of particular interest in this study is the SKCS 4100 because of its potential as a rapid and accurate measurement technique that utilizes a small quantity of sample for single kernel hardness measurement. Psotka (1997) reported that the SKCS provides a hardness index that does not significantly change with changes in moisture content. Psotka cited an example wherein the hardness index did not change when the moisture content increased from 10 to 15% moisture. The single kernel hardness measurements allow for differentiation of wheat samples that are mixed with soft and hard wheats. The SKCS 4100 provide the best phenotypic measure of the material properties of the wheat endosperm manifested by the action of the Hardness loci (Morris et al 1999). However, as with the other hardness measures, this is a destructive test because kernels are crushed during measurement.

Also of interest in this study is NIRS, which can measure some attributes nondestructively. NIRS has been used to measure protein (Williams and Thompson 1978; Williams 1979), hardness using ground samples (Williams 1979; Norris et al 1989; Ohm et al 1998), moisture content (Williams and Thompson 1978), vitreousness (Dowell 2000), and color (Delwiche and Massie 1996; Dowell 1998). One of the current approved methods for wheat hardness measurement is wheat hardness as determined by near-infrared spectroscopy (Approved Method 39-70A). This method involves grinding samples before the spectra are taken, so this is also a destructive method.

The application of optical transmittance properties for measuring single-kernel wheat hardness was investigated by Delwiche (1993). Results indicated that measurement capability was largely based on the extent of correlation between hardness and vitreousness. Soft wheats have more variation than hard wheats. Delwiche concluded that intact-kernel transmittance measurements may lack the sensitivity needed to directly measure the biochemical component (presumably, a low molecular weight protein) that determines hardness.

A study to measure whole grain hardness by near infrared reflectance was done by Manley et al (1996), who investigated the potential of using multiplicative scatter correction (MSC), area under the second derivative curve (Area), and principal component analysis (PCA) for measuring hardness of whole wheat grain by NIRS. The study was based on the ability of NIRS to measure hardness based on light scattering in ground wheat samples, hypothesizing that this light scattering effect could be used to predict hardness in whole grains. Pure scatter as measured in MSC and Area is inadequate to describe hardness of whole wheat; the first and second principal components yielded the best results ($r = 0.68$).

The objective of this study was to determine whether single kernel visible and near-infrared reflectance spectroscopy could be used as a nondestructive, automated, rapid, and accurate bulk hardness measurement and classification technique. There are numerous applications, breeding programs for example, where a hardness measurement method that is nondestructive, rapid, and accurate is needed.

MATERIALS AND METHODS

Wheat Samples

Two sets of samples (calibration and validation) were obtained. The calibration sample set (Table I) consisted of the U.S. National Institute of Standards and Technology (NIST) wheat hardness reference samples and five additional soft wheat samples. The cultivars were (1) Tam 105, (2) Arapahoe, (3) Newton, (4) Yecora Rojo, (5) Len, (6) Cardinal, (7) Titan, (8) Madsen, (9) Malcolm, and (10) Tres. Samples 1–5 are classified as hard wheat, while samples 6–10 are soft wheat. Three sets of NIST samples differentiated by the original moisture content (MC), before equilibration to <14%, were used. Set 1 refers to NIST samples that were <13%; Set 2 had 13.0≤MC≥≤14.5 and Set 3 had MC>14.5 The five additional samples with lower hardness values than the NIST soft wheat samples were obtained from the USDA Soft Wheat Quality Laboratory (SWQL), Wooster, OH. These included (1) Houser, (2) Clark 1, (3) Clark 2, (4) Sawyer, and (5) Caldwell. Approximately 100 kernels were randomly obtained from each of the 35 samples. Each 100-kernel sample was loaded in the sample hopper of the SKCS4170 for singulated and automated measurements of spectra, single kernel hardness, and single kernel moisture content.

The validation samples consisting of 30 wheat samples (15 hard and 15 soft) were obtained from the FGIS, Kansas City, MO, Perten Instruments, Springfield, IL and SWQL, Wooster, OH. The 15 hard wheat samples included (1) HRW Nekota Pukwana, (2) HRSWPB926R, (3) HRS Madison, (4) HRW Dodge 1007, (5) HRS Verde Bristol, (6) HRS Sharp Bristol, (7) HRW FGIS57564, (8) HRS Berthoud, (9) HRS Nordic, (10) HRS Bristol, (11) HRS Kulm, (12) HRS Waverly, (13) HRW FGIS54570, (14) HRW FGIS54843, and HRS Gus GWB5. The 15 soft wheat samples were (1) SRW/SRS FGIS2, (2) SRW FGIS56477, (3) SRW PRM Wynee, (4) P4 Idaho 144002, (5) Pioneer 2548, (6) P4 Idaho 1440024, (7) P4 Idaho 014005, (8) Coker 9733, (9)P4 Idaho 1440013, (10) P4 Idaho 1440011, (11) P4 Idaho 1430007, (12) Arthur, (13) P4 Idaho 1430011, (14) P4 Idaho 1430021, and (15) P4 Idaho 1430002. Approximately 100 kernels were randomly obtained from each of the 30 samples for spectra collection at as-is MC.

Spectra Measurement

The Perten Single Kernel Characterization System (SKCS) 4100 equipped with Perten Diode Array (DA) 7000 spectrophotometer, also known as SKCS 4170, was used to automatically measure spectra of each kernel. The DA 7000 consists of a silicon (400–950 nm) and InGaAs (950–1700 nm) sensor. The silicon sensor measures reflectance at 7-nm intervals, and the InGaAs sensor measures reflectance at 11-nm intervals. All data is then interpolated to 5 nm. For each kernel, 8 spectra were taken, then an average spectrum for each kernel is saved onto a spectral multifile. The same settings used to collect the 100 kernel spectra for calibration samples were used for the 30 validation samples. Validation samples were processed one day after collecting the spectra of the calibration samples.

Baseline spectra were obtained using a Spectralon reference standard. Wheat kernels were individually and automatically fed using the SKCS 4170 singulator for spectra collection using the DA 7000 component. This was immediately followed by grain hardness measurement using the SKCS 4100 component.

Moisture Content and Single Kernel Grain Hardness Measurements

The MC of bulk samples were obtained using the Approved Method 44-15A (AACC 2000). The SKCS 4100 component of the SKCS 4170 (Perten Instruments, Springfield, IL) was used to measure MC and hardness values of individual kernels. The single kernel hardness measurements served as the actual or reference

TABLE I
Mean and Standard Deviation of Hardness Values of 35 Wheat Calibration Samples Obtained Using the SKCS 4100[a]

Sample	Set 1		Set 2		Set 3	
	Mean	SD	Mean	SD	Mean	SD
Hard						
TAM 105	73.7	14.1	74.8	14.2	73.3	16.3
Arapahoe	61.4	16.4	64.1	14.6	63.7	14.3
Newton	65.3	15.3	65.2	15.3	68.3	13.5
Yecora Rojo	58.0	13.1	61.7	13.6	61.5	13.2
Len	80.3	14.7	80.3	12.2	83.8	12.6
Soft						
Cardinal	21.2	15.9	21.4	14.2	21.9	13.5
Titan	17.2	15.1	21.0	16.1	23.4	13.5
Madsen	32.2	12.7	27.2	15.0	30.6	15.7
Malcolm	31.3	13.2	30.2	14.4	29.7	14.5
Tres	32.0	14.6	31.8	12.8	33.7	13.4
Caldwell	–6.5	15.0	. . .	. . .	. . .	. . .
Houser	4.9	20.8	. . .	. . .	. . .	. . .
Sawyer	5.9	15.0	. . .	. . .	. . .	. . .
Clark 1	12.8	16.4	. . .	. . .	. . .	. . .
Clark 2	11.3	17.4	. . .	. . .	. . .	. . .

[a] Set 1, Set 2, and Set 3 are NIST samples with varying MC levels before equilibration to approximately <14%.

hardness values of single kernels or individual spectra. Martin et al (1993) described the SKCS 4100 and its operating principles for measuring kernel hardness, moisture content, weight, and diameter; the hardness index or value is calculated from measurements of the force required to crush each kernel.

Perten Instruments (1999) summarize the SKCS 4100 hardness classification logic diagram used for classifying soft, hard, and mixed wheat as officially approved by the Grain Inspectors, Packers and Stockyard Association, United States Department of Agriculture (GIPSA-USDA). This is the same logic diagram that was used for classifying predicted samples. Hardness classification is determined from the average hardness index of the sample and the distribution of individual kernel hardness measurements within four hardness ranges. Classification initially involved determination of mean hardness score. If the mean hardness score was >46, the sample was more likely to be classified as hard; if it was ≤46, the sample was more likely to be classified as soft. Then, a four-part histogram based on predetermined hardness ranges was created that grouped the number of kernels successively into the classification number of the form AAA-BBB-CCC-DDD-EE. AAA refers to percent kernels with hardness index ≤33; BBB is percent kernels with hardness index >33 and ≤46; CCC is percent kernels with hardness index >46 and ≤59; DDD is percent kernels with hardness index >59; EE refers to classification 01 and 02 for hard, 03 for mixed, 04 and 05 for soft. The frequency of samples in each category, based on preset cutoffs, determines the final classification of a sample.

TABLE II
Classification Results for Wheat Hardness Reflectance Spectra (550–1690 and 950–1690 nm) Using PLS Analysis

Model/Statistic[a]	550–1,690 nm	950–1,690 nm
1-kernel		
R^2	0.49	0.40
SECV	20.94	22.68
RPD	1.40	1.29
Factors	8	7
N1	3,430	3,430
N2	3,430	3,430
5-kernel average		
R^2	0.83	0.76
SECV	10.65	12.91
RPD	2.45	2.02
Factors	7	5
N1	3,325	3,325
N2	665	665
10-kernel average		
R^2	0.86	0.84
SECV	9.51	10.43
RPD	2.70	2.46
Factors	5	5
N1	3,150	3,150
N2	315	315
20-kernel average		
R^2	0.90	0.87
SECV	8.27	9.28
RPD	3.08	2.75
Factors	5	4
N1	2,800	2,800
N2	140	140
30-kernel average		
R^2	0.91	0.88
SECV	7.70	8.67
RPD	3.3	2.93
Factors	5	4
N1	3,150	3,150
N2	105	105
50-kernel average		
R^2	0.91	0.88
SECV	7.57	8.83
RPD	3.35	2.87
Factors	5	4
N1	3,430	3,430
N2	70[b]	70[b]

[a] R^2, coefficient of determination; SECV, standard error of cross-validation; RPD, ratio of standard deviation of reference data to SECV; Factors, number of PLS factors used; N1, number of individual spectra (kernels) used; N2, number of averaged spectra used.

[b] Randomly selected 50-kernel samples within N1 with Rep 2 possibly containing a few kernels from Rep 1.

Data Analysis

Spectra were analyzed with partial least squares (PLS) regression (Martens and Naes 1989) utilizing a commercial software (PLSPlus/IQ for Grams/32, Galactic Industries, Salem, NH). Two wavelength ranges (550–1690 and 950–1690 nm) were considered for all PLS regressions of the calibration data set. Reflected energy at <550 nm was noisy and did not give meaningful data, thus that region was not used in any analyses. The 950–1690 region was studied to eliminate any visible wavelength influences on classifications, and to mimic the use of only an InGaAs sensor that is being used in related studies. Statistical measures included 1) coefficient of multiple determination (R^2), 2) standard error of cross validation (SECV), 3) relative predictive determinant (RPD) (Williams 1997), and 4) number of PLS factors.

While 100 kernels were initially set as the sample size, there were differences in actual sample size across wheat samples. These are attributed to instances when a spectrum was collected but the hardness data was rejected because of improper weight or when a mispositioned kernel resulted in a poor spectrum, even though the hardness data was valid. Thus, an array basic macro was written to remove outlier spectra obtained when a spectrum was collected but no kernel was present or when a kernel was mispositioned. The array basic macro rejected spectra that have span values >0.30. This span value was obtained from earlier tests (results not shown) where span values of empty bucket and various mispositioned kernels were identified.

TABLE III
Mean and Standard Deviation Hardness Values of Reference SKCS 4100 and Predicted NIR for Hard and Soft Wheat Validation Samples

	Reference SKCS 4100		Predicted NIR	
Sample ID[a]	Mean	SD[b]	Mean	SD[b]
HRW Nekota Pukwana	56	13.1	52	25.7
HRS WPB926R	59	12.0	62	16.7
HRS Madison	61	13.4	62	21.3
HRW Dodge 1007	63	17.3	55	23.8
HRS Verde Bristol	63	15.1	70	23.4
HRS Sharp Bristol	64	14.0	61	22.7
HRW FGIS57564	65	22.1	66	20.5
HRS Berthoud	66	17.5	56	30.3
HRS Nordic	67	13.2	61	28.1
HRS Bristol	68	14.7	62	24.3
HRS Kulm	70	17.3	75	22.6
HRS Waverly	71	15.7	77	24.6
HRW FGIS54570	72	15.1	68	24.2
HRW FGIS54843	73	15.2	60	27.7
HRS Gus GWB5	83	14.4	89	18.4
SRW/SRS 2	–6	14.5	–13	21.1
SRW FGIS56477	4	13.6	–9	23.3
SRW PRM Wynne	16	20.5	24	21.9
P4 Idaho 1440002	16	10.8	24	18.0
Pioneer 2548	17	13.1	29	23.0
P4 Idaho 1440024	17	12.8	23	20.4
P4 Idaho 014005	20	14.8	12	27.9
Coker 9733	24	18.3	27	30.6
P4 Idaho 1440013	24	13.2	27	18.2
P4 Idaho 1430011	24	12.1	34	20.8
P4 Idaho 1430007	27	16.4	38	21.1
Arthur	29	15.2	47	22.2
P4 Idaho 1430011	29	14.0	46	17.2
P4 Idaho 1430021	30	20.1	45	15.7
P4 Idaho 1430002	33	17.2	38	17.9

[a] Three replicates for each sample set.

[b] Average of three SD values for 30 single kernel measurements.

After removing outliers, the total number of individual spectra (N1) available for analysis was 3430; the number of averaged spectra used (N2) varied from 70 to 665 (see Table II) depending on the number of kernels used for averaging. The macro then computed an average spectrum of randomly picked kernels for each predetermined mass averaged spectra sample size. A discussion of the mathematical basis of averaging of samples from the spectral and reference perspectives can be found in Delwiche and Hruschka (2000). PLS regression models were developed correlating mass-averaged NIR spectra and the corresponding mass-averaged reference hardness measurements.

Model Validation

A calibration created using the 30-kernel (550–1690 nm) model was used to predict the hardness values from spectra collected for the 30 validation samples (15 hard and 15 soft wheat samples). The predicted hardness values from the 30-kernel averages were then compared with the hardness values obtained using the averaged 30-kernel SKCS 4100 hardness reference values based on mean hardness values and standard deviations. The potential of the 30-kernel (550–1690 nm) model for classifying the 30 validation samples wheat as soft or hard was evaluated following the approved GIPSA-USDA classification definition used in the SKCS 4100.

From the hard and soft validation sample sets, three samples were chosen to represent low, medium, and high hardness wheat. The samples identified were 1) HRW Nekota Pukwana, SD, low hardness hard wheat; 2) HRS Kulm, medium hardness hard wheat; 2) HRS Gus GWB5, ND, high hardness hard wheat; 4) SRW/SRS2, low hardness soft wheat; 5) Pioneer 2548, IN, medium hardness soft wheat, and 6) P4 Idaho 1430002, high hardness soft wheat. The nine hardness combinations selected were 1) low hardness hard wheat + low hardness soft wheat; 2) low hardness hard wheat + medium hardness soft wheat; 3) low hardness hard wheat + high hardness soft wheat; 4) medium hardness hard wheat + low hardness soft wheat; 5) medium hardness hard wheat + medium hardness soft wheat; 6) medium hardness hard wheat + high hardness soft wheat; 7) high hardness hard wheat + low hardness soft wheat; 8) high hardness hard wheat + medium hardness soft wheat; and 9) high hardness hard wheat + high hardness soft wheat.

Mixtures were simulated for each combination: 1) 10% hard + 90% soft; 2) 20% hard + 80% soft, 3) 30% hard + 70% soft, 4) 40% hard + 60% soft, 5) 50% hard + 50% soft, 6) 60% hard + 40% soft, 7) 70% hard + 30% soft, 8) 80% hard + 20% soft, and 9) 90% hard + 10% soft.

A SAS program was created to combine the Monte Carlo technique and SKCS 4100 hardness classification decision logic. This involves random number generation of 30-kernel mixtures of hard and soft wheat, computation of means, and hardness classification utilizing the IF and THEN/ELSE commands with reference to the logic diagram. One thousand randomly generated 30-kernel samples were generated for each of these combinations and mixtures for a total of 81,000 simulated mixtures. The accuracy of the prediction model was then computed based on the number of times that a mixture was correctly classified as a mixture taking the reference method (SKCS 4100) to be 100% accurate.

RESULTS AND DISCUSSION

Calibration Wheat Samples

The NIST hard and soft wheat samples had MC values ranging from 10.9 to 12.5% and 11.2 to 12.7%, respectively. Except for one sample in the hard wheat category that had MC standard deviation (SD) of 0.63, combined hard and soft wheat samples had MC SD ranging from 0.19 to 0.29. The hardness index generated by the SKCS 4100 has been reported as not being significantly affected by changes in moisture content (Psotka 1997).

Table I summarizes the mean and SD of the hardness values of the 35 calibration samples obtained using the SKCS 4100. The hard wheat samples had mean hardness values ranging from 58 to 84 and SD ranging from 12.2 to 16.4. Mean hardness values of soft wheat samples ranged from –7 to 34, with SD ranging from 12.7 to 20.8.

Wheat Hardness Measurement: PLS Analyses of Calibration Samples

Table II presents the classification results for wheat hardness reflectance spectra (550–1690 and 950–1690 nm wavelengths) using PLS analysis. A total of 12 models were generated and the corresponding statistics were compared with determine which model best predicts bulk wheat hardness.

The R^2 values for single, 5, 10, 20, 30, and 50-kernel mass averaged samples for the 550–1690 nm wavelengths were 0.49, 0.83, 0.86, 0.90, 0.91, and 0.91, respectively. Substantial improvement in predicting hardness was obtained when spectral mass averaging was done. An increasing trend in R^2 was observed as the number of kernels used in spectral mass averaging increased, although at a decreasing rate. The improvement in R^2 from the 20-kernel to 30-kernel spectral mass-averaged samples was only 1%. No improvement in R^2 was observed between 30-kernel and 50-kernel mass-averaged samples. For the 950–1690 nm, R^2 was 0.40, 0.76, 0.84, 0.87, 0.88, and 0.88 for single, 5, 10, 20, 30, and 50-kernel spectral mass-averaged samples, respectively. A comparison of the R^2 values between the 550–1690 and 950–1690 nm wavelength ranges showed slight but consistently higher values for the 550–1690 nm prediction models.

The SECV, which is a method for determining the best number of independent variables in building a calibration equation, decreased with increased number of kernels used for mass averaging. SECV values at 550–1690 nm were 21, 11, 10, 8, 8, and 8 for single, 5, 10, 20, 30, and 50-kernel mass-averaged samples, respectively and for 950–1690 nm were 23, 13, 10, 9, 9, and 9, respectively. The SECV values were slightly but consistently higher in the 950–1690 nm compared with the 550–1690 nm wavelengths.

The RPD, which is the ratio of standard deviation of reference data in the prediction sample set to the standard error of cross validation (Williams 1997) is used as an indicator of the usefulness of a calibration. Results show that both the single-kernel and the 5-kernel models that included only NIR wavelengths had RPD values of ≈2.0 or less. Likewise, the single-kernel 550–1690 nm model also had RPD that was <2.0. This indicates that these models are of little use even for rough screening. All other models had RPD values of ≈2.5 or greater, indicating that they can be used to distinguish between hard and soft wheat. RPD values increased when models included more kernels in mass-averages although at a declining rate, and when visible wavelengths were included.

The number of PLS factors was determined after a concurrent examination of the predicted residual error sum of squares (PRESS), relationship of actual versus predicted and corresponding R^2 value, and beta coefficient results generated using Grams 32 (Galactic Industries, Salem, NH). Results showed that the number of factors was highest (8) in the 550–1690 nm model for single-kernel model; the PLS factor was 5 for 10, 20, 30, and 50-kernel models. The 550–1690 nm model generally required more PLS factors compared with the 950–1690 nm. While the complexity of a model is lower at lower PLS factors, the 550–1690 nm model was chosen considering that the 550–690 nm wavelengths contributed to improving other statistical measures.

Plots of reflectance spectra of two representative soft and two representative hard wheat samples are shown in Fig. 1. These plots were prepared by averaging spectra of 30 single kernels. The absorbance or logarithm (base 10) of the reciprocal of reflectance values (i.e., log 1/R) for hard wheat was greater than that for soft wheat. With an increase in hardness index, the log 1/R generally increased at every wavelength in the 500 to 1700 nm spectra. The hard wheat cultivar Len (SKCS 4100 hardness index = 80) had higher absorbance compared with Newton (SKCS 4100 hardness index = 65), which were both substantially higher compared with the two soft wheat samples (Titan and Houser with SKCS 4100 hardness values of 21 and 5, respectively). These results may be explained by the intracellular space that exists around the starch granules of soft wheat, forming a discontinuity in the starch-protein matrix (Glenn and Saunders 1990). This physical discontinuity probably scatters more light back to the sensor, which is seen as less absorption in softer kernels.

Figure 2 provides an indication of the important wavelengths for grain hardness prediction based on PLS regression beta coefficients. The absolute values of the PLS beta coefficients show the contribution of each wavelength to the calibration model. The peaks around 650 to 700 nm are related to color differences. The peaks and valleys at ≈1100, 1200, 1380, 1450, and 1670 nm are related to the protein and starch structure (Williams and Norris 1987). Additionally, this may be attributed to the degree of adhesion between starch granules and endosperm matrix being higher for hard wheat compared with soft wheat, as indicated by Simmonds et al (1973) and Barlow et al (1973).

Another factor that allows for measurement of kernel hardness by optical measurement is its extent of correlation with vitreousness (Delwiche 1993). Dowell (2000) showed that NIR spectroscopy can be used to quantify the vitreousness of durum wheat, possibly because of protein, starch, or scattering effects of NIR absorption. It is thus probable that hardness is being quantified to the extent of the level of relationship between hardness and vitreousness.

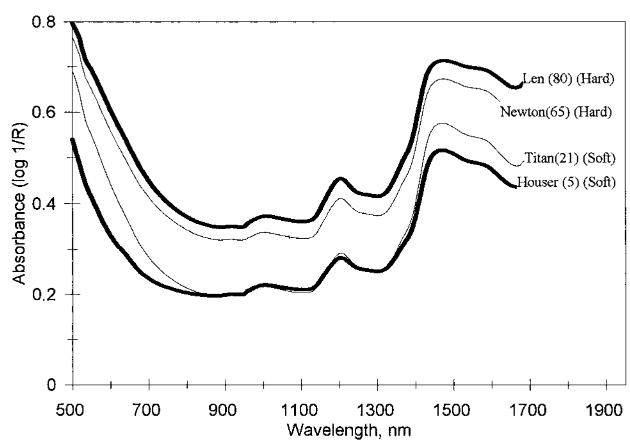

Fig. 1. Plot of average absorbance spectra when analyzing reflected energy for representative hard and soft wheat samples. Note: Numbers in parentheses immediately following wheat cultivar are mean hardness values of wheat cultivar based on the SKCS 4100 and traces provided are the average of 30 single kernel spectra.

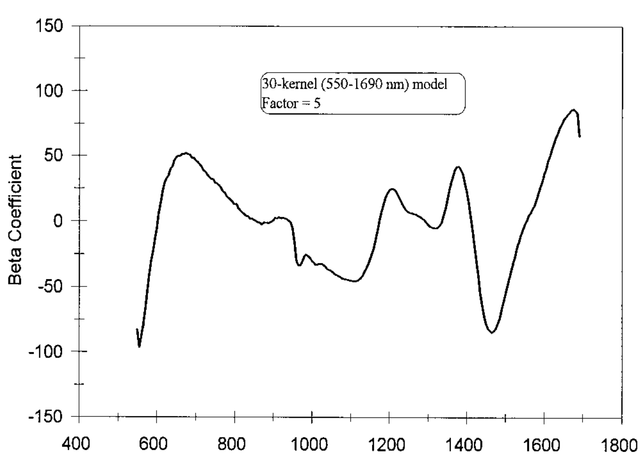

Fig. 2. Partial least squares regression beta coefficients indicating important wavelengths for grain hardness predictions from visible and NIR spectra.

Model Validation

Quantifying wheat hardness. Table III shows the SKCS 4100 reference and visible and near infrared reflectance (visNIR) 30-kernel predicted mean hardness values and SD for each validation sample. For SKCS 4100 measurements, mean hardness ranged from –6 to 83; SD ranged from 10.8 to 22.1. For visNIR model

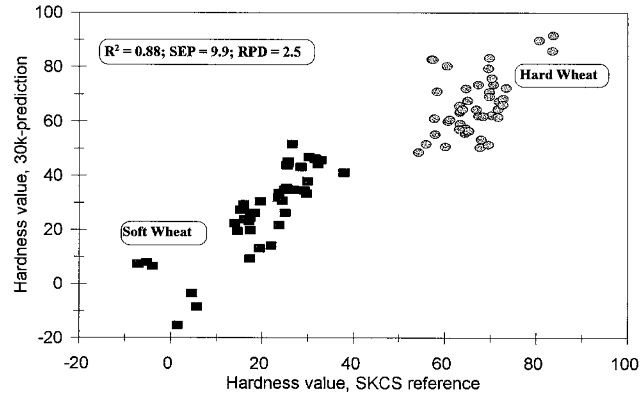

Fig. 3. Validation test results showing hardness values for 30 kernel average of SKCS 4100 reference values vs. predictions using 30 kernel spectra average of hard and soft wheat validation samples.

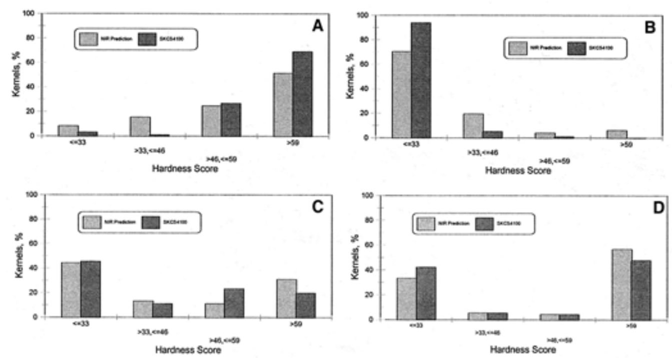

Fig. 4. Validation test results for wheat classification showing four-part histogram of representative hard (**A**), soft (**B**), and two mixed (**C** and **D**) wheat samples for SKCS 4100 and visible and NIR prediction.

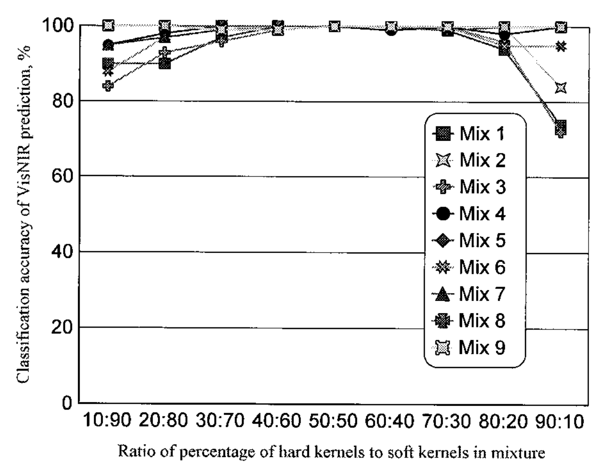

Fig. 5. Classification accuracy of VisNIR for predicting wheat mixtures in comparison with 100% accuracy of reference method (SKCS 4100). Each point represents 1,000 30-kernel samples generated using Monte Carlo simulation in SAS. Mix 1, 2, and 3: mixtures of low hardness hard kernels blended with low, medium, and high hardness soft kernels. Mix 4, 5, and 6: mixtures of medium hardness hard kernels blended with low, medium, and high hardness soft kernels. Mix 7, 8, and 9: mixtures of high hardness hard kernels blended with low, medium, and high hardness soft kernels.

prediction, mean hardness ranged from –13 to 89; SD ranged from 15.7 to 30.6. The SD of prediction was higher than the reference SKCS 4100, except for one hard wheat and one soft wheat samples. Regression of mean SKCS 4100 reference and mean 30-kernel (550–1690 nm) predicted hardness values of hard and soft wheat samples resulted in an $R^2 = 0.88$. This relationship is shown in Fig. 3. The RPD of 2.5 indicates that the model is useful for screening purposes.

Quantifying Wheat Hardness

Hard wheat. The mean SKCS 4100 hardness values of hard wheat samples ranged from 56 to 83 with SD ranging from 12.0 to 22.1. The mean predicted hardness values obtained using the 30-kernel (550–1690 nm) model ranged from 52 to 89 (SD = 16.7 to 30.3) for hard wheat. The overall difference in mean SKCS 4100 reference and 30-kernel (550–1690 nm) model prediction hardness for hard wheat is 1.6 with reference hardness being slightly higher than the predicted hardness. Seven hard wheat samples had predicted values higher than the actual values by 1 to 7 hardness values; the remaining 8 samples had predicted values lower than the actual by 3 to 13 hardness values. The SD values for the predicted values were generally higher than those for the reference data.

Soft wheat. The mean SKCS 4100 hardness values of soft wheat ranged from –6 to 33 with SD ranging from 10.8 to 20.5. The mean predicted hardness values for soft wheat ranged from –13 to 47 (SD = 15.7 to 30.6). Only three of the 15 soft wheat samples had lower mean predicted hardness values compared with the reference SKCS 4100 hardness value; the model generally have higher predicted than reference hardness values. The predicted hardness values had a higher overall difference of 6 hardness number compared with SKCS 4100. Similar to the hard wheat samples, the SD values for the predicted values were generally higher than those for the reference data. The higher variation in soft wheat prediction compared with hard wheat agrees with the finding of Delwiche (1993) on single kernel hardness prediction using the transmittance mode.

Classifying hard, soft or mixed wheat. The SKCS 4100 hardness definition was used to determine hard, soft, or mixed wheat classification of validation samples. Figure 4 shows sample histograms generated for classifying soft, hard, or mixed wheat samples.

The prediction model correctly classified 97% of the validation samples as soft or hard. There was one soft wheat sample that was predicted as hard wheat primarily because the mean hardness exceeded the cutoff (46 hardness number) by 1 hardness number.

The Monte Carlo technique generated 81,000 simulated mixtures, which were then individually classified as hard, soft, or mixed wheat samples based on the SKCS 4100 hardness definition. Figure 5 summarizes the classification accuracy of the prediction model, which ranged from 72 to 100%, taking the reference method (SKCS 4100) to be 100% accurate. Mixtures containing at least 20% of a contrasting hardness had 90 to 100% correct classification. Mixtures containing 10% of contrasting hardness were not predicted as well. Samples that contained 10% hard wheat and 90% soft wheat had correct classifications ranging from 84 to 100%. On the other hand, samples containing 10% soft wheat mixed with 90% hard wheat had correct classifications ranging from 72 to 100%. Mixtures that were misclassified as hard wheat are mixtures containing higher percentage of hard wheat in the mixture; mixtures that were misclassified as soft were mixtures containing higher percentage of soft wheat in the mixture.

Mixtures 1, 2, and 3, which had relatively lower correct classifications compared with all other mixtures (74, 84, and 72%, respectively), were the mixtures that contained low hardness hard wheat kernels. Misclasssification may be attributed to mean predicted hardness value (52) of Mixtures 1, 2, and 3 being close to the mean hardness value cutoff of 46 coupled with a high SD (25.7). Mixtures containing medium hardness hard wheat (Mix 4, 5, and 6) had 88 to 100% correct classification. Mixtures containing high hardness hard wheat (Mix 7, 8, and 9) had 95 to 100% correct classification. These results generally followed the expected trend where mixtures of highly opposing hardness values are better predicted compared with mixtures containing samples with closer hardness values.

CONCLUSIONS

The absorbance (log 1/R) values for hard wheat were greater than those for soft wheat; indicating that spectra will be useful in identifying wheat hardness. A single-kernel model did not provide a good model for predicting single-kernel hardness values. Use of spectral mass averaging improved the bulk hardness measurement model for both the 550–1690 and 950–1690 nm wavelengths. Additionally, increasing the number of kernels used for mass-averaging resulted in improved model performance. Visible and NIR reflectance spectra (550–1690 nm) analyzed using partial least squares (PLS) regression provided slightly better wheat hardness predictions than NIR spectra (950–1690 nm) alone. This shows that including the visible wavelength range improved measurements. The wavelengths (650 to 700 nm, 1100, 1200, 1380, 1450, and 1670) that contributed most to the ability of the model to predict hardness were likely related to protein, starch, and color differences. The 30-kernel (550–1690 nm) quantitatively predicted hardness values of soft and hard wheat ($R^2 = 0.88$). The RPD = 2.5 indicates that the model is useful for screening purposes. Mixtures containing at least 20% of a contrasting hardness had 90 to 100% correct classification. Mixtures containing 10% hard wheat + 90% soft wheat had correct classifications ranging from 84 to 100%. Mixtures containing 10% soft wheat + 90% hard wheat had correct classifications ranging from 72 to 100%.

These results show that single kernel visible and near-infrared reflectance spectra are applicable for hardness measurements of bulk wheat. The current proposed technique is a non-destructive, automated, and rapid bulk hardness measurement utilizing whole, single wheat kernels. Its potential may be attributed to the apparent capability of this technique to distinguish between the strength of adhesion between starch and protein, which varies across hard and soft wheat, and which is manifested with absorbance (log 1/R) being higher for hard wheat than in soft wheat. Additionally, the prediction capability of NIR may probably be related to the extent of the level of relationship between hardness and vitreousness.

NIR using whole kernels has already proven effective for measuring numerous grain attributes, such as protein, moisture content, vitreousness, internal insects, bunt, etc. The same instrument used to measure these attributes can be used for predicting hardness and for classifying pure or mixed wheat samples. This may reduce the number of instruments or steps required for evaluating grain attributes.

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