Determining Weight and Moisture Properties of Sound and Fusarium-Damaged Single Wheat Kernels by Near-Infrared Spectroscopy

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ABSTRACT

Single kernel moisture content (MC) is important in the measurement of other quality traits in single kernels because many traits are expressed on a dry weight basis. MC also affects viability, storage quality, and price. Also, if near-infrared (NIR) spectroscopy is used to measure grain traits, the influence of water must be accounted for because water is a strong absorber throughout the NIR region. The feasibility of measurement of MC, fresh weight, dry weight, and water mass of single wheat kernels with or without *Fusarium* damage was investigated using two wheat cultivars with three visually selected classes of kernels with *Fusarium* damage and a range of MC. Calibration models were developed either from all kernel classes or from only undamaged kernels of one cultivar that were then validated using all spectra of the other cultivar. A calibration model developed for MC when using all kernels from the wheat cultivar Jagalene had a coefficient of determination (R^2) of 0.77 and standard error of cross validation (SECV) of 1.03%. This model predicted the MC of the wheat cultivar 2137 with R^2 of 0.81 and a standard error of prediction (SEP) of 1.02% and RPD of 2.2. Calibration models developed using all kernels from both cultivars predicted MC, fresh weight, dry weight, or water mass in kernels better than models that used only undamaged kernels from both cultivars. Single kernel water mass was more accurately estimated using the actual fresh weight of kernels and MC predicted by calibrations that used all kernels or undamaged kernels. The necessity for evaluating and expressing constituent levels in single kernels on a mass/kernel basis rather than a percentage basis was elaborated. The need to overcome the effects of kernel size and water mass on single kernel spectra before using in calibration model development was also highlighted.

Moisture content (MC) of grains is one of the most important characteristics affecting grain quality and price. When other quality parameters such as protein content and oil content of grains are measured, the values should be interpreted on a dry weight basis or in terms of the MC (e.g., 13.4% protein content at 12% moisture content) for comparison of constituents (Anon 2007). Seed viability and storage characteristics are also severely affected by the grain MC. Grain MC is also an important factor in pricing at grain marketing (Wilson and Dahl 2002). Hence, determination of MC is of paramount importance in quality evaluation, handling, storage, and marketing of grains intended for food, feed, or seed.

Near-infrared spectroscopy (NIRS) is a widely used technique in food and feed analysis (Shenk et al 2001). Water is a very strong absorber in the NIR region, and analysis of water content by NIRS was the first successful application of this technology in food analysis (Norris 1964). Thereafter, it was used for analysis of many constituents in food and agricultural commodities. The influence of water should be included in NIR calibrations because it is such a strong absorber. NIR spectrometric methods have been developed for quality analysis of wheat including grain, milling, flour, dough, and baking quality parameters (Bramble et al 2006; Dowell et al 2006b; Jirsa et al 2008).

Quality evaluation of single kernels is gaining increased attention due to its ability to overcome sampling error problems associated with the methods of evaluation of grain quality by bulk sampling methods (Tonning et al 2008). Single kernel techniques have been developed for evaluating many wheat quality parameters using an automated kernel-feeding, single kernel NIR (SKNIR) system (Dowell et al 2006a). Techniques have also been developed for measurement of mycotoxin levels such as deoxynivalenol (DON) in single kernels of *Fusarium*-damaged wheat using the SKNIR system (Dowell et al 1999). When kernel samples are evaluated for *Fusarium* damage and DON levels, samples often include both sound (undamaged) and *Fusarium*-damaged kernels (FDK, or scabby kernels), which are significantly different in both chemical composition and physical properties. Such samples are composed of kernels with varying size, weight, and MC. Seitz and Bechtel (1985) showed that hard red winter (HRW) wheat kernels with increasing degrees of visible *Fusarium* head blight infection had higher ergosterol and DON levels, lower protein content, and lower kernel weight. Though kernel samples with varying MC are used in measuring constituent levels, the constituent levels are expressed as the percentage of the constituent that comprises the total sample. Hence, concomitant MC determination is important for estimation of constituent values at a given MC, thus enabling comparison of the levels of constituents among single kernels or bulk samples with varying MC.

The SKNIR system (Dowell et al 2006a) illuminates an area of the sample viewing trough that is larger than a kernel. The whole kernel is illuminated with visible and NIR light and scanned during spectrum acquisition. Therefore, the weight of the kernel, as well as the mass of water it contains, has a marked influence on the magnitude of NIR absorbance and the shape of the spectrum from the kernel, particularly in regions where NIR absorption bands of water and other major kernel dry matter components are located. The quantity of water and dry matter content of the kernel depends on the size of kernel, but it is not necessarily related to the % MC or % dry matter. Therefore, it might be reasonably expected that an NIR spectrum of a single kernel could be used to estimate single kernel weight (fresh weight or dry weight) as well as the mass of water the kernel contains.

An instrument with a calibration for single kernel MC and weight determination could be programmed to provide a constituent level at a given MC for single kernels or for bulk samples based on single kernel evaluation. Grain samples to be evaluated for *Fusarium* damage have both sound kernels and kernels with varying degrees of *Fusarium* damage and are very diverse in terms of kernel physical and chemical properties. However, there is no literature reporting NIR techniques for determination of weight, water mass, or MC in single wheat kernels or wheat kernels with or without *Fusarium* damage.

Therefore, the objective of this study was to predict single kernel fresh weight, dry weight, water mass, and MC of wheat kernels with or without *Fusarium* damage using NIR spectroscopy to express any constituent value at a given moisture level.

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MATERIALS AND METHODS

Two commercial HRW wheat cultivars, Jagalene and 2137, were used for calibration development and validation. Seven 10-g samples of each cultivar containing visually sound (brown plump), intermediate (brownish white shrunken), and scabby (white tombstones) kernels were placed in small (50 mL) plastic containers. A sample in an open container was placed in a low humidity chamber. Tap water was added to each of the other six containers at a rate of 0, 0.1, 0.2, 0.3, 0.4, and 0.5 mL/10 g, and the samples were thoroughly mixed by shaking the closed container to get single kernels with a gradient of MC. Containers were left for stabilization for three days before taking samples for scanning. Four kernels for each class (sound, intermediate, and scabby) were randomly selected from each container for scanning. Each kernel was weighed using a balance (Ohaus Discovery, Pine Brook, NJ) with a readability of 0.01 mg and immediately scanned using the SKNIR system (Dowell et al 2006a). Two spectra were recorded for each single kernel at two random positions in the 950–1,650 nm spectral range at 5-nm intervals. Consequently, a dataset of a single cultivar had 168 spectra (i.e., seven different MC levels, three classes of kernels, four kernels per class per MC level, and two random spectra per kernel).

Scanned kernels were dried in an oven at 130°C for 19 hr (ASABE 2008) and dry weights were recorded. Oven-dried MC of single kernels were computed on a fresh weight basis. Water mass in each kernel was computed by the difference in fresh weight (FW) and dry weight (DW) of kernels.

Mean-centered spectra (950–1,650 nm) with corresponding single kernel MC, fresh weight, dry weight, and water mass values were used to develop calibration models using the partial least squares (PLS) regression technique in the GRAMS/AI 8.0 software package (Thermo Fisher Scientific, Billerica, MA).

Two types of calibration models were developed for each cultivar: calibrations using spectra of all three types of kernels (2137-All or Jagalene-All) and calibrations using spectra of only sound kernels (2137-Sound or Jagalene-Sound) for predicting single kernel fresh weight, dry weight, MC, and water mass. Calibrations developed for one cultivar were validated using all spectra (2137-All or Jagalene-All) or spectra of individual types of kernels (2137-Sound, 2137-Intermediate, 2137-Scabby, Jagalene-Sound, Jagalene-Intermediate or Jagalene-Scabby) of the other cultivar. Validation datasets of one cultivar were predicted by both types of calibrations of the other cultivar. The paired residuals from these predictions were subjected to a *t*-test (v.9.1, SAS Institute, Cary, NC) to determine whether the differences in standard error of prediction (SEP) values from the two types of calibrations were statistically different. The ratio of the SEP to the standard deviation (RPD) was calculated as RPD = SD_{val}/SEP where SD_{val} is the standard deviation of the trait in the validation dataset (Williams and Norris 2001). The validation statistics of SEP, bias, R^2, and RPD were used for the evaluation of fitness and adequacy of the calibration models.

The water mass of single kernels was estimated by using actual fresh weight and MC predicted by the calibrations and compared against direct water mass predictions. The optimum number of PLS factors for each calibration model were selected based on the predicted residual error sums of squares (PRESS) statistic of cross-validations.

TABLE I
Variability and Distribution of Moisture Content (MC), Fresh Weight, Dry Weight, and Water Mass/Kernel (mg) in Different Classes of Kernels from Wheat Cultivar 2137

	Min	Max	Range	Avg	SD	CV%
MC %						
All	6.3	15.3	9.1	11.3	2.2	19.7
Sound	7.5	15.0	7.5	11.3	2.0	18.1
Intermediate	7.9	14.8	6.8	11.4	2.0	17.1
Scabby	6.3	15.3	9.1	11.3	2.7	23.6
Fresh weight (mg)						
All	10.0	42.1	32.1	23.8	7.0	29.5
Sound	20.3	37.2	16.9	28.0	4.1	14.6
Intermediate	18.4	42.1	23.7	27.1	5.8	21.6
Scabby	10.0	24.0	14.0	16.2	3.4	21.0
Dry weight (mg)						
All	8.7	37.0	28.3	21.1	6.2	29.4
Sound	17.3	33.6	16.3	24.8	3.6	14.7
Intermediate	16.3	37.0	20.7	23.9	5.1	21.3
Scabby	8.7	20.8	12.1	14.4	3.0	20.9
Water mass (mg)						
All	1.0	5.1	4.1	2.7	1.0	36.2
Sound	1.8	4.2	2.4	3.2	0.7	22.8
Intermediate	1.8	5.1	3.3	3.1	0.9	30.1
Scabby	1.0	3.2	2.3	1.8	0.6	34.1

TABLE II
Variability and Distribution of Moisture Content (MC), Fresh Weight, Dry Weight, and Water Mass/Kernel (mg) in Different Classes of Kernels from Wheat Cultivar Jagalene

	Min	Max	Range	Avg	SD	CV%
MC %						
All	6.2	14.8	8.5	11.4	2.2	19.1
Sound	6.9	14.1	7.1	11.2	2.1	18.3
Intermediate	7.3	14.3	7.0	11.4	2.0	17.8
Scabby	6.2	14.8	8.5	11.6	2.4	21.1
Fresh weight (mg)						
All	8.4	36.5	28.1	24.1	6.8	28.3
Sound	18.3	35.3	17.1	27.9	4.1	14.7
Intermediate	22.1	36.5	14.4	28.0	4.3	15.4
Scabby	8.4	25.6	17.2	16.5	4.2	25.6
Dry weight (mg)						
All	7.5	32.3	24.9	21.4	6.1	28.3
Sound	15.9	30.5	14.6	24.7	3.5	14.2
Intermediate	19.0	32.3	13.4	24.8	3.9	15.9
Scabby	7.5	22.1	14.6	14.6	3.6	24.6
Water mass (mg)						
All	0.7	4.8	4.1	2.8	0.9	34.4
Sound	1.6	4.8	3.2	3.1	0.8	26.1
Intermediate	2.2	4.7	2.5	3.2	0.7	22.6
Scabby	0.7	3.7	3.0	2.0	0.7	38.2

RESULTS AND DISCUSSION

Moisture Content and Weight of Kernels

Kernel weight and moisture properties are presented in Tables I and II for 2137 and Jagalene, respectively. The MC of 2137 kernels was 6.3–15.3% with a mean MC of 11.3% (Table I), while the Jagalene kernels had a slightly narrower range of MC at 6.2–14.8% with a mean MC of 11.4%. The MC of the kernels from the two cultivars used in this study had the same standard deviation of 2.2 and the range of MC reasonably represented the typical MC of harvested and processed grains. With regard to the kernel class, scabby kernels had a higher range and variability of MC values compared to sound and intermediate types of kernel, even though the average MC was approximately the same across all classes of kernels. All types of kernels were together in the containers during the moistening of kernels; therefore, the higher variability in MC of scabby kernels may be due to high variability in physical properties of scabby kernels in relation to kernel hydration.

Fresh weight of the 2137 kernels was 10.0–42.1 mg, while dry weight was 8.7–37.0 mg (Table I). Jagalene kernels had a lower range of weight with fresh weight at 8.4–36.5 mg and dry weight at 7.5–32.3 mg (Table II). Average fresh weight and dry weight of Jagalene kernels were slightly higher compared to 2137 kernels. In general, both fresh weight and dry weight of 2137 and Jagalene kernels and the variability in weight were approximately the same. In addition, the variability in the weight of scabby kernels was higher compared to that of sound or intermediate types of kernels. Consequently, scabby kernels had lower water mass and the variability in the water mass of scabby kernels was also higher compared to other types of kernel.

Water mass in each single kernel plotted against respective MC of each type of kernel of both cultivars are given in Fig. 1. The mass of water in a single kernel is directly related to both the weight and MC of the kernel. These results indicated that kernels with the same MC had a wider range of water mass due to differences in kernel weight. Scabby kernels are light in weight compared to sound or intermediate types of kernel (Tables I and II). Therefore, scabby kernels tend to possess a low water mass compared to sound or intermediate types of kernel at a given MC and this could affect the MC predictions.

The NIR spectra of scabby kernels are somewhat flat compared to the spectra of sound or intermediate types of kernel in the spectral regions at ≈970, 1190, and 1450 nm, while sound or intermediate kernel spectra showed distinct broad peaks (Fig. 2). Water is a strong absorber of NIR radiation. Pure water has a strong, broad NIR absorption peak at ≈1450 nm (Socrates 2001) due to the first overtone vibrations of the O-H stretch. There are also NIR water absorption bands at 970 nm due to the second overtone of the O-H stretch and at 1190 nm due to the combination of the first overtone of O-H stretch and O-H bending (Luck 1974; Buning-Pfaue 2003). Sound and intermediate kernel spectra also showed a noticeable rise in slope at ≈1360 nm compared to the scabby kernel spectrum. This could be attributed to the C-H combination vibrations of -CH3 groups of structural and food reserve components of kernels; sound and intermediate types of kernels have more NIR absorbance due to the higher weight and presence of more food reserves compared to scabby kernels. In addition, the absorption band at ≈1190 nm is also influenced by the C-H second overtone of the -CH3 groups (Shenk et al 2001). These results indicated that the NIR spectra of single kernels are significantly influenced by the mass of water present in kernels as well as the weight of the kernel. Thus, it would be advantageous if the influence of the kernel weight and water mass on the spectrum is somehow adjusted before spectra are used in calibration development for other constituents. The effect of kernel weight and water mass not only create a baseline shift in spectra, but also influence the shape of spectra. Therefore, derivative processing of spectra alone will not help to remove the effects of weight and water mass of kernels.

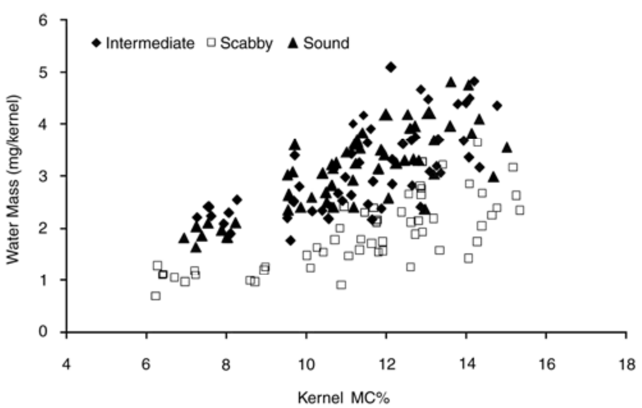

Fig. 1. Water mass in each single kernel plotted against respective MC of each type of kernel of both cultivars.

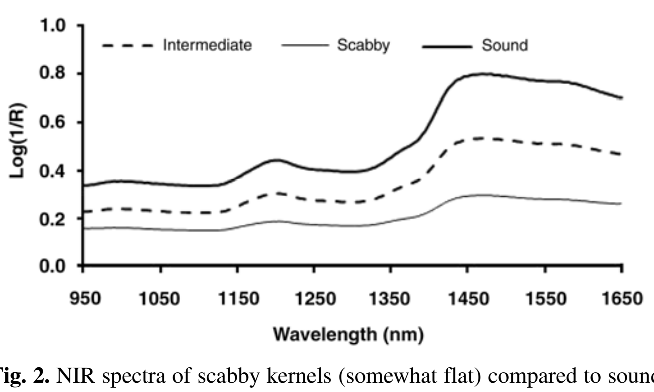

Fig. 2. NIR spectra of scabby kernels (somewhat flat) compared to sound or intermediate kernel spectra (distinct broad peaks).

It is also evident that in single kernel analysis, it is perhaps not appropriate to express constituent levels on a percentage basis. For example, a kernel evaluated to have 12% MC may have ≈1.5–5.5 mg of water per kernel (Fig. 1) depending on the weight of the kernel. Thus, it would be rather helpful if the level of constituent is expressed as a mass per kernel basis. If the weight of the kernel could be provided, this would also improve the accuracy of estimating small bulk sample constituent levels using single kernel analysis by estimating the mass of constituents in single kernels of the sample. Having the kernel weight individually is important, especially when the size and weight of the kernels in or among samples vary widely as in FHB-infected kernel samples.

Calibration Models

The number of PLS factors, coefficient of determination (R^2), and standard error of cross validation (SECV) of calibration models separately for single kernel MC, fresh weight, dry weight, or water mass of the two cultivars of kernels are given in Table III. The optimum number of PLS factors for the models were selected based on the *F*-test of the *F*-ratio between the PRESS value of *i*th PLS factor and the minimum PRESS value observed when each PLS factor was added to the model. Optimum number of PLS factors was determined when addition of a PLS factor to the model caused the *F*-test probability level to fall at or below 0.75.

Models that use all kernels always had higher number of PLS factors and higher SECV values compared to respective models that used only sound kernels. The R^2 of MC calibrations derived only using sound kernels were higher than MC calibration models developed using all kernels perhaps because kernel size for sound kernels was much more uniform than when including all kernels. Thus, the MC calculation would be less influenced by kernel size variability. However, R^2 values of the calibration models using all kernels for fresh weight, dry weight, and water mass were equal

TABLE III
Statistics for Calibrations Developed from All Kernels or Sound Kernels for Determination of Moisture Content (MC), Fresh Weight, Dry Weight, and Water Mass of Single Kernels

	PLS Factors	R^2	SECV
2137-All			
MC %	6	0.79	1.01
Fresh weight (mg)	4	0.76	3.41
Dry weight (mg)	5	0.78	2.88
Water mass (mg)	6	0.81	0.42
Jagalene-All			
MC %	6	0.77	1.03
Fresh weight (mg)	5	0.77	3.27
Dry weight (mg)	5	0.77	2.89
Water mass (mg)	7	0.83	0.39
2137-Sound			
MC %	4	0.87	0.73
Fresh weight (mg)	3	0.33	3.33
Dry weight (mg)	4	0.37	2.88
Water mass (mg)	4	0.75	0.36
Jagalene-Sound			
MC %	5	0.87	0.73
Fresh weight (mg)	3	0.60	2.57
Dry weight (mg)	3	0.52	2.40
Water mass (mg)	4	0.83	0.34

or markedly higher than that of the models using only sound kernels. Calibration models with all kernels for water mass had the highest R^2 values compared to models for MC, fresh weight, or dry weight. The increase in R^2 values in models using all kernels may be due to greater range in reference values in such models.

Validation of Calibration Models

The calibration models of one cultivar were validated by predicting traits of all spectra of the other cultivar. The R^2, bias, and RPD values of validations for Jagalene-All and 2137-All calibrations are given in Table IV. The validation results of Jagalene-Sound and 2137-Sound calibrations are presented in Table V. The SEP values for calibrations and the *t*-test probability values for the paired residuals of predictions are presented in Table VI.

The Jagalene-All calibration for MC (R^2 = 0.77, SECV = 1.03% with six PLS factors) (Table III) predicted the MC of 2137 kernels with a R^2 = 0.81, SEP = 1.02%, RPD = 2.2, and a bias = 0.43% (Tables IV and VI). However, prediction of the MC in scabby kernels increased the SEP value to 1.49%, while MC in sound and intermediate kernels were predicted with SEP values of 0.74 and 0.59%, respectively. Likewise, a higher SEP value (1.34) was also observed when the 2137-All calibration model was used to predict the MC of Jagalene-scabby kernels (Table VI). The same pattern was observed when the MC calibration model developed from sound kernels predicted the MC of the kernels in the other cultivar (Table VI). MC calibrations developed from sound kernels (R^2 = 0.87 and SECV = 0.73% with four or five PLS factors) (Table III) predicted the MC of sound kernels of the other cultivar with R^2 = 0.83–0.88, SEP = 0.74–0.87%, RPD = 2.4–2.7 and a bias = –0.10–0.28% (Tables V and VI) showing that NIRS could be used to predict the MC of sound kernels reasonably well. However, SEP is markedly increased (1.73–1.85%) when sound kernel calibrations predicted MC in scabby kernels with a corresponding decrease in RPD values (1.4–1.5). Calibration models that used all kernels could predict the MC in scabby kernels with SEP = 1.34–1.49% (Table VI) with RPD = 1.8 (Table V). Therefore, to predict MC in single kernels of *Fusarium*-damaged wheat samples that include all types of kernels (sound to intermediate to scabby tombstones), the calibration must be developed using all types of kernels.

When calibration models for fresh weight and dry weight were validated, it was noted that the R^2 of validations were significantly lower in predicting the weight of individual types of kernels compared to the prediction of all kernels. The highest and the lowest R^2 values of 0.79 and 0.74 were noted when the calibrations developed from all kernels predicted kernel fresh weight or dry weight of the other cultivar, but the R^2 values dropped to the highest and lowest of 0.62–0.15 when the calibrations using all kernels predicted the fresh weight or dry weight of individual types of kernels (Table IV). Predictions of fresh weight or dry weight of individual types of kernels by models developed from sound kernels also showed lower R^2 values, with the highest and lowest R^2 values = 0.66 and 0.08 (Table V). SEP values of validations of fresh weight and dry weight of scabby kernels by calibration models did not follow the trend shown by validations of MC calibration models. In contrast, scabby kernel weights were predicted with low SEP values compared to predictions of sound or intermediate types by calibrations developed from sound kernels or all kernels.

TABLE IV
Prediction Statistics for All Kernels or for Individual Type of Kernels When Using Calibrations Developed from All Kernels of the Other Cutlivars

Validation	Kernel Type	Calibration R^2	Calibration Bias	Calibration RPD
2137		Jagalene-All		
MC %	All	0.81	0.43	2.2
	Sound	0.87	0.52	2.7
	Intermediate	0.91	0.48	3.4
	Scabby	0.84	0.29	1.8
Fresh weight (mg)	All	0.79	1.18	2.2
	Sound	0.47	1.25	1.4
	Intermediate	0.51	0.70	1.4
	Scabby	0.60	1.59	1.4
Dry weight (mg)	All	0.79	0.96	2.2
	Sound	0.47	1.01	1.4
	Intermediate	0.51	0.59	1.4
	Scabby	0.61	1.29	1.4
Water mass (mg)	All	0.83	0.17	2.5
	Sound	0.81	0.21	2.2
	Intermediate	0.68	0.10	1.7
	Scabby	0.76	0.19	1.9
Jagalene		2137-All		
MC %	All	0.78	–0.29	2.1
	Sound	0.90	–0.26	1.9
	Intermediate	0.92	–0.40	2.9
	Scabby	0.81	–0.20	1.8
Fresh weight (mg)	All	0.74	–0.87	1.9
	Sound	0.60	–0.49	1.5
	Intermediate	0.15	–1.75	1.0
	Scabby	0.36	–0.38	1.2
Dry weight (mg)	All	0.78	–0.81	2.2
	Sound	0.62	–0.72	1.6
	Intermediate	0.29	–1.09	1.2
	Scabby	0.41	–0.61	1.3
Water mass (mg)	All	0.83	–0.17	2.3
	Sound	0.84	–0.20	2.3
	Intermediate	0.63	–0.19	1.6
	Scabby	0.76	–0.12	1.8

TABLE V
Prediction Statistics for All Kernels or for Individual Types of Kernels When Using Calibrations Developed from Sound Kernels of the Other Cultivar

Validation	Kernel Type	Calibration R^2	Calibration Bias	Calibration RPD
2137		Jagalene-Sound		
MC %	All	0.74	–0.06	1.7
	Sound	0.88	0.28	2.7
	Intermediate	0.91	–0.34	2.6
	Scabby	0.87	–0.11	1.5
Fresh weight (mg)	All	0.65	3.45	1.5
	Sound	0.40	1.05	1.3
	Intermediate	0.46	1.49	1.3
	Scabby	0.30	7.81	1.2
Dry weight (mg)	All	0.58	3.50	1.4
	Sound	0.34	0.93	1.2
	Intermediate	0.40	1.71	1.3
	Scabby	0.27	7.85	1.2
Water mass (mg)	All	0.78	0.20	2.0
	Sound	0.75	0.14	1.9
	Intermediate	0.71	0.02	1.6
	Scabby	0.54	0.45	1.4
Jagalene		2137-Sound		
MC %	All	0.61	–0.28	1.7
	Sound	0.83	–0.10	2.4
	Intermediate	0.83	0.29	2.3
	Scabby	0.73	0.66	1.4
Fresh weight (mg)	All	0.45	2.05	1.3
	Sound	0.66	–1.15	1.7
	Intermediate	0.08	–0.52	1.0
	Scabby	0.32	7.83	1.2
Dry weight (mg)	All	0.61	0.81	1.5
	Sound	0.56	–0.93	1.5
	Intermediate	0.12	–0.97	1.0
	Scabby	0.26	4.34	1.2
Water mass (mg)	All	0.60	0.17	1.5
	Sound	0.80	–0.18	0.6
	Intermediate	0.56	–0.03	1.5
	Scabby	0.48	0.73	1.3

Calibrations for water mass had four to seven PLS factors with R^2 = 0.75–0.83 and SECV = 0.34–0.42 mg (Table III). Calibrations developed from all kernels predicted water mass with R^2 = 0.63–0.84 and lowest and highest SEP values were 0.31–0.53 mg with RPD = 1.6–2.5, while bias values were –0.20 to 0.21 mg (Tables IV and VI). In comparison, models developed from sound kernels had R^2 = 0.48–0.78, SEP = 0.37–0.62 mg, RPD = 0.6–2.0 and bias of –0.18 to 0.73 mg. Therefore, models developed from all kernels seem to predict water mass in kernels better than the models developed from only sound kernels.

The results of the *t*-tests for paired residuals when a trait was predicted by two calibrations using all kernels or only sound kernels showed that calibrations derived from all kernels generally predict traits of kernels better with significantly lower or equal SEP values than calibrations derived from only sound kernels (Table VI). The Jagalene-All and Jagalene-Sound calibrations each predicted MC of 2137-Sound kernels with an equal SEP (0.74%), while the SEP values were lower for all other traits of all or individual types of kernels for the Jagalene-All calibration when compared to the Jagalene-Sound calibration. The same trend was observed when 2137-All calibration predicted traits of Jagalene kernels, with only one exception where 2137-Sound calibration predicted MC of Jagalene-Sound kernels with a significantly lower SEP = 0.87% with a *P* value of 0.0176. However, it is not unusual to expect a calibration derived from only sound kernels to predict traits of sound kernels much better than a calibration using all kernels.

The RPD values for calibration models that use all kernels are shown in Table IV. In general, the MC of kernels was predicted with a higher RPD (1.8–3.4) compared to the RPD values of 1.0–2.5 obtained for predicting fresh weight, dry weight, and water mass. The RPD values were lower for all traits when calibrations derived from only sound kernels were used (Table V).

The results of the above validations showed that NIRS can be used to estimate MC of single wheat kernels reasonably well. Determination of fresh weight, dry weight, or water mass of single wheat kernels by NIRS requires further improvement in calibration models. However, because water mass is more important than the MC in single kernels, the water mass of kernels was also estimated by using the predicted MC and actual fresh weight of kernels using models developed from all kernels or from only sound kernels. The prediction statistics were greatly improved when compared to the direct prediction of water mass (Fig. 3; Table VII). For example, water mass of 2137-All kernels were predicted by Jagalene calibration developed from all kernels with R^2 = 0.83, SEP = 0.40 mg, RPD = 2.5, and bias = 0.17 mg (Tables IV and VI). When water mass of kernels was estimated using predicted MC and actual fresh weight of kernels, prediction statistics were R^2 = 0.96, SEP = 0.21 mg, RPD = 4.8, and bias = 0.11 mg (Table VII). This trend was observed when water mass of all kernels or individual types of kernels was estimated using the MC

TABLE VI
SEP for Calibrations of Two Cultivars with Corresponding *t*-Test Probability Values for Paired Prediction Residuals

Validation	Kernel Type	SEP Calibration		
		Jagalene		
2137		Sound	All	Pr > | *t* |
MC %	All	1.26	1.02	0.0092
	Sound	0.74	0.74	0.0528
	Intermediate	0.78	0.59	0.2488
	Scabby	1.85	1.49	<0.0001
Fresh weight (mg)	All	4.70	3.22	<0.0001
	Sound	3.15	2.97	0.3957
	Intermediate	4.45	4.09	0.0503
	Scabby	2.87	2.36	<0.0001
Dry weight (mg)	All	4.49	2.84	<0.0001
	Sound	2.97	2.66	0.9838
	Intermediate	4.06	3.59	0.0145
	Scabby	2.59	2.08	<0.0001
Water mass (mg)	All	0.49	0.40	0.0032
	Sound	0.36	0.32	0.6037
	Intermediate	0.57	0.53	0.8779
	Scabby	0.43	0.31	<0.0001
		2137		
Jagalene		Sound	All	Pr > | *t* |
MC %	All	1.26	1.07	0.8293
	Sound	0.87	1.09	0.0176
	Intermediate	0.86	0.69	0.8225
	Scabby	1.73	1.34	0.0703
Fresh weight (mg)	All	5.35	3.52	<0.0001
	Sound	2.40	2.78	0.5970
	Intermediate	4.21	4.11	0.5568
	Scabby	3.49	3.43	<0.0001
Dry weight (mg)	All	4.00	2.82	<0.0001
	Sound	2.36	2.20	0.3162
	Intermediate	3.79	3.35	0.0232
	Scabby	3.12	2.84	<0.0001
Water mass (mg)	All	0.62	0.40	<0.0001
	Sound	1.26	0.35	0.8293
	Intermediate	0.48	0.45	0.3265
	Scabby	0.56	0.39	<0.0001

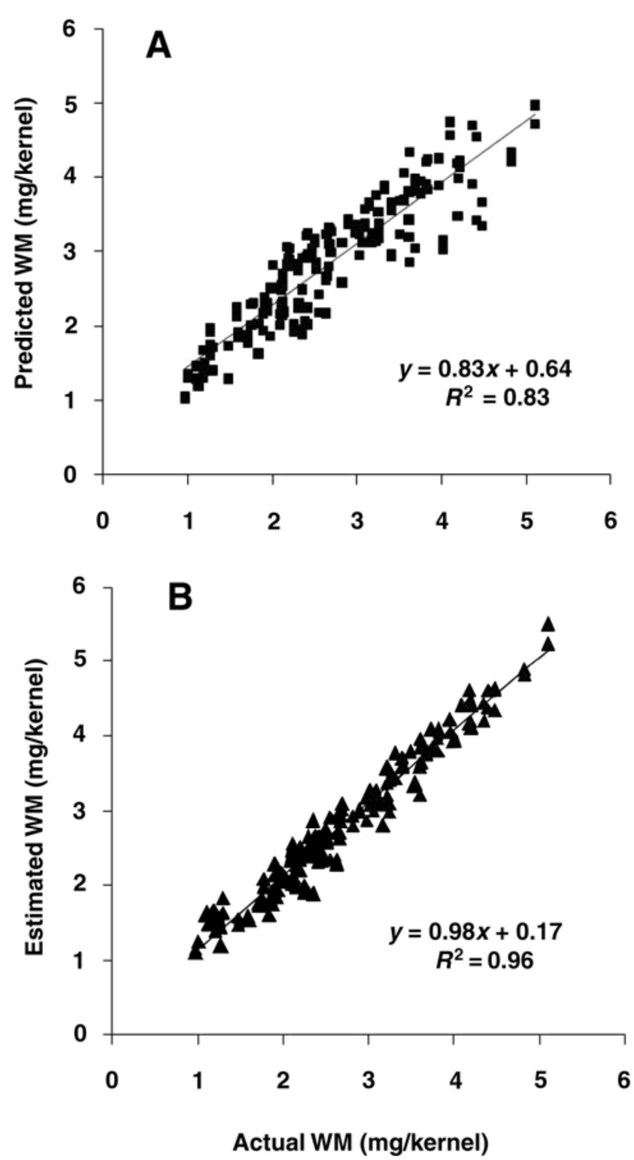

Fig. 3. Water mass of kernels by (**A**) direct NIRS prediction and (**B**) NIRS-predicted MC and actual fresh weight of kernels.

TABLE VII
Prediction Statistics for Water Mass Estimated by Predicted MC and Actual Fresh Weight of All or Individual Type of Kernels When Using Calibrations Developed from All Kernels or from Only Sound Kernels

Validation	Kernel Type	Calibration R^2	SEP	Bias	RPD	R^2	SEP	Bias	RPD
		Jagalene-All				Jagalene-Sound			
2137	All	0.96	0.21	0.11	4.8	0.94	0.25	–0.02	4.0
	Sound	0.92	0.20	0.15	3.5	0.93	0.19	0.08	3.7
	Intermediate	0.97	0.15	0.12	6.0	0.96	0.22	–0.10	4.1
	Scabby	0.86	0.24	0.05	2.5	0.81	0.31	–0.03	1.9
		2137-All				2137-Sound			
Jagalene	All	0.94	0.25	–0.07	3.6	0.93	0.26	0.05	3.5
	Sound	0.92	0.31	–0.07	2.6	0.92	0.24	–0.02	3.3
	Intermediate	0.96	0.19	–0.10	3.7	0.87	0.26	0.10	2.7
	Scabby	0.94	0.21	–0.05	3.3	0.90	0.26	0.09	2.7

predicted by calibrations from both cultivars. These results suggest that when actual weight of kernels can be measured by incorporating a measuring device to the instrument, constituent levels may be estimated more accurately in terms of mass or kernel basis. When such weight and constituent mass data is available, constituent levels in small bulk samples may be accurately estimated by single kernel analysis of those samples. These results also warrant the testing of this methodology for estimation of other important grain quality traits such as protein content, starch content, etc., which are presently estimated in terms of percentage basis.

CONCLUSIONS

The results of this study showed that NIR spectroscopy can be used to measure single kernel properties such as water mass or MC of sound or *Fusarium*-damaged single wheat kernels using the SKNIR system. Addition of spectra from scabby and intermediate types of kernels in calibration models increased the SECV of models. However, for predicting kernel properties in kernel samples with various stages of *Fusarium* damage, it is important to have a calibration model derived of spectra from sound, intermediate, and scabby kernels. The higher prediction errors associated with the scabby kernels may be due to high variability in chemical properties such as starch, protein, and lipid content, along with kernel weight and physical properties such as size, shape, surface texture, density, etc., of the scabby kernels in relation to the degree of *Fusarium* infection compared to sound kernels.

It appears that in single kernel analysis, it is important to measure constituents in terms of mass to kernel basis rather than percentage of constituent basis. Calibrations for measurement of constituents on a percentage basis had higher SEP and lower R^2 values. It may be possible to improve calibration performances if the influence of kernel size on spectra is removed by any spectral pretreatment process before using it for calibration development. The water mass of kernels could be estimated by direct prediction, by using the predicted fresh weight and the predicted MC of kernels, or by using the actual fresh weight and the predicted MC of kernels. If the actual fresh weight of the kernels could be provided by a weighing device attached to the instrument, then the water mass of single kernels can be predicted with very high accuracy.

LITERATURE CITED

Anon. 2007. Specifications, tolerances, and other technical requirements for weighing and measuring devices. Handbook 44. National Institute of Standards and Technology. U.S. GPO: Washington, DC.

ASABE. 2008. Moisture Measurement—Unground Grain and Seeds. ASAE S352.2 APR1988 (R2008). The Association: St. Joseph, MI.

Bramble, T., Dowell, F. E., and Herman, T. J. 2006. Single-kernel near-infrared protein prediction and the role of kernel weight in hard red winter wheat. Appl. Eng. Agric. 22:945-949.

Buning-Pfaue, H. 2003. Analysis of water in food by near infrared spectroscopy. Food Chem. 82:107-115.

Dowell, F. E, Ram, M. S., and Seitz, L. M. 1999. Predicting scab, vomitoxin, and ergosterol in single wheat kernels using near-infrared spectroscopy. Cereal Chem. 76:573-576.

Dowell, F. E., Maghirang, E. B., Graybosch, R. A., Baenziger, P. S., Baltensperger, D. D., and Hansen, L. E. 2006a. An automated near-infrared system for selecting individual kernels based on specific quality characteristics. Cereal Chem. 83:537-543.

Dowell, F. E., Maghirang, E. B., Xie, F., Lookhart, G. L., Pierce, R. O., Seabourn, B. W., Bean, S. R., Wilson, J. D., and Chung, O. K. 2006b. Predicting wheat quality characteristics and functionality using near-infrared spectroscopy. Cereal Chem. 83:529-536.

Jirsa, O., Hruskova, M., and Svec, I. 2008. Near-infrared prediction of milling and baking parameters of wheat varieties. J. Food Eng. 87:21-25.

Luck, W. A. P. 1974. Structure of water and aqueous solutions. Verlag Chemie: Weinheim, Germany.

Norris, K. H. 1964. Design and development of a new moisture meter. Agric. Eng. 45:370-372.

Seitz, L. M., and Bechtel, D. B. 1985. Chemical, physical, and microscopical studies of scab infected hard red winter wheat. J. Agric. Food Chem. 33:373-377.

Shenk, J. S., Workman, J. J., and Westerhaus, M. O. 2001. Application of NIR spectroscopy to agricultural products. Pages 419-474 in: Handbook of Near-Infrared Analysis, 2nd Ed. D. A Burns and E. W. Ciurczak, eds. Taylor and Francis: Boca Raton, FL.

Socrates, G. 2001. Infrared and Raman Characteristics Group Frequencies, 3rd Ed. John Wiley: Chichester, England.

Tonning, E., Nogaard, L., Engelson, S. B., Pedersen, L., and Esbensen, K. H. 2008. Single kernel near infrared analysis of bulk wheat heterogeneity—A theory of sampling reference study. NIR News 19:4-7.

Williams, P., and Norris, K. 2001. Near-Infrared Technology in the Agricultural and Food Industries, 2nd Ed. AACC Int.: St. Paul, MN.

Wilson, W. W., and Dahl, B. L. 2002. Marketing of wheat on a constant and nil moisture basis. Agribusiness and Applied Economics Report No 496. North Dakota State University: Fargo, ND.

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