Single Wheat Kernel Size Effects on Near-Infrared Reflectance Spectra and Color Classification

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ABSTRACT

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An optical radiation measurement system was used to measure reflectance spectra of single wheat kernels from 400 to 2,000 nm. Five classes of wheat were used for this study. Three kernel sizes (large, medium, and small) were used to determine how wheat kernel size affects visible and near-infrared (NIR) reflectance spectra and single wheat kernel color classification. Mean kernel weights ranged from 35.6 to 57.2 mg for large kernels, from 26.9 to 45.0 mg for medium kernels, and from 17.6 to 31.4 mg for small kernels. The results showed that wheat kernel size significantly affects visible and NIR reflectance spectra. Determination of the color class of red kernels increased in accuracy and that of white kernels decreased in accuracy as kernel size decreased. Data pretreatments such as multiplicative scatter correction (MSC), first or second derivatives, and first or second derivatives with MSC reduced the effect of kernel size on reflectance spectra and color classification.

Many factors affect the accuracy of near-infrared (NIR) reflectance spectroscopy for the analysis of agricultural products such as grains. Although NIR spectroscopy is relatively simple when compared with other types of analyses (there are no reagents to prepare and weighing is not required for some analyses), nearly 40 sources of error have been identified (Hruschka 1990). Of these, sample factors and operational factors are the two major sources of error affecting measurement accuracy. Williams (1975) and Williams and Thompson (1978) indicated that the most important sample factors affecting the accuracy of NIR reflectance spectroscopy for the analysis of ground wheat samples were the mean particle size and particle size distribution. Sample factors are influenced by growing environment, which causes large variations in kernel size, kernel size distribution, kernel density, and kernel color. These variations affect spectral measurement by influencing the absorption of radiation by the wheat sample (Watson et al 1977).

Norris and Williams (1984) studied the effect of particle size of ground hard red spring wheat samples on spectral measurements. They found that sample-induced errors in NIR reflectance spectral analysis could be caused by the variation of the sample surface. They reported that the log (1/*R*) values were significantly affected by particle size; coarse samples had higher absorption values. The effect of particle size was greater at longer wavelengths. They found that the second derivative divided by the second derivative at optimum wavelengths gave the best performance in predicting protein content of hard red spring wheat samples that varied widely in particle size.

NIR spectroscopy research has shifted from ground and bulk sample analysis to single-kernel analysis and from NIR transmittance to NIR reflectance (Delwiche and Massie 1996). Part of the reason for the shift from transmittance to reflectance is that reflectance is easier to automate. To meet these changes and obtain high levels of accuracy, the effect of grain's physical properties, such as kernel size, on single kernel spectra must be understood.

Kernel size can influence the measurement of single-kernel NIR spectra by affecting the amount of light (*F*) reaching the sensor, which is determined by the equation $F = f/D$, in which f is the focal length and D is the diameter of the illuminated object, if it is smaller than the field of view. The radiant flux (ϕ_c) increases as the inverse of the square of F: $\phi_c = 1/F^2$.

The smaller the value of *F*, the more radiant flux is collected by the lens. To obtain high levels of accuracy during the measurement, the focal length *f*, or distance from the kernel to the fiber, must be kept constant. However, the variation in kernel size can cause the focal length to change during measurement. Also, differences in kernel sizes will result in different illumination and reflectance areas and direction of reflected light. The objectives of this study were to: 1) identify the effect of wheat kernel size on single wheat kernel reflectance spectra and color classification, and 2) find methods to reduce the effect of kernel size on the reflectance spectra.

MATERIALS AND METHODS

Five U.S. market classes of wheat, hard red spring, hard red winter, soft red winter, hard white wheat, and soft white wheat, were supplied by the Grain Inspection, Packers, and Stockyards Administration (GIPSA) Technical Center (Kansas City, MO) (Table I). Single wheat kernel reflectance spectra from 400 to 2,000 nm at 2-nm intervals were collected with an optical radiation measurement system (Oriel, Stratford, CT), which has been described by Wang et al (*in press*). A single wheat kernel was suspended horizontally from the germ end by a vacuum tube, and the crease side of the kernel was viewed. Two experiments were conducted. The first experiment studied the effect of kernel size on NIR spectra. For this experiment, 375 kernels (25 small, medium, and large kernels from each class) were selected. The second experiment studied the effect of kernel size on color classification. For this experiment, 375 kernels (25 from three cultivars of each class) were selected randomly. These kernels were used as a calibration set to develop calibration equations that were used to predict the color of kernels used in the first experiment. Wang (1997) gave a complete discussion of all samples used in these experiments. To keep the distance constant between the reflectance surface of a wheat kernel and the end of the optical fiber, a three-dimensional multi-axis precision translator (Newport, Irvine, CA) was used to adjust kernel position. To maintain proper orientation, a waveplate/polarizer holder (Newport) was used to adjust kernel orientation from 0 to 360°.

The spectral data were first transferred to log (1/*R*) form by using the software package Grams/32 (Galactic Industries, Salem, NH). The spectral data were smoothed by the method of Savitsky and Golay (1964). A fifth-degree polynomial with 25 points was used for smoothing the spectral region from 400 to 1,450 nm, and a fifth-degree polynomial with 45 points was used for smoothing the spectral region from 1,450 to 2,000 nm. Derivatives were used to correct for overlapping peaks and large baseline variations. A

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fifth-degree polynomial with 25 points was used to calculate the first and second derivatives by the Savitsky-Golay method. Mean centering was used to remove some variation from the data and tended to scale the data such that the mathematics of the spectral decomposition and correlations performed better. Multiplicative scatter correction (MSC) was used to reduce the effect of kernel size on the reflectance spectra. MSC is designed to correct indeterminate path length effects caused by scattering and is calculated by regressing each training spectrum against an ideal spectrum (the average of all the spectra) and by removing the slope and offset effects.

Data were analyzed by partial least squares (PLS) regression (Galactic Industries). Three-class PLS models were developed to identify the effects of kernel size on the reflectance spectra. In each wheat class, large kernels were assigned a constant value of 1.0, medium kernels were assigned a constant value of 2.0, and small kernels were assigned a constant value of 3.0. For single wheat kernel color classification tests, two-class PLS models were developed to identify the effect of kernel size on color classification. Red and white wheat kernels were assigned constant values of 1.0 and 2.0, respectively. During the model testing, a kernel was considered to be correctly categorized if the predicted value lay on the same side of the midpoint as its assigned value. NIR model performance was reported as the multiple coefficient of determination (r^2) and standard error of calibration of each calibration. The number of factors reported was based on the *t* test and was the minimum required to give the maximum multiple coefficient of determination.

TABLE I
Samples Used to Determine the Effect of Wheat Kernel Size on Reflectance Spectra

Wheat Class[a] Kernel Size	No. of Kernels	Width (mm) Mean	Width (mm) SD	Length (mm) Mean	Length (mm) SD	Weight (mg) Mean	Weight (mg) SD
HRS							
Large	25	3.62	0.13	6.83	0.32	56.6	5.4
Medium	25	2.94	0.07	6.47	0.25	31.8	4.6
Small	25	2.36	0.08	5.78	0.31	18.8	2.9
HRW							
Large	25	3.20	0.07	6.83	0.17	35.6	2.9
Medium	25	2.79	0.03	6.43	0.24	26.8	2.4
Small	25	2.43	0.18	5.64	0.25	17.6	2.4
SRW							
Large	25	3.71	0.06	7.45	0.21	45.8	3.0
Medium	25	3.25	0.03	6.89	0.12	34.7	3.0
Small	25	2.78	0.04	5.94	0.11	22.1	2.0
HWW							
Large	25	3.87	0.07	7.45	0.21	57.2	3.8
Medium	25	3.38	0.05	7.00	0.20	45.0	3.7
Small	25	2.89	0.06	6.48	0.19	31.4	4.1
SWW							
Large	25	3.93	0.08	7.45	0.27	55.9	4.6
Medium	25	3.35	0.08	6.81	0.29	39.1	2.1
Small	25	2.93	0.10	6.17	0.21	28.0	2.8

[a] HRS = hard red spring; HRW = hard red winter; SRW = soft red winter; HWW = hard white wheat; and SWW = soft white wheat.

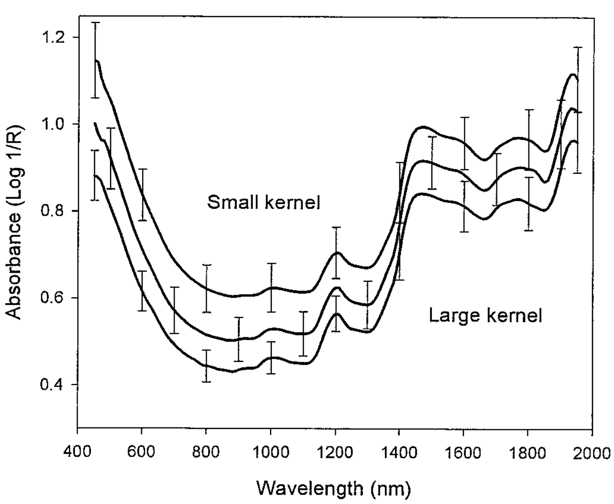

Fig. 1. Effect of wheat kernel size on the absorbance spectra in the wavelength region of 400–2,000 nm. Each vertical bar represents one standard deviation.

RESULTS AND DISCUSSION

Effect of Kernel Size on Reflectance Spectra

In single-kernel reflectance measurement, the illumination area is one of the most important factors affecting the errors caused by kernel size variation. The average spectral curves showed that the amount of radiation absorbed by wheat kernels decreased as kernel size increased (Fig. 1). Small kernels had a smaller reflectance area, which resulted in a large value of *F* (see equation) and less radiant flux than medium and large kernels. Therefore, small kernels lost some radiation energy to the background during the spectral measurement.

TABLE II
Summary of the Coefficient of Determination (r^2) and Percentage of Correct Classification of Kernel Size Using a Three-Class Partial Least Squares Classification Model[a]

Region and Pretreatment	r^2 Red Wheat[b]	r^2 White Wheat[c]	Average Correct Classification (%)
NIR region (750–1,900 nm)			
Log (1/*R*)	0.77a	0.72a	80
MSC[d] on log (1/*R*)	0.65b	0.57b	69
First derivative	0.61b	0.59b	70
MSC on first derivative	0.55c	0.47c	67
Second derivative	0.50d	0.48c	65
MSC on second derivative	0.41e	0.40d	58
Visible region (550–750 nm)			
Log (1/*R*)	0.62a	0.60a	66
MSC on log (1/*R*)	0.09c	0.22b	41
First derivative	0.22b	0.25b	46
MSC on first derivative	0.09c	0.22b	43
Second derivative	0.14b	0.27b	44
MSC on second derivative	0.03c	0.15c	39

[a] Values in a column followed by different letters are significantly different at ($P < 0.05$).
[b] Averages of hard red spring, hard red winter, and soft red winter.
[c] Averages of hard white and soft white.
[d] Multiplicative scatter correction.

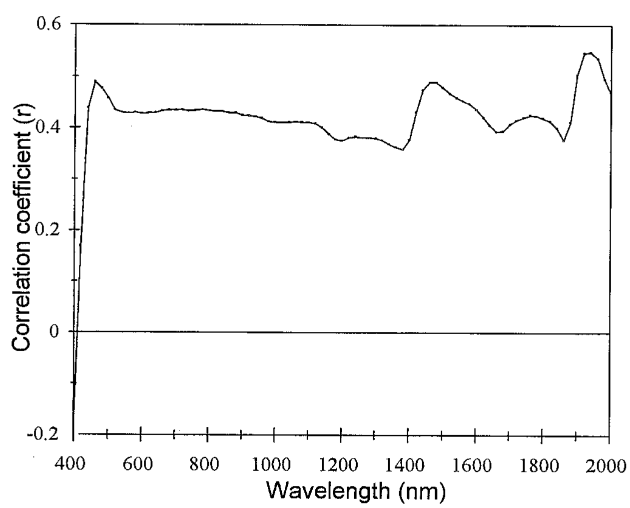

Fig. 2. Correlation coefficient (*r*) between kernel size and absorbance, log (1/*R*), in the wavelength region of 400–2,000 nm.

The effect of kernel size on the spectra was determined by the multiple linear relationship between kernel size and absorbance, log (1/*R*), by using the three-class PLS classification models. Table II shows the values of r^2 averaged across the five wheat classes and the percentage of correctly classified kernels by size. In the NIR region, the results indicated that kernel size had a linear correlation with absorbance for both red (r^2 = 0.77) and white (r^2 = 0.72) wheats when the log (1/*R*) values were used. In the visible region, the results also indicated that the effects of kernel size on the reflectance spectra were significant; r^2 = 0.62 for red wheats and 0.60 for white wheats. The percentage of all kernels correctly classified by size was higher for the NIR region (80%) than for the visible region (66%). These results indicate that kernel size had a greater effect on the NIR region than on the visible region. A plot of the coefficients of determination indicates that a linear relationship between kernel size and absorbance exists throughout the entire wavelength region (Fig. 2). The greatest correlations occurred at ≈490, 1,460, 1,760, and 1,920 nm. The correlation peaks at ≈1,460 and 1,760 nm were dominated by a functional group of amino (NH), which related to protein. The correlations at ≈1,920 nm were dominated by water.

The effects of kernel size on absorbance spectra can be reduced by data pretreatments such as MSC, first derivative, second derivatives, and combinations of MSC and the first derivative and second derivatives (Table II). All of the data pretreatments significantly ($P < 0.05$) reduced the effect of kernel size on the absorbance spectra, regardless of the individual pretreatment or the combination of the pretreatments. However, the second derivative and second derivative with MSC were the best methods for reducing the effect of kernel size on the NIR spectra. In general, log (1/*R*) with MSC and the first and second derivatives with MSC were the most effective methods for reducing the effect of kernel size on the reflectance spectra in the visible region.

TABLE III
Effect of Kernel Size on Predicting Wheat Color Class[a] when Kernel Absorbance (log 1/*R*) is Used Over the Wavelength Region of 500–1,700 nm

Class	Prediction Values	Red Kernels[b]			White Kernels[c]		
		Large	Med.	Small	Large	Med.	Small
Red	<0.55	0	0	5	0	0	0
	0.56–0.75	0	4	11	0	0	0
	0.76–1.05	14	32	35	0	0	0
	1.06–1.25	27	27	21	0	0	2
	1.26–1.45	24	10	3	0	1	1
	1.46–1.50	3	1	0	1	1	1
White	1.51–1.55	2	1	0	0	0	4
	1.56–1.75	3	0	0	8	7	14
	1.76–2.05	2	0	0	27	35	28
	2.06–2.25	0	0	0	14	6	0

[a] Red class, ≤1.5; and white class, >1.5.
[b] Includes hard red spring, hard red winter, and soft red winter. n = 75 for each kernel size.
[c] Includes hard white wheat and soft white wheat. n = 50 for each kernel size.

Effect of Kernel Size on Single Wheat Kernel Color Classification

The effect of kernel size on single wheat kernel color classification was tested by using the PLS calibration equations described by Wang (1997). Table III shows the effect of kernel size on the prediction of wheat kernel color class. For red wheat, the large kernels had larger predicted class values and more misclassified kernels than medium and small kernels. For white wheat, the small kernels had smaller predicted class values and more misclassified kernels than large and medium kernels.

Some data pretreatments can be used to reduce the effect of kernel size on single wheat kernel color classification. Table IV shows the effect of data pretreatments on kernel color classification. The second derivative yielded a higher percentage of misclassified kernels than log (1/*R*). Therefore, the test results from the second derivative are not presented. In the visible region, when the log (1/*R*) values were used for single wheat kernel color classification, the average percentage of misclassified kernels was 4%; after the first derivative was used to reduce kernel size effects, the percentage of misclassified kernels was 2.4%. In the visible and NIR regions, when the log (1/*R*) values were used for single wheat kernel color classification, the percentage of misclassified kernels was 4%; after MSC was used on log (1/*R*) to reduce kernel size effects, the percentage of misclassified kernels was 2.1%. Thus, several types of spectral pretreatments reduced the effect of kernel size on color classification.

CONCLUSIONS

Wheat kernel size had a significant effect on visible and NIR reflectance spectra. The amount of radiation absorbed by the wheat kernel decreased as kernel size increased. Therefore, kernel size may introduce variation into absorbance spectra and reduce the accuracy of analysis. Kernel size had a stronger effect on spectra in the NIR region than in the visible region, indicating that kernel size had a significant effect on wheat constituent analysis. Data pretreatments such as MSC, first or second derivatives, and the first or second derivatives with MSC can reduce the effect of kernel size on spectra. Generally, the best methods were the second derivative and second derivative with MSC.

Kernel size had a significant effect on single wheat kernel color classification. Predicted values of color class increased as kernel size increased. The accuracy of determining red kernels increased and of white kernels decreased as kernel size decreased. These effects were reduced significantly by some data pretreatments. When

TABLE IV
Effect of Kernel Size, Data Pretreatments, and Wavelength Range on Single Wheat Kernel Color Classification

Range (nm) Pretreatment	Red Kernels[a] Misclassified (%)				White Kernels[b] Misclassified (%)				Mean Across Color Classes
	Large	Medium	Small	Mean	Large	Medium	Small	Mean	
500–750									
Log (1/*R*)	10.7	0	0	3.6	0	2.0	12.0	4.6	4.0
MSC,[c] log (1/*R*)	5.3	6.8	1.3	4.9	0	0	6.0	2.0	3.7
First derivative	5.3	1.3	0	2.2	2.0	0	6.0	2.7	2.4
First derivative, MSC	5.3	1.3	0	2.2	4.0	0	6.0	3.3	2.7
500–1,700									
Log (1/*R*)	10.7	0	0	3.6	0	2.0	12.0	4.6	4.0
MSC, log (1/*R*)	5.3	0	0	1.8	2.0	0	6.0	2.6	2.1
First derivative	4.0	0	0	1.3	2.0	0	12.0	4.7	2.7
First derivative, MSC	6.7	0	0	2.2	4.0	0	6.0	3.3	2.7

[a] Includes hard red spring, hard red winter, and soft red winter. For each kernel size, number of kernels = 75.
[b] Includes white hard white wheat and soft white wheat. For each kernel size, number of kernels = 50.
[c] Multiplicative scatter correction.

MSC was applied to log (1/*R*) to reduce kernel size effects, the accuracy of color classification increased from 96 to 97.9%. Thus, MSC should be useful in other single-kernel NIR studies to reduce the effect of kernel size on classification results.

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