Automated Color Classification of Single Wheat Kernels Using Visible and Near-Infrared Reflectance

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ABSTRACT

Modification of an existing single kernel wheat characterization system allowed collection of visible and near-infrared (NIR) reflectance spectra (450–1,688 nm) at a rate of 1 kernel/4 sec. The spectral information was used to classify red and white wheats in an attempt to remove subjectivity from class determinations. Calibration, validation, and prediction results showed that calibrations using partial least squares regression and derived from the full wavelength profile correctly classed more kernels than either the visible region (450–700 nm) or the NIR region (700–1,688 nm). Most results showed >99% correct classification for single kernels when using the visible and NIR regions. Averaging of single kernel classifications resulted in 100% correct classification of bulk samples.

Wheat (*Triticum aestivum* L., and *T. compactum* Host.) undergoes many environmental stresses that can affect kernel color. For example, excessive rain, frost, insects, and disease can alter the appearance of kernels, causing genetically red kernels to appear white and genetically white kernels to appear red. This makes it difficult, and sometimes impossible, for inspectors to determine color class. Currently, Federal Grain Inspection Service inspectors visually examine kernels and determine color class along with other quality characteristics (Federal Register 1987). Color class is important to determine accurately because functionality traits of genetically red and white wheats cause them to mill and bake differently. Thus, millers and bakers need accurate information on color class to meet customer specifications. In addition, the current process of determining color class is labor-intensive, and color class can be difficult to determine if samples contain mixtures of red and white wheat.

Other researchers have attempted to develop methods of objectively determining color class. McCaig et al (1993) used a near-infrared (NIR) and visible (VIS) spectrophotometer to classify red and white wheat, but the instrument could not classify single kernels and had difficulty with samples that were not obviously red or white. Several researchers (Chen et al 1972, Bason et al 1995) used a tristimulus color meter to classify red and white samples with good accuracy, but they did not consider difficult-to-classify samples nor could they handle single kernels. Ronalds and Blakeney (1995) used NIR-VIS information to classify bulk samples with good success. However, they had some overlap of predicted samples. The instrument also had no single kernel capability, which is needed to detect mixed samples. Other researchers used color machine vision (Neuman et al 1989a,b) and monochromatic machine vision (Neuman et al 1987) to classify single wheat kernels, but with considerable overlap of color classes. Delwiche and Massie (1996) measured reflectance of single kernels over the range of 550–750 nm and 1,100–2,500 nm. They showed classification accuracies from 80 to 100%, with the best red and white wheat classifications resulting from visible wavelength information.

The above-mentioned studies did not include automated classification and either could not accommodate single kernels or the experimental designs did not include samples that were not obviously red or white. The objective of this research was to determine the accuracy of an automatic single kernel color classification system, particularly when classifying kernels difficult for inspectors to classify. The automated classification system is part of related research to integrate color and protein measurements into an existing single kernel wheat characterization system (SKWCS).

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MATERIALS AND METHODS

Instrumentation

A SKWCS was integrated with a DA-7000 diode-array spectrometer (Perten Instruments, Reno, NV) using fiber optics to allow automated collection of VIS and NIR spectra. The SKWCS consists of a singulator wheel, a weighing bucket, and crushing mechanism. Martin et al (1993) gives a complete description of the SKWCS. The modified kernel singulator delivered kernels at a rate of 1 kernel/4 sec. The crushing mechanism of the SKWCS was bypassed to preserve samples for confirmation of color class as needed. The parallel-channel DA-7000 spectrometer has silicon sensors spaced at 7-nm intervals that measure VIS and NIR radiation at 400–950 nm. Indium-galium-arsenide sensors spaced at 11-nm intervals measure NIR radiation at 950–1,700 nm. A stationary grating disperses broadband energy to the sensors where all data is collected simultaneously. A light chopper allows automatic rejection of ambient light. All data were interpolated to 2-nm intervals. The DA-7000 collects spectra at a rate of 30/sec.

Sample Selection

Calibration, validation, and prediction sets were carefully selected from samples representing hard red winter, soft red winter, hard red spring, soft white and hard white wheat. The calibration and validation samples originated from all growing areas in the United States and had protein, moisture, and hardness values representing crop averages and ranges reported in the Federal Grain Inspection Service's 1992, 1993, and 1994 market survey reports. Samples originated from commercial sources and from breeders and were stored in air-tight containers at room temperature before and during testing. Of the 112 samples selected, 88 were considered obviously red or white classes, whereas the remaining 24 samples were difficult for inspectors to determine color class. A NaOH test (DePauw and McCaig 1988) confirmed the color class of all suspect samples. The prediction set also represented the same growing areas and classes listed above, but came from different locations from the calibration set. Table I shows the number of samples for each sample set.

Data Collection

Samples were randomly analyzed by placing ≈20 kernels from one sample in the SKWCS and allowing the system to automatically feed the kernels and collect spectra, resulting in 20 sequentially numbered spectra per sample. The spectrum stored for each kernel consisted of an average of six spectra. A new baseline consisting of a spectrum of an optically flat black material was collected after ≈100 spectra. All data were electronically stored. Ten even-numbered spectra from each sample were placed in the calibration file. Kernels not fully in the field of view during data collection were discarded and replaced by their closest odd-numbered neighbors. The remaining odd-numbered spectra made up the validation set. The prediction set was collected similarly, but not divided into odd or even samples.

Data Analysis

All data were analyzed using partial-least squares (PLS) regression, which is a spectral decomposition technique, performed by a module of GRAMS/32 software (Galactic Industries, Salem, NH). PLS uses concentration information during the decomposition process and tries to get as much information as possible into the first few loading vectors. PLS takes advantage of the correlation relationship between the spectral data and the concentration of the biological parameter, which in this case is color. Martens and Naes (1989) give a complete description of PLS. All spectra were mean-centered before analysis. PLS was chosen over principal components techniques because related unpublished research shows PLS is superior because it takes advantage of concentration information.

For the calibration file, all red class kernels were assigned a value of 1, and all white class kernels were assigned a value of 2. In predictions, kernels with a predicted value of <1.5 were classed as red and all other kernels classed as white. Statistical significance of differences in classification percentages and coefficients of determinations (r^2) were calculated using least significant differences and homogeneity of correlation coefficients procedures described by Steel and Torrie (1980).

TABLE I
Number of Samples and Kernels Used in Wheat Kernel Color Classification Studies[a]

	Calibration and Validation Sets			Prediction Set	
	No. Samples	Calibration Kernels	Validation Kernels	No. Samples	Kernels
Red					
Obvious red	43	430	278	na[b]	na
DTC[c]	12	120	101	na	na
Total	55	550	379	30	300
White					
Obvious white	45	450	376	na	na
DTC	12	120	101	na	na
Total	57	570	477	30	300
Grand total	112	1,120	856	60	600

[a] Maximum of 10 calibration or validation kernels drawn from each sample.
[b] Not applicable. Prediction set samples not divided into obvious red and white or difficult-to-classify sets.
[c] Difficult-to-classify. Samples were difficult for trained inspectors to determine color class.

TABLE II
Classification of Single Wheat Kernels Using Visible (VIS) (450–700 nm) and Near-Infrared (NIR) (700–1,688 nm) Spectra[a]

Wavelength Range	% Correct Red	% Correct White	% Correct Total	r^2	Standard Error[b]
Calibration set					
VIS	99.6	97.5	98.6a	0.825a	0.209
NIR	99.1	97.9	98.5a	0.807a	0.220
VIS+NIR	100.0	99.8	99.9b	0.875b	0.177
Validation set					
VIS	96.6	95.0	95.7a	0.765a	0.222
NIR	96.6	96.0	96.3a	0.749a	0.233
VIS+NIR	98.2	98.1	98.1b	0.819b	0.200
Prediction set					
VIS	98.7	98.0	98.3a	0.807a	0.226
NIR	95.7	98.3	97.0a	0.762b	0.256
VIS+NIR	98.7	99.7	99.2b	0.832a	0.220

[a] Values in columns followed by the same letter for a given set are not significantly different as determined by the least significant difference and homogeneity of correlation coefficients tests at the $P = 0.10$ level.
[b] Based on correctly classifying red kernels as <1.5 and white kernels as ≥1.5.

RESULTS AND DISCUSSION

System Performance

The automated system collected spectra at a rate of 1 kernel/4 sec. Approximately one kernel out of every 10 either was absent or not fully in the field of view when a spectrum was collected. This resulted in spectra of only the background, or of only part of the kernel. These spectra were excluded from the calibration, validation, and prediction sets by culling spectra that had absorbance values above a predetermined cutoff point. Observations of kernel spectra and correlation spectra revealed excessive noise below 450 nm, thus data below this value were excluded. In addition, the interpolation algorithm resulted in truncation of values above 1.688 nm. Thus, the wavelength ranges used in all subsequent analysis were within the 450–1,688 nm region.

Classification Results

Results are presented separately for the calibration, validation, and prediction sets. The color class of the kernels used in each of these three sets was predicted by using the calibration model developed from the kernels in the calibration set. Correlation spectra showed most information useful for correct classification lay in the 450–650 nm region. However, results revealed that best classification resulted from using the entire 450–1,688 nm region. The number of PLS factors used for the calibrations were 22, 20, and 24 for the VIS (450–700 nm), NIR (700–1,688 nm), and VIS+NIR region,

TABLE III
Classification of Obviously Red and White and Visually Difficult-to-Classify Wheat Kernels Using Visible (VIS) (400-700 nm) and Near-Infrared (NIR) (700-1,688 nm) Spectra[a]

	% Correct					
	Difficult-to-Classify			Obvious Red or White		
	Red	White	Total	Red	White	Total
Calibration set						
VIS	100	96.7	98.3a	99.5	97.8	98.6a
NIR	100	98.3	99.2ab	98.8	97.8	98.3a
VIS+NIR	100	100	100b	100	99.8	99.9b
Validation set						
VIS	99.0	91.1	95.0a	95.7	96.0	95.9a
NIR	96.0	99.0	97.5a	96.8	95.2	95.9a
VIS+NIR	96.0	97.0	96.5a	98.9	98.4	98.6b

[a] Values in columns followed by the same letter for a given set are not significantly different at the $P = 0.10$ level.

TABLE IV
Classification of Samples if the Color Class of All Kernels from Each Sample is Averaged to Determine Color Class

Wavelength Range[a]	% Correct
Calibration Set	
VIS	100
NIR	100
VIS+NIR	100
Validation Set	
VIS	99.1
NIR	100
VIS+NIR	100
Prediction Set	
VIS	100
NIR	96.7
VIS+NIR	100

[a] VIS = visible (450–700 nm). NIR = near-infrared (700–1,688 nm).

respectively. The number of factors selected was based on the *F*-ratio of the predicted residual error sum of squares (PRESS). When the ratio of the current PRESS to all previous PRESS values fell below 0.75, then this was considered the optimum number of factors. Using a few more or less factors than those reported did not significantly change classification accuracies.

A calibration based on the entire VIS and NIR spectrum resulted in 99.9% correct classification of the kernels in the calibration set (Table II). This classification rate was significantly higher than the rates achieved when using the VIS or NIR regions separately. Some differences were not significant at the $P = 0.05$ level, but were significant at the $P = 0.10$ level. Thus, the $P = 0.10$ level was used for all statistical comparisons. The r^2 for the combined VIS+NIR region (0.875) was also significantly higher than results achieved when using either the VIS or NIR region separately. The high classification rate achieved for any of the wavelength regions examined (98.5–99.9%) was expected because these predicted kernels were the same as those used to develop the calibration.

Table II shows classification rates for the validation set as being only slightly lower than the calibration set rates. This was expected because kernels in the validation set came from the same samples as kernels from the calibration set. Classification results were slightly lower probably because some sampling error was introduced due to kernel-to-kernel variability within samples. The VIS+NIR region still classified kernels significantly better (98.1% correct) than either the VIS (95.7%) or NIR (96.3%) regions alone. The r^2 value of 0.819 was also significantly better than the other regions. The similarity in classification rates between the validation and calibration set shows the validity of the calibration model.

The true test of the calibration is demonstrated by predicting the color class of samples derived from sources independent of the calibration set. Results from this prediction set (Table II) indicated that classification rates were similar to those for the calibration and validation sets. The correct prediction of kernels using the VIS+NIR calibration was significantly higher than the other regions. The correct classification rate of 99.2% and r^2 of 0.832 was similar to the values achieved with the calibration and validation sets, which illustrates the robustness of the calibration. Thus, this calibration should be valid across growing regions and crop years since the prediction set represented nine states and seven crop years and no attempt was made to cull difficult samples from the prediction set.

Table III separates the kernels into difficult-to-class and obviously red or white categories. The table illustrates that the calibration performs similarly for both the difficult kernels and obvious red or white kernels.

Table IV shows classification results if the color classes of individual kernels are averaged to give an average color class for the sample. In all tests, the VIS+NIR calibration classified 100% of the samples correctly. The VIS and NIR calibrations each misclassed a few samples, which again illustrates that both regions are needed for maximum classification accuracy.

These results compare favorably with those reported by Delwiche and Massie (1996) where they reported 97.5% and 98.8% correct classification for white and red wheat kernels, respectively. They achieved their results using only the 551–750 nm region, but did not included difficult-to-classify kernels. The inclusion of kernels that were not obviously red class or white class in the research reported here likely explains the need for NIR to improve classifications. It was also encouraging to see that results from the present tests were similar to those reported by Delwiche and Massie, considering that kernels of the present study were automatically fed and randomly placed in the viewing area, whereas kernels of their study were hand-placed and always oriented in the same direction. Thus, error due to kernel placement was likely higher in the present tests, yet excellent classification was still achieved.

All results show that NIR information is needed in addition to visible wavelength information. This suggests that there is some difference in the molecular structure of red and white wheat classes. NIR spectra are primarily influenced by combination and overtone bond vibrations involving carbon, hydrogen, nitrogen, and oxygen bonds. However, the presence of other atoms can influence the spectra. Thus, to fully explain why NIR is important in classifying red and white wheat classes, further chemometric analysis is needed to identify what physical and biochemical properties are unique to each color class.

In summary, results from this study show that an automated system can remove subjectivity in classifying red and white wheat kernels by measuring spectral reflectance from 450–1,688 nm. A correct classification rate for individual kernels of 99.2% on samples independent of the calibration set was achieved. When averaging single kernel predictions, 100% of all bulk samples were correctly classified. Thus, this system will reduce inspector subjectivity in determining color class in samples of wheat and identify mixtures of red and white wheat classes by predicting single kernel color class. Future work is recommended on identifying specific wavelengths contributing to correct classification and on determining the chemical components responsible for the uniqueness of red and white color classes.

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