Effect of NaOH on Visible Wavelength Spectra of Single Wheat Kernels and Color Classification Efficiency

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ABSTRACT

A diode-array system, which measures spectral reflectance from 400 to 700 nm, was used to quantify single wheat kernel color before and after soaking in NaOH as a means of determining color class. Wheat color classification is currently a subjective determination and important in determining the end-use of the wheat. Soaking kernels in NaOH and classifying the soaked kernels with the diode-array system resulted in more difficult-to-classify kernels correctly classified (98.1%) than the visual method of classifying kernels (74.8%). Kernel orientation had a slight effect on correct classification, with the side view correctly classifying more kernels than the dorsal or crease view. The diode-array system provided a means of quantifying kernel color and eliminated inspector subjectivity when determining color class.

Licensed inspectors grade most wheat (*Triticum aestivum* L.) produced and marketed in the United States and all wheat shipped in foreign commerce. These grades include estimates of moisture content, test weight, damage, defects, dockages, foreign material, heat-damage, other grains, shrunken and broken kernels, spring or winter class, hard or soft class, and red or white class (GIPSA/FGIS 1993). Rapid and objective means exist for measuring some factors such as moisture content and test weight. However, a rapid and objective method for determining other factors such as red or white wheat class does not exist. Currently, inspectors view kernels and attempt to classify the kernels into red or white wheat classes. However, factors such as weather conditions, soil conditions, cultivar, disease, and insect damage can influence kernel color by stressing the seed or otherwise affecting the visual appearance of the seed. These factors can make it impossible to visually determine color class. Color class is important because red and white wheat have different milling, baking, taste, and, of course, visual characteristics. These color class identification problems are also encountered by wheat breeders, other grain scientists, industry workers, and others that need to correctly determine color class.

Kernels can be soaked in a solution of sodium hydroxide (NaOH) to assist inspectors in determining color class. Genetically red kernels tend to turn deep red after soaking, whereas genetically white kernels tend to turn light cream in color (Chmelar and Mostovoj 1938, Quartley and Wellington 1962, DePauw and McCaig 1988, Kimber 1971, Coles and Wrigley 1976, Lamkin and Miller 1980). While the determination of red or white wheat class is less subjective after soaking in NaOH than before soaking, this evaluation still requires some judgment by the inspector. This may be especially true when comparing samples grown in different crop years and under different environmental conditions that may affect the kernels reaction to NaOH. Determining red and white wheat class can be a problem before and after soaking in NaOH if the inspector is color blind, which occurs in 8% of males (Padgham 1965). Some uniform means of quantifying the NaOH reaction on wheat is needed. Besides helping inspectors determine color class, a means is needed to determine color class consistently and accurately to provide a calibration set for related research to automate the red and white classification process.

Two basic means exist to quantify color: 1) map the color to a point in a three-dimensional color space or 2) measure the reflectance at specific wavelengths throughout the visible spectrum (MacAdam 1985). Converting perceived color to a point in a three-dimensional color space using an instrument such as a colorimeter is commonly used in many industries, including the grain industry (Chen et al 1972; Johnston et al 1980; Neuman et al 1989a,b; Bason et al 1995). A more accurate means of quantifying color is to use a spectrophotometer to measure the reflectance of an object at many different wavelengths. This gives more information about the color characteristics of an object and is also used in many other areas, including the grain industry (Massie and Norris 1965; Hawk et al 1970; McCaig et al 1992, 1993; Ronalds and Blakeney 1995, Delwiche and Massie 1996). While other researchers have used colorimeters and spectrophotometers to quantify kernel color, most research was on bulk samples and there is no published literature reporting the use of this technology to assist in classifying single kernels soaked in NaOH. Quantifying the color of single kernels versus a bulk sample is necessary to determine whether the bulk sample has a mixture of red and white wheat classes. The objective of this research was to evaluate the use of a spectrophotometer to quantify the color of single wheat kernels after soaking in NaOH as a means of determining red and white wheat class.

MATERIALS AND METHODS

Ten samples were collected from each of the five wheat classes: hard red winter, soft red winter, hard red spring, soft white, and hard white (Table I). In addition, 10 samples were selected from wheats determined to be difficult to classify (DTC) as red or white by the USDA Federal Grain Inspection Service (FGIS). Ten kernels were randomly selected from each of the 60 samples and stored with a unique identification number, resulting in a total of 600 kernels. The FGIS Board of Appeals and Review (BAR) provided the original classification on all bulk samples.

The color of each kernel was quantified using a diode-array spectrometer (DA-7000, Perten Instruments, Reno, NV) that measures reflectance from 400 to 1,700 nm using an array of silicon sensors (400–950 nm, 7-nm spacings) and indium-galium-arsenide sensors (950–1700 nm, 11-nm spacings). Only information from the visible portion (400–700 nm) was used in this research. The diode-array system (DAS) collected spectra at a rate of 30 per second. White light illuminated a single kernel through

[1]Grain and Marketing Production Research Center, ARS, USDA, Manhattan, KS 66502. Mention of trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.

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an 8 × 60 mm (diameter by length) fiber optic bundle. A 2 × 90 mm fiber optic bundle carried the kernel reflectance to the diode array.

Kernels were individually placed in the trough using tweezers to prevent skin contaminates from affecting spectra or NaOH tests. A reflectance reading was collected from the crease side (0° from vertical), crease at 90°, and dorsal side (180°), resulting in three spectra per kernel. All kernels were placed in the trough with the germ end pointing in the same direction. To reduce noise in the spectra, 10 spectra were automatically averaged per view by the DAS. All data were stored in a GRAMS/32 (Galactic Industries Corporation, Salem, NH) format. Red and white classes were alternately run to minimize variations due to time.

All data were analyzed using partial least squares (PLS), a spectral decomposition technique similar to principle component regression (Marten and Naes 1989). PLS uses concentration information during the decomposition process and tries to include as much information as possible into the first few loading vectors. PLS takes advantage of the correlation relationship between the spectral data and the constituent concentrations, which in this case, is color. The only data pretreatment applied to the spectra was mean centering, which consists of calculating the average spectrum of all spectra in the training set and then subtracting the result from each spectrum. The number of factors reported is the minimum required to give the maximum correct classification.

A calibration was developed by selecting only known red and white kernels and assigning a value of 1 or 2 for red and white kernels, respectively. If either PLS or inspectors had difficulty classifying a kernel into the red or white class, that kernel was not used in the calibration set. After culling questionable kernels, the calibration set consisted of 491 kernels. The same kernels were used for the before-soaking and after-soaking calibrations. The remaining kernels (n = 107) were used in the prediction set, resulting in a total of 598 kernels of the original 600 kernels (two kernels were lost during data collection). The original color class was not known for 10 of the 107 kernels in the prediction set. A calibration set was then developed using the wavelengths from 400 to 700 nm, and the color class of the prediction set was estimated. In the prediction set, a kernel was classed as red if two or more views of a kernel had a value of <1.5. All other kernels were classed as white.

TABLE I
Samples Used to Quantify the NaOH Color Test[a]

Hard Red Winter	Soft Red Winter	Hard Red Spring Spring	Hard White	Soft White	Difficult To Classify
Mesa Smits (93)	Cardinal (94)	21A[b] (92)	13C[b] (91)	7C[b] (93)	FGISWH[b] (95)
FGIS[b] (95)	18A[b] (90)	Len (94)	Klasic (91)	Madsen (94)	HWHR[b] (95)
TAM 105 (94)	Cardinal (94)	21C[b] (94)	14B[b] (91)	Tres[c]	Snowhite (95)
12A[b] (92)	Titan (94)	Yecora Rojo (94)	KS84HW196 (95)	Malcolm (94)	5B[b] (95)
BIN126HRW (94)	Cardinal (90)	22B[b] (93)	Klasic (94)	9A[b] (94)	Bleached[b] (96)
Abilene Knievel (93)	Titan (94)	22C[b] (95)	Klasic (89)	9B[b] (90)	TAM107[c]
Arapahoe (94)	Cardinal (94)	23A[b] (91)	Klasic (94)	9C[b] (90)	TAM107[c]
13B[b,c]	20A[b] (90)	23B[b] (93)	16A[b] (91)	10A[b] (90)	TAM107[c]
17B[b] (93)	Caldwell (90)	23C[b] (95)	16B[b] (91)	Malcolm (94)	NDHV[b] (95)
Newton (94)	Cardinal (90)	Len (94)	Klasic (90)	Tres (94)	NDHV[b] (95)

[a] Cultivar name is followed by the crop year. Unknown cultivars are assigned a sample name.
[b] Cultivar name not known.
[c] Crop year unknown.

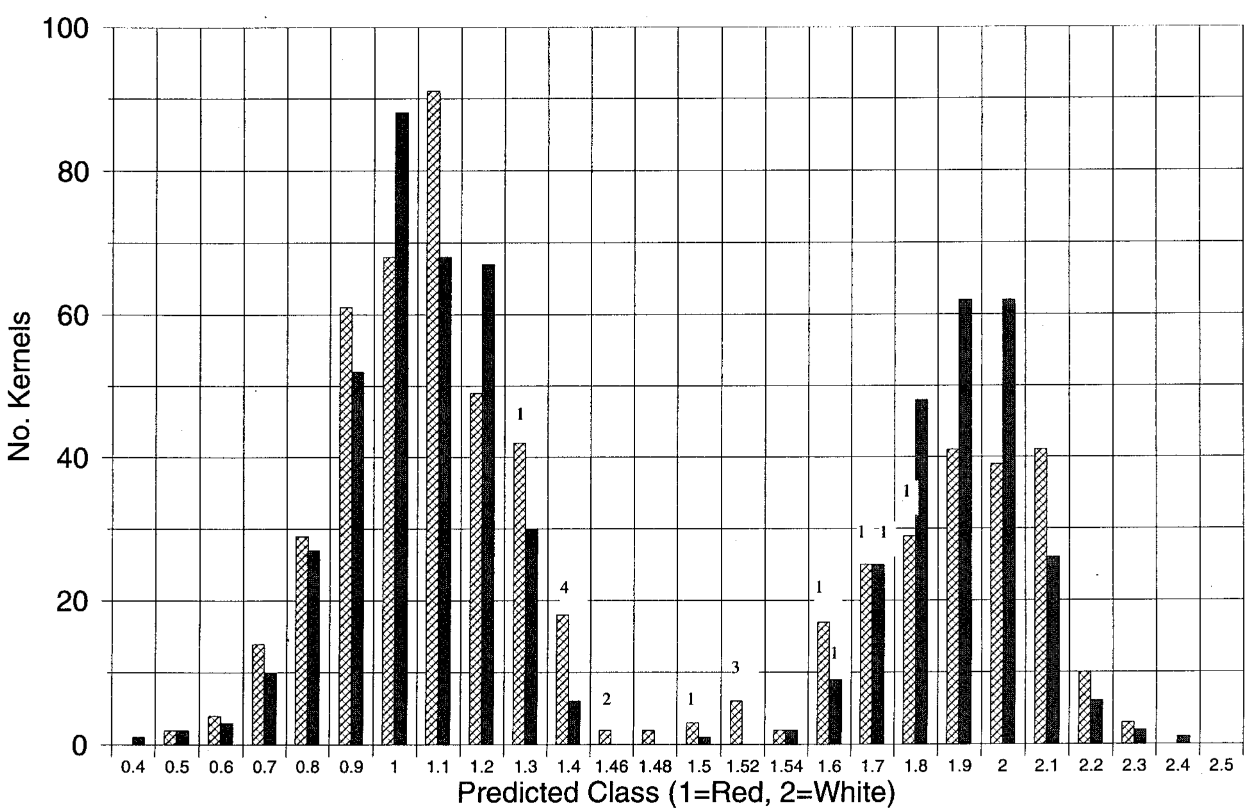

Fig. 1. Predicted color class for wheat kernels (dorsal view). Hatched bars represent kernels before soaking in NaOH. Solid bars represent kernels after soaking. Numbers indicate kernels missclassed in each range.

After collecting all spectra from the original samples, the kernels were individually soaked in 15 mL of a 5% (w/v) solution of NaOH at room temperature (≈23°C) for 1 hr. Literature gives no consensus on optimum soaking time, and the reported soaking time listed by other researchers ranges from 5 min to 2 hr. One hour was selected because most literature states this is adequate and a longer soaking time may cause the kernel to disintegrate, thus preventing subsequent reflectance readings. After soaking for 1 hr, kernels were immediately blotted dry and reflectance measured as described above. Surface moisture was removed since it could contribute to measurement errors by causing specular reflectance. The standard error of predictions (SEP), correlation coefficients (Steel and Torrie 1980), and classification accuracies for the known red and white kernels were compared for the before-soaking and after-soaking tests to determine whether the NaOH test improved the ability to predict color class. The homogeneity of correlation coefficients test and the equality of variances test was used to determine statistical differences in the SEP and correlation coefficients. Because FGIS uses kernel morphological characteristics and the NaOH test to determine color class of questionable samples, these two parameters were used as the final correct kernel classifications. Photographs were taken of all kernels before and after soaking.

RESULTS AND DISCUSSION

The number of kernels falling into specific prediction intervals for the dorsal view of all kernels is presented in Figure 1. Fewer kernels stayed in the borderline red-white area (a predicted value of ≈1.5) after soaking in NaOH. The number of kernels in the dorsal view in the interval between 1.45 and 1.55 decreased from 15 to three after soaking. The procedure does help move kernels from the borderline area. Other views gave similar results. Table II shows that the R^2 increases and SEP decreases after soaking kernels in NaOH, which also shows that the separation between the red and white classes gets more distinct. Most before and after NaOH R^2 values and SEP were significantly different ($P = 0.10$). Thus, the data show that soaking kernels in NaOH helps differentiate kernels into red and white classes, although a few (<0.7%) remain close to the borderline.

Table III shows a summary of kernel classes when classified by FGIS using: 1) visual identification, 2) DAS before soaking in NaOH, and 3) DAS after soaking in NaOH. A visual identification after soaking was not done because no official guidelines exist for classifying NaOH-soaked red and white wheat. When classifying unsoaked kernels, the DAS misclassified fewer difficult-to-classify kernels (15.0%) than the visual method (25.2%). A perfect classification using the DAS on unsoaked kernels was not expected because the DAS only duplicates what the human eyes sees, but it should classify kernels more consistently than the eye. Much greater improvement in classification accuracy was seen when the DAS was used to classify kernels soaked in NaOH. Only two white kernels (1.9% of difficult-to-classify kernels) and zero red kernels were misclassified after the kernels were soaked. It is also important to note that, after soaking in NaOH, the DAS correctly classified the 10 kernels that the visual inspection could not class.

The before-soaking DAS disagreed with the original before-soaking visual classification on five white class kernels and six red class kernels. The DAS was correct on four of the five white class kernels and on four of the six red class kernels. There were seven other kernels that the visual classification and DAS both incorrectly classified as red before soaking, but they were correctly classified as white after soaking by the DAS. The DAS correctly classified one kernel before soaking but incorrectly classified it after soaking.

Kernel orientation affected the classification accuracy with the 90° view having a significantly ($P = 0.10$) better R^2 and standard error of prediction (Table II). The DAS misclassified the crease side of white kernels more often than other views because the crease tended to be darker. This error diminished after the kernel was soaked. The DAS misclassified the dorsal side of red kernels more often than other views because the dorsal side tended to be lighter than the rest of the kernel. The dorsal misclassification occurred before and after soaking.

In summary, soaking kernels in NaOH accentuates the difference between red and white wheat classes and the DAS helped quantify this difference. PLS provided an objective means of classifying the kernels into red and white classes. Results showed the visual method misclassified 25.2% of the difficult-to-classify kernels, whereas the combination of soaking kernels in NaOH and classifying with the DAS misclassified only 1.9% of those kernels. Further research should include wavelengths outside the visible range to determine whether similar classification accuracy can be achieved without soaking kernels in NaOH.

TABLE II
Correlation Coefficients (R^2) and Standard Error of Predictions (SEP) for Classification of Known Red and White Wheat Kernels Before and After Soaking in NaOH[a]

	R^2		SEP	
View	**Before**	**After**	**Before**	**After**
Dorsal	0.85a	0.87a	0.189a*	0.175a*
Side	0.88b*	0.90b*	0.170b*	0.154b*
Crease	0.85a*	0.89a*	0.187c*	0.161c*

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

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TABLE III
Classification of Single Wheat Kernels Visually with a Diode Array System (DAS) Before NaOH and After NaOH Soaking

			All Kernels		Difficult Kernels
	Red Kernels	**White Kernels**	**% Misclassed**	**% Unclassed**	**% Misclassed**
Visual identification	398 (23)[a]	190 (4)	4.5	0.2	25.2
DAS identification					
Before soaking	397 (14)	201 (2)	2.7	nd[b]	15.0
After soaking	371 (0)	227 (2)	0.3	nd	1.9

[a] Values in parentheses are number of red or white kernels misclassified. Of 598 total kernels, 491 were obviously red or white and 107 were difficult to classify.
[b] None detected.

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