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AUTOMATED SINGLE WHEAT KERNEL QUALITY MEASUREMENT USING  
NEAR-INFRARED REFLECTANCE

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**Summary:**

An automated system for measuring protein, moisture, color class, bunted kernels, and hidden insect infestations of single wheat kernels was developed by integrating a single kernel characterization system with a near-infrared diode-array spectrometer. The system collected spectra over the range of 400-1700 nm at a rate of one kernel per four seconds. The automated system accurately predicted all factors with a high degree of accuracy.

**Keywords:**

Wheat, near-infrared, protein, insects, moisture, quality, spectrophotometer, inspection, grading, color.

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## INTRODUCTION

The Grain Inspection, Packers and Stockyards Administration currently inspects wheat samples (ca. 250 g) to estimate grain quality, storability, and end-product yield. Factors measured during inspection include protein, moisture, color class, fungal damage, and insect infestation (USDA, 1991). Some measurements, such as moisture and protein, are determined on a bulk sample. This average value does not indicate the distribution of single kernel values comprising the average. Thus, a lot containing a mixture of high and low moisture or protein kernels can not be detected. Knowledge of single kernel moisture and protein values may help handlers and millers improve end-product yield by segregating mixed lots or adjusting mills to handle mixed lots.

Other quality factors, such as color class, damage, and insect infestation, require visual examination. Accurate color classification is essential to ensure the lot reaches the appropriate processing line since genetically red or white wheats mill, bake, and taste different regardless of visual color. Environmental conditions can cause red kernels to appear white or white kernels to appear red, resulting in incorrect color classification. Visual examination of samples for insect infestation indicates the presence of insects or insect damaged kernels, but cannot determine if kernels contain hidden infestations (insects developing inside kernels). Storey et al. (1982) showed that 16% of 1-kg samples collected from elevators contained larvae inside single kernels, as indicated by incubating samples, even though sieving for adult insects indicated that only 4% of the samples were infested upon original inspection. Wheat can be damaged by fungal diseases that significantly reduce the crop value (Meronuck, 1997). Scab, caused by *Fusarium graminearum*, and karnal bunt (*Tilletia indica* Matte) are two severe wheat quality problems related to fungal invasion and identification of these quality problems are crucial to ensuring good food quality and maintaining our export markets. Thus, some means of automatically and objectively detecting single kernel protein, moisture, color class, damaged kernels and hidden insect infestation is needed.

Near-infrared reflectance spectroscopy (NIRS) offers a means of detecting quality characteristics such as protein, moisture, color class, damage, and hidden insect infestations (Murray and Williams, 1990). Delwiche (1995, 1996) measured single wheat kernel protein using NIR transmittance and reflectance. Delwiche and Massie (1996) determined single kernel color class using NIR reflectance. Ridgway and Chambers (1996) detected larvae in single wheat kernels using NIR reflectance. Lamb and Hurburgh (1991) measured single soybean seed moisture using NIR transmittance. Thus, previous research shows that NIRS can measure single kernel quality attributes. However, all previous research used hand-placed kernels of fixed orientation. Martin et al. (1993) developed a single kernel wheat characterization system (SKWCS) that singulates kernels for subsequent weight, size, moisture, and hardness measurements at a rate of two kernels per second. The objective of this research was to determine the accuracy of automatically measuring single kernel protein, moisture, color class, fungal damage, and hidden infestations using a NIR reflectance spectrometer integrated with the SKWCS.

## PROCEDURES

### Instrumentation

A SKWCS integrated with a DA-7000 diode-array spectrometer (DAS) (Perten Instruments, Springfield, IL) automatically collected visible (VIS) and NIR spectra (400-1700 nm) from randomly oriented single wheat kernels. The SKWCS consists of a singulator wheel, a weighing bucket, and crushing mechanism. The crushing mechanism of the SKWCS was bypassed in this research to preserve samples for subsequent tests. The modified kernel singulator delivered kernels at a rate of one kernel per four seconds to the viewing area of the spectrometer. The DAS illuminates the kernel and measures reflectance using fiber optic probes and collects spectra at a rate of 30 per second. Six spectra of each kernel were collected and averaged to reduce noise.

### Data Analysis

All data were analyzed using partial-least squares (PLS) regression on mean-centered spectra. PLS is a spectral decomposition technique and available as a module of GRAMS/32 software (Galactic Industries Corporation, Salem, New Hampshire). PLS uses chemical 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 constituent concentrations. Martens and Naes (1989) give a complete description of PLS.

### Protein Tests

Ten kernels were selected from each of ten samples of hard red winter wheat with bulk protein values ranging from 9.4 to 16.2% and equilibrated to the same ambient moisture content (ca. 12% wet basis) for a minimum of one week. Each kernel was run through the automated system 10 times and then hand-placed in the viewing area with the crease-side facing the reflectance probe for an additional 10 spectra, resulting in a total of 1100 spectra from 100 kernels. Kernels were uniquely identified and the nitrogen content determined by the combustion method using a Leco model FP-428 nitrogen analyzer (St. Joseph, MI). Prior to nitrogen analysis, kernels were dried 19 h at 130°C, weighed to the nearest 0.01 mg, allowed to regain ambient moisture for a few days, then weighed again immediately before combustion. Separate calibrations were obtained from spectra originating from randomly-oriented and hand-placed kernels. Protein content (Nx5.7) was mathematically corrected to a 12% mc.

### Moisture Content Tests

Samples from five classes of wheat, hard red winter (HRW), soft red winter (SRW), hard red spring (HRS), soft white (SW), and hard white (HW), were equilibrated to about 15% mc by adding a known quantity of water to samples and allowing kernels to equilibrate in a sealed container for several weeks. Four kernels were selected from each sample and weighed to 0.1 mg. Each kernel was run through the automated system 10 times and spectra collected and stored. These kernels were then allowed to lose about 2% mc by air or oven drying at 32°C and weighed. The process was repeated until the kernel moisture reached about 7%. Kernel moisture during the drying process was estimated by measuring the moisture of a few kernels using a

SKWCS. Kernels were then oven dried (19 h, 130°C) at the end of the test and the correct mc for each step calculated using the final dry weight and the weight at each moisture determination. The final data set consisted of 1000 spectra (4 kernels x 5 classes x 5 mc x 10 reps).

### Color Classification Tests

For color classification tests, calibration and prediction sets were selected from samples representing all five wheat classes. The samples for the calibration set originated from all growing areas in the U.S. 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. Of the 112 samples selected, 88 were considered obviously red or white classes, whereas the remaining 24 samples were more difficult for inspectors to determine color class. A NaOH test (DePauw and McCaig, 1988) confirmed the color class of all suspect samples. The prediction data set, consisting of 30 red and 30 white samples, also represented the same growing area and classes listed above, but came from different locations than the calibration samples to ensure independence in populations. Samples were randomly run by placing about 20 kernels from one sample in the automated system, resulting in 20 sequentially numbered spectra per sample.

### Bunted Kernels

Spectra from approximately 60 kernels infested with varying degrees of common bunt (*Tilletia tritici* and *T. laevis*) were automatically collected and compared to 600 sound kernels. The degree of infestation varied from only slight fungal damage to kernels completely covered with the fungus.

### Hidden Insect Infestation Tests

Wheat samples were selected to test the effect of class, protein content within a class, moisture content within a class, and different insect species infesting a single class on the accuracy of detecting hidden insect larvae of different sizes by the automated system. This study included five classes (HRW, SRW, HRS, HW, SW), three moistures (10, 11.3, 13.2%), two protein levels (11.3%, 16.2%), and three insect species (rice weevil, *Sitophilus oryzae* (L.))(Coleoptera: Curculionidae); lesser grain borer, *Rhyzopertha dominica* (F.)(Coleoptera: Bostrichidae); and Angoumois grain moth, *Sitotroga cerealella* (Olivier)(Lepidoptera: Gelechiidae)).

To obtain wheat kernels with different size larvae of the rice weevil, 20 g samples of wheat were placed in clear, plastic vials (16 dram, 3.2 x 8 cm) with screen lids, and equilibrated for two weeks by adding the appropriate amount of water and placing samples over a saturated solution of sodium bromide at 25°C. Four female rice weevils (ca. 1 wk-old) were removed from stock cultures and placed in each vial. The weevils were removed after 14 days, and another equilibrated 20 g wheat sample was infested with four new female weevils. For the other two species, 10 adult lesser grain borer or 10 Angoumois grain moths were placed on 20 g samples of equilibrated wheat in the vials. The adults were removed at weekly intervals and fresh samples were infested each succeeding week for four weeks. Using these infestation methods, after four weeks we obtained all possible ages of larvae for detection analysis.

Wheat kernels from each treatment were X-rayed (Throne, 1994), and radiographs were examined on a light table to identify infested kernels. About 100 infested kernels containing approximately equal numbers of small, medium, or large larvae were selected from each treatment. The length and width of each larva was measured on the radiograph using a 7X micrometer. We also X-rayed and selected kernels not exposed to insects (ca. 20) and kernels exposed to insects but not infested with larvae (ca. 20) for each treatment to use in the detection analysis. Each measured kernel was uniquely identified and NIR spectra collected using the automated system within eight hours of X-raying to minimize changes in larval growth or kernel moisture content. Calibration and validation sets were formed by selecting odd-numbered spectra for one set and even-numbered spectra for the other. A total of 577 spectra were collected.

## RESULTS AND DISCUSSION

### Protein Results

When analyzing spectra from kernels randomly placed by the automated system, a 19-factor PLS calibration derived from absorbance values over the range of 700-1688 nm resulted in a standard error of cross validation (SECV) of 0.75% protein and  $r^2=0.94$  (Table 1). When kernels were hand-placed in a fixed orientation, the SECV and  $r^2$  improved slightly to 0.60% protein and 0.96, respectively, from a calibration using 13 factors. Thus, the random placement of the automatically-fed kernels increases noise in the spectra and slightly reduces the prediction accuracy. The error is slightly higher than that reported by Delwiche (1996) who reported a standard error of calibration (SEC) of 0.49%,  $r^2=0.97$ , and a standard error of performance (SEP) of 0.54%, using 11 PLS factors derived from reflectance spectra averaged from 32 scans over the range of 1100-1400 nm. The error and number of factors reported in this research is likely higher than that reported by Delwiche because a wider wavelength range was used and kernel orientation was not as precisely controlled. Kernel size did not affect protein measurement which also agrees with Delwiche (1996). These results for single kernel protein values fall within the range reported in literature for bulk wheat NIR protein analysis where Williams (1975), Krischenko (1990), and Williams et al. (1985) reported standard errors (SE) ranging from 0.21 - 0.75% and  $r^2$  ranging from 0.85 to 0.96.

### Moisture Content Results

The automated system predicted single kernel moisture content (17 factors, 700-1688 nm) with a SECV=0.43% and  $r^2=0.97$ . Use of first derivatives of the raw spectra offered little improvement in predictions with the SECV improving to 0.41% and  $r^2$  to 0.98. Predictions improved (SECV=0.31%) when kernels outside the range common to most samples (8-14% mc) were excluded from the statistical analysis (Table 1). The precision (repeated measuring of one kernel) of predicting single kernel moisture within each class ranged from a SE=0.15% to a SE=0.24%. Thus, the variation in predictions based on multiple kernels was similar to the variation of multiple spectra from a single kernel. These results are similar to those derived from bulk samples where Williams et al. (1985) and Williams (1975) reported SE ranging from 0.17 to 0.62% and  $r^2$  ranging from 0.92 to 0.97.

### Color Classification Tests

Results showed calibrations derived from the full wavelength profile (450-1688 nm) correctly classed more kernels than either the visible region (450-700 nm) or the NIR region (700-1688 nm). Most results showed greater than 99% correct classification for single kernels when using the visible and NIR regions and greater than 20 PLS factors. Averaging of single kernel classifications resulted in 100% correct classification of bulk samples since official procedures require only 97% of single kernels be of one class. When predicting the color class of kernels derived from samples not included in the calibration set, a classification rate of 99.2% and  $r^2=0.83$  was achieved (Table 1). These results from automated data collection compare favorably to those achieved by Delwiche and Massie (1996) using hand-placed kernels where they correctly classed about 98% of red and white kernels using a 7-factor PLS model over the visible region of 550-750 nm.

### Bunted Kernel Tests

Spectra from bunted kernels differed significantly from sound kernels. This was expected since portions of the kernel are replaced with the fungus. It was also noted that kernels infested internally with bunt, but with no external evidence of the fungus, were readily detected by the system. Thus, sufficient radiation is penetrating the kernel to allow absorption of radiation by the fungus. 93% of all bunted kernels were correctly identified and no sound kernels were improperly classed as bunted. These results may have significant implications in developing a automated, objective system to detect kernal bunt or scab in U.S. wheat.

### Hidden Infestation Tests

Kernels infested with larvae of any species were easily detected. The final calibration (17 factors, 1000-1350 and 1500-1680 nm) included all samples and accounted for moisture, protein, and wheat class effects. For all species, 99.1% of all kernels infested with larger larvae were correctly identified (Table 1). The minimum detectable larva size varied with the species. Although smaller larvae were detected with less confidence, infested samples should contain larvae of all sizes. Thus, the system should detect infested samples with a high degree of confidence. These results from the automated system are similar to those reported by Ridgway and Chambers (1996) using hand-placed kernels.

In summary, an automated system for determining quality attributes of single wheat kernels was developed which measures protein, moisture, color class, fungal damaged kernels and identifies hidden insect infestation with accuracies similar to bulk-sample instruments and similar to results achieved from research where kernels were hand-placed. Thus, an existing SKWCS can be modified to accurately measure additional quality attributes of wheat. The system collected six absorbance spectra per kernel (400-1700 nm) at a rate of one kernel per four seconds. Additional research is needed to increase the speed to two kernels per second, which is the maximum speed of the SKWCS. Additional research is also needed to see if classifications can be improved through other data analysis techniques.

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## REFERENCES

- Delwiche, S. R. 1995. Single wheat kernel analysis by near-infrared transmittance: protein content. *Cereal Chem.* 72(1):11-16.
- Delwiche, S. R. 1996. Single kernel protein content in wheat by near-infrared reflectance. ASAE Paper No. 963031. St. Joseph, MI: ASAE.
- Delwiche, S. R. and D. R. Massie. 1996. Classification of wheat by visible and near-infrared reflectance from single kernels. *Cereal Chemistry* 73(3):399-405.
- DePauw, R. M. and R. M. McCaig. 1988. Utilization of sodium hydroxide to assess kernel color and its inheritance in eleven spring wheat varieties. *Can. J. Plant Sci.* 68:323-329.
- Krischenko, V. P. 1990. Use of near-infrared reflectance spectrophotometry in investigations in the USSR. In P. C. Williams and K. H. Norris (eds.), *Near Infrared Technology in the Agricultural and Food Industries*. American Association of Cereal Chemists, Inc., St. Paul, MN.
- Lamb, D. T. and C. R. Hurburgh, Jr. 1991. Moisture determination in single soybean seeds by near-infrared transmittance. *Transactions of the ASAE* 34(5):2123-2129.
- Martens, H. and T. Naes. 1989. *Multivariate calibrations*. John Wiley & Sons Ltd., Guildford, UK.
- Meronuck, R. A. 1997. Effect of wheat scab and karnal bunt on quality. Proc. of the International Wheat Quality Conference, Manhattan, KS (In Press).
- Martin, C. R., R. Rousser and D. L. Brabec. 1993. Development of a single kernel wheat characterization system. *Transactions of the ASAE* 36: 1399-1404.
- Murray, I. and P. C. Williams. 1990. Chemical principles of near-infrared technology, pages 17-34. In P. C. Williams and K. H. Norris [eds.], *Near-Infrared Technology in the Agricultural and Food Industries*. American Association of Cereal Chemists, Inc., St. Paul, MN.
- Ridgway, C. and J. Chambers. 1996. Detection of external and internal insect infestation in wheat by near-infrared reflectance spectroscopy. *J. Sci. Food Agric.* 71: 251-264.
- Storey, C. L., D. B. Sauer, O. Ecker and D. W. Fulk. 1982. Insect infestations in wheat and corn exported from the United States. *J. Econ. Entomol.* 75: 827-832.
- Throne, J. E. 1994. Life history of immature maize weevils (Coleoptera: Curculionidae) on corn stored at constant temperatures and relative humidities in the laboratory. *Environ. Entomol.* 23: 1459-1471.
- USDA. 1991. *Inspecting grain. Practical procedures for grain handlers*. Federal Grain Inspection Service, Washington, DC.
- Williams, P. C. 1975. Application of near infrared reflectance spectroscopy to analysis of cereal grains and oilseeds. *Cereal Chem.* 52:561-576.
- Williams, P. C., K. H. Norris and D. C. Sobering. 1985. Determination of protein and moisture in wheat and barley by near-infrared transmission. *J. Agric. Food Chem.* 33:239-244.

Table 1. Summary of statistics from using an automated near-infrared system for measuring protein and moisture, and detecting red and white color class and hidden insect infestation in single wheat kernels.

Quality Attribute Measured	$r^2$	SE (%)	% Correctly Classified <sup>a</sup>
Protein	0.94	0.75	--
Moisture			
All Samples	0.97	0.43	
8-14% range only	0.96	0.31	--
Color Class	0.83	--	99.2
Hidden Infestation	0.70	--	99.1 <sup>b</sup>

<sup>a</sup> Results from the validation set.

<sup>b</sup> Percentage of kernels with 2nd instar or larger larvae that were correctly identified as infested.