

## An Automated Near-Infrared System for Selecting Individual Kernels Based on Specific Quality Characteristics

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### ABSTRACT

Cereal Chem. 83(5):537–543

An automated sorting system was developed that nondestructively measured quality characteristics of individual kernels using near-infrared (NIR) spectra. This single-kernel NIR system was applied to sorting wheat (*Triticum aestivum* L.) kernels by protein content and hardness, and proso millet (*Panicum miliaceum* L.) into amylose-bearing and amylose-free fractions. Single wheat kernels with high protein content could be sorted from pure lines so that the high-protein content portion was 3.1 percentage points higher than the portion with the low-protein kernels. Likewise, single wheat kernels with specific hardness indices could be removed from pure lines such that the hardness index in the

sorted samples was 29.4 hardness units higher than the soft kernels. The system was able to increase the waxy, or amylose-free, millet kernels in segregating samples from 94% in the unsorted samples to 98% in the sorted samples. The portion of waxy millet kernels in segregating samples was increased from 32% in the unsorted samples to 55% after sorting. Thus, this technology can be used to enrich the desirable class within segregating populations in breeding programs, to increase the purity of heterogeneous advanced or released lines, or to measure the distribution of quality within samples during the marketing process.

Most grain quality characteristics are measured on a composite of multiple kernels from bulk samples and therefore information on characteristics of single kernels are lost. For example, wheat (*Triticum aestivum* L.) grain protein content is measured using bulk samples, resulting in one average value for that sample. However, the protein content standard deviation among single kernels from one field can be 0.5 to 1.4 percentage points (Malloch and Newton 1934; Levi and Anderson 1950). This range in protein content is influenced by the environment and genetics, with the influence of genetics ranging from 15% (Sunderman et al 1965) to 83% (Stuber et al 1962). An accurate measure of the uniformity of protein content within a sample is an important factor in determining breadmaking potential, with higher protein content generally yielding larger loaf volumes (Bushuk et al 1969). Because protein content is related to product quality, uniform protein content within lots is important for making specific, high-quality products. Thus, average protein content is important for determining specific end use markets, but there has been no method to determine whether protein content within samples is uniform, or whether a bulk lot is a blend of high- and low-protein content wheat.

Wheat hardness is also not uniformly distributed within blended lots, or even within highly inbred lines or cultivars, and can have a standard deviation of  $\approx 20$  hardness units within a sample (Martin et al 1993). Hardness is influenced by the environment and genetics (Huebner and Gaines 1992; Morris et al 1999) and affects flour yield, starch damage, milling energy requirements (Evers and Bechtel 1988), and baking properties (Pomeranz et al

1984). If hard and soft lots are blended, a small amount of soft kernels can have a large detrimental effect on baking quality (Morris 1992). Thus, uniformity in hardness can have a positive effect on baking quality. Chung et al (2004) showed that when samples within an advanced line were sorted into four hardness fractions, the bread quality from the four fractions increased with hardness.

There are many other grain quality attributes with variability between kernels within a sample that is similar to or greater than protein content or hardness. These can include starch content, amylose content, fusarium damage, insect damage, kernels that are nonvitreous, sprout damage, kernel color, etc. For those attributes influenced by genetics, nondestructively measuring quality characteristics of individual kernels and subsequent sorting could help breeders select specific characteristics in segregating populations and in heterogeneous advanced lines in their breeding programs and to study the effects of genetics and environment on selected characteristics. The ability to sort and recover seed with specific quality characteristics also would assist breeders engaged in the introgression of characteristics from one market class to another. For example, wheat breeders interested in introducing a disease resistance gene from soft to hard wheats could select hard-grained seed from early generation samples, and then discard soft kernels or use them for soft wheat breeding programs. This selection would greatly help a hard (or a soft) wheat breeder select lines with the hard (or soft) kernels. Also, the kernels with specific attributes could be removed from samples and planted in different environments to study the influence of environment and genetics on this selected characteristic. The technology could also be used at all stages of grain marketing, handling, and storage systems to determine distributions of quality factors within pure or blended lots.

There has been research reporting technology for nondestructive measurement of single-kernel attributes such as protein content (Delwiche and Hruschka 2000; Shadow and Carrasco 2000; Rittiron et al 2004), insect damage (Dowell et al 1998), hardness (Maghirang and Dowell 2003), vitreousness (Dowell 2000), class (Zayas et al 1996), density (Nielsen et al 2003), and color (Dowell 1998). While some of this previous research included automated feeding and scanning, none included a means to automatically sort the kernels based on desired attributes. Pasikatan and Dowell (2004) reported segregating large samples by protein content using a commercial high-speed color sorter with a combination of color and NIR filters, but shifts in protein content were small and likely due to differences in color or vitreousness.

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\*The e-Xtra logo stands for “electronic extra” and indicates that the online version contains a color version of Fig. 1 not included in the print edition.

The objective of this research was to develop an automated system to measure attributes of single kernels, develop calibrations for selected quality parameters, and then sort single kernels based on user-defined parameters. Sample applications are given for sorting wheat by hardness and protein content, and for sorting waxy (amylose-free) kernels from proso millet (*Panicum miliaceum* L.).

## MATERIALS AND METHODS

### SKNIR System

A system to feed single kernels to a near-infrared (NIR) spectrometer and then sort the kernels based on user-defined criteria was developed by the USDA ARS Grain Marketing and Production Research Center, Engineering Research Unit, Manhattan, KS, and commercialized by Perten Instruments (Stockholm, Sweden) (Fig. 1). This single-kernel NIR (SKNIR) system used a vacuum wheel from the SKCS 4100 (Perten Instruments, Stockholm, Sweden) to feed kernels to the spectrometer viewing area. The vacuum wheel consisted of a nearly vertical wheel  $\approx 15$  cm in diameter with eight evenly spaced 0.7-mm vacuum ports located 0.6 cm from the edge of the wheel. The vacuum wheel picked up one kernel at a time from a kernel bin and deposited it into a V-shaped trough ( $\approx 12$  mm long, 10 mm wide, and 5 mm deep). The kernel was illuminated with white light through a fiber optic bundle. Reflected energy was transmitted to a spectrometer (Control Development, South Bend, IN) that used an indium-gallium-arsenide sensor and measured absorbance at 950–1650 nm. The spectrometer integration time was 6.3 msec, and 24 spectra were averaged from each kernel. The time required to collect, average, and store the spectra was  $\approx 0.5$  sec for each kernel. The resulting spectrum was then used to determine kernel attributes through user-defined calibrations. After the kernel was analyzed, the trough was able to be rotated counterclockwise by a stepper motor to drop

the kernel back into the original kernel bin for a repeated measurement, or rotated clockwise so that the kernel dropped through a series of gates that led to one of four sorting bins based on predefined sort settings. A Spectralon diffuse-reflectance standard (Labsphere, North Sutton, NH) was mounted on the reverse side of the trough and was presented to the spectrometer for collecting baselines by rotating the trough  $180^\circ$ . Kernels were scanned and sorted at a speed of  $\approx 1$  kernel/2 sec. If kernel placement, scanning, and sorting were optimized, then a two- to fourfold increase in speed may be possible.

The system software allowed the user to sort kernels using calibrations developed with software such as Grams (Galactic Industries, Salem, NH) or Unscrambler (CAMO, Woodbridge, NJ). The user could also set reject criteria so that spectra from kernels that may not be fully in the field of view, poorly positioned kernels, spectra from broken kernels, etc., could be rejected. The reject criteria consisted of accepting only spectra with absorbance between specific values at a selected wavelength, or having a line slope between specific values between two selected wavelengths. The rejected kernels were returned to the original kernel bin. The user selected the sorting parameters for placing kernels in the four sorting bins, and a real-time histogram displayed the number of kernels in each bin during the sorting process. If reject criteria were properly selected, and if reference data were readily available, then calibrations could be completed in about one day.

A camera captured images of each kernel positioned in the viewing trough and displayed the image on the computer screen. This assisted the user in ensuring the system was functioning correctly. The images were stored for subsequent color and dimensional analyses, but this information could not presently be used for real-time sorting.

The user selected the number of kernels to sort so that when this value was reached, the system stopped. The user also selected criteria so that the system automatically terminated after the

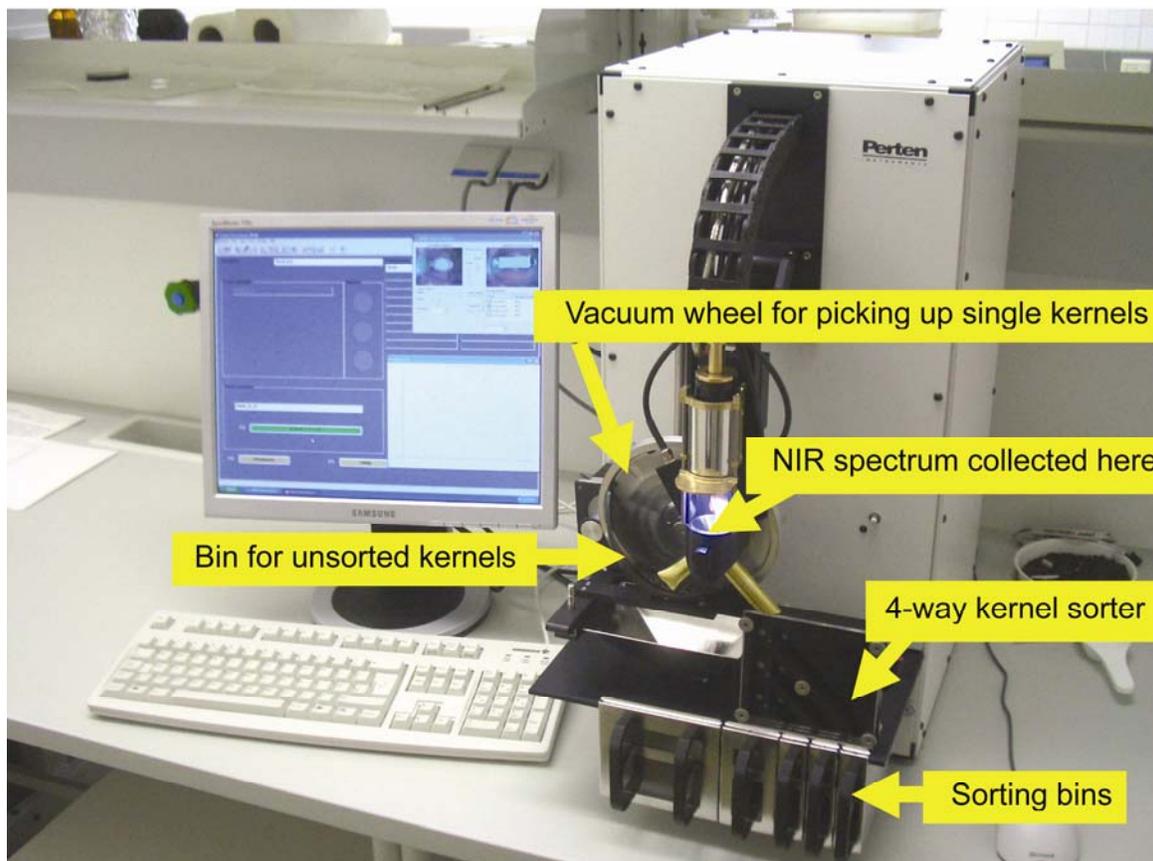


Fig. 1. Automated system for sorting single kernels using near-infrared spectroscopy.

spectrometer had recorded a specified number of invalid spectra, which occurred when the spectrometer repeatedly measured spectra of the empty viewing area because no more kernels were available for sorting.

### Development of Wheat Calibration Models

Calibration models were created for single-kernel protein content and hardness by using partial least squares (PLS) regression (Martens and Naes 1989) and Grams software (PLSPlus/IQ, Galactic Industries, Salem, NH). The number of factors when the *F*-ratio probability level was  $\leq 0.75$  was used for the calibration model. The regression equation, referred to as beta coefficients in Grams, was used to determine the wavelengths that were most important in the final calibration models.

The calibration set for protein content consisted of 97 hard red winter wheat samples obtained from the Federal Grain Inspection Service in 2004. These samples had an average protein content of 12.75% with a standard deviation of 1.71%. Spectra were collected from 100 kernels from each sample and then averaged to give one spectrum per sample. The bulk protein content (12% moisture content basis) was determined by Approved Method 39-25 (AACC International 2000), the NIR method for protein content in whole-grain wheat using the Foss NIRSystems 6500 (Foss North America, Inc., Silver Springs, MD) equipped with a natural product sample cell. The protein content was then assigned to the average spectrum. In a preliminary study using crop year 2000 wheat, we compared 1) a calibration created from 1,000 hard red winter single kernel spectra (10 kernels from 100 samples) and corresponding single-kernel protein reference measurements (6.2–21.2% protein content); and 2) a calibration created from spectra of 100 kernels averaged from each of 100 samples and the corresponding bulk protein reference measurement (8.8–18.1% protein content). The calibrations were then used to predict the bulk protein of 120 independent samples (9.6–15.3% protein content) using 100 kernels from each sample. The standard error of prediction of both methods was  $\approx 0.43$ . Thus, to simplify the calibration method for the results reported herein, bulk protein measurements were assigned to spectra averaged from each sample. The repeatability for the protein reference method is  $\approx 0.20\%$  protein (Hunt et al 1977), and the protein content results should be within  $\approx 0.50\%$  (Osborne and Fearn 1983) of the Kjeldahl method (Approved Method 46-11A, AACC International 2000), the crude protein-improved Kjeldahl method, copper catalyst modification.

The hardness calibration was created using 10 U.S. National Institute of Standards and Technology wheat hardness reference samples and 23 additional hard and soft wheat samples for a total of 14 soft and 19 hard samples. The average hardness index for this set was 46.8 with a standard deviation of 26.9. For each sample, spectra were collected from 100 kernels and then averaged and assigned the average hardness index obtained using the SKCS 4100 (Approved Method 55-31, AACC International 2000), the single-kernel characterization system for wheat kernel texture.

### Wheat Test Sets

The wheat test set consisted of different crosses of 22  $F_2$  winter wheat crosses grown by Stephen Baenziger, University of Nebraska, in Yuma, AZ, in 2004. These samples were from single and three-way crosses involving various parents as would be expected

in a traditional hard winter wheat breeding program. Some crosses included hard and soft kernel type parents and were made to increase the disease resistance and agronomic performance of subsequent selections for the desired hard kernel types. Four samples were sorted into four protein content levels, and 18 samples were sorted into four hardness levels. The sorting criteria chosen for binning into four hardness or protein content levels were set so that  $\approx 25\%$  of each sample was sorted into each of the four bins. Sorting was terminated when  $\approx 40$  g were sorted for each bin. Protein content and hardness of the unsorted and sorted wheat test set samples were measured as described above.

### Proso Millet Calibration Models and Test Sets

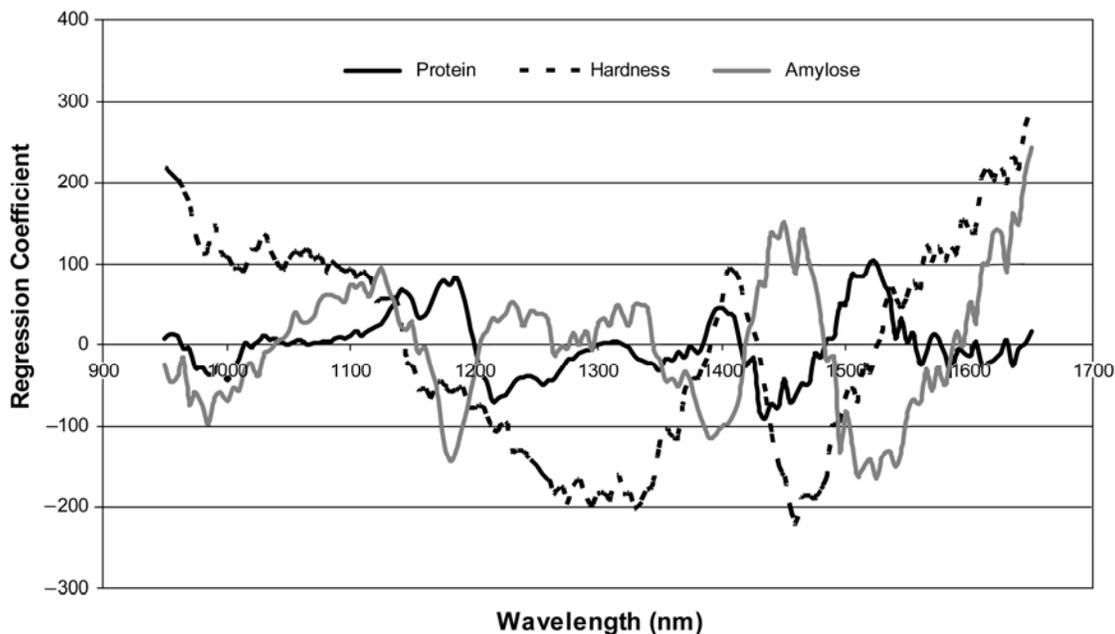
Proso millet (*Panicum mileaceum* L.) (also known as proso or broomcorn millet) samples were selected at random from several  $F_3$  populations. Populations were derived from the following matings: Earlybird/PI 436626, PI 436625/Earlybird, PI 170597/PI 436626, Huntsman/PI 436626, or Sunrise/PI 436626. PI 436225 and PI 436226 are waxy selections, originally from China and now housed in the USDA-ARS North Central Regional Plant Introduction Center at Ames, IA. PI 170597 is a wild-type (nonwaxy) selection from Turkey. The remaining lines are wild-type (nonwaxy) proso cultivars developed by the University of Nebraska and adapted to dryland conditions of the western Great Plains area. The  $F_3$  populations were field-grown in 2004 at the University of Nebraska Panhandle Research and Extension Center, Scottsbluff, NE (Table I). Plant selections from  $F_3$  populations derived from waxy/wild-type proso crosses are either true-breeding (pure) waxy, true-breeding (pure) wild-type, or segregating waxy/wild-type. However, it is difficult to obtain 100% pure waxy samples. Proso seed is small, and mechanical mixtures from harvest machinery are difficult to avoid. Also, while proso largely is self-pollinated, natural outcrossing can occur at frequencies of up to 10% (Baltensperger and Cai 2004). Preliminary genetic studies (Graybosch and Baltensperger 2004) have determined the waxy trait of proso is determined by the presence of duplicate recessive genes. The presence of a dominant gene at one of the two postulated waxy loci is enough to confer wild-type phenotype. Thus, segregating plants from  $F_3$  populations may produce wild-type/waxy progeny in ratios of either 15:1 (or 93.75% wild-type) or 3:1 (75% wild-type), depending on whether the heterozygous individual carries one or two dominant genes.

The following single plant selections were harvested and threshed individually: 23 white seeded samples composed of  $>95\%$  waxy kernels; 17 white seeded samples containing 80–95% waxy kernels; eight red seeded waxy samples; 38 white seeded wild-type individuals; 31 white seeded segregating samples, or samples that had a large percentage of waxy and wild-type kernels; and seven red seeded segregating samples. The frequency of waxy kernels in each sample was verified by the rapid iodine staining method (Pedersen et al 2004) on 48 kernels from each sample. Only the 23 white seeded waxy samples that were  $>95\%$  pure and the 38 wild-type samples were used in the calibration set. The remaining 25 white and red waxy, and the 38 white and red segregating samples containing waxy and wild-type kernels were used as a test set.

For the calibration development, 100 seeds from the 23 white waxy and 38 wild-type calibration samples were scanned. Spectra

TABLE I  
Number of Bulk Samples Used for Calibration and Test Sets for Sorting Single Proso Millet Seeds  
Using a Single-Kernel Near-Infrared Sorting System

Sample Set	White Samples		Waxy Red Samples	Segregating Samples		Wild-Type Samples
	$>95\%$ Waxy	80–95% Waxy		White	Red	White
Calibration set	23	–	–	–	–	38
Test set	–	17	8	31	7	–



**Fig. 2.** Regression coefficients resulting from a partial least squares regression analysis of near-infrared spectra when measuring protein content and hardness of single wheat wheat, and when sorting proso millet containing amylose from amylose-free kernels. The absolute value of the regression coefficient at each wavelength shows the influence of each wavelength on the prediction equation.

**TABLE II**  
Results From Using a Single-Kernel Near-Infrared System to Sort Samples into Four Protein Content (PC) Ranges<sup>a</sup>

Sample ID	Unsorted Samples (PC %)	Sorted Samples (PC %)			
		Bin 1	Bin 2	Bin 3	Bin 4
548	14.0	12.7	13.3	13.7	15.4
549	13.6	12.0	12.9	13.5	14.9
550	15.4	13.2	14.5	15.2	17.1
551	14.3	12.6	13.1	13.9	15.6

<sup>a</sup> Reported on a 12% moisture content basis.

resulting from misplaced kernels that may not have been removed by the SKNIR reject criteria were removed manually, and the resulting spectra for each sample were averaged into one spectrum representing each sample. The calibration was developed using a PLS analysis and was subsequently used to attempt to select waxy kernels from all waxy and segregating samples. For the PLS calibration, the waxy samples were assigned a value of “1” and the wild-type were assigned a value of “2”. The sorting criterion was adjusted to place  $\approx 50\%$  of the seeds in bins 1 and 2, and 50% in bins 3 and 4. Samples were sorted until about 10 g of seed was obtained by combining bins 1 and 2. Twenty kernels were obtained from each bin to confirm the sorting accuracy by iodine staining.

## RESULTS AND DISCUSSION

### Sorting Wheat by Protein Content

The single-kernel protein content calibration model had  $R^2 = 0.92$  and  $SECV = 0.47\%$  when using five PLS factors. The reference sample had a protein content range of 9.4–16.3% (Avg = 12.8%; SD = 1.73%), while the validation samples had a protein content range of 12.8–15.4% (Avg = 13.9%; SD = 0.69%). This agrees well with the single-kernel protein content study reported by Delwiche and Hruschka (2000) where they achieved  $R^2 = 0.91$  and  $SECV = 0.37\%$  for hard red winter wheat with a protein content range of 8.4–16.4%. The regression coefficients of the calibration equation occurring at 985, 1140, 1185, and 1435 nm (Fig. 2) agree with those identified by Williams (2001) as some of the principal protein content absorption bands.

Table II shows the protein content of the four unsorted and sorted samples. The average difference between the bins with the highest and lowest protein contents (bins 1 and 4) was  $\approx 3.1$  percentage points. For each sample, the average difference between consecutive bins was  $\approx 1$  percentage point. Thus, the system was effective at sorting samples into four protein content fractions.

### Sorting Wheat by Hardness

The hardness calibration model had  $R^2 = 0.85$  and  $SECV = 10.4$  hardness units when using five PLS factors. The regression equation showed the most important wavelengths occurred at  $\approx 950, 1310, 1405, 1460,$  and  $1650$  nm (Fig. 2). Maghirang and Dowell (2003) reported slightly better results with  $R^2 = 0.88$  and  $SECV = 8.8$  hardness units when using a wider wavelength range of 550–1700 nm. They also reported similar important absorption regions occurring at 1405, 1460, and 1650 nm. These wavelengths likely relate to the protein and starch interface because the degree of adhesion between starch and protein may influence hardness (Simmonds et al 1973).

The hardness indices of the unsorted and sorted samples are given in Table III. The average difference in the hardness of bin 1 and 4 was 17 units, with the smallest and largest differences being 2.8 and 29.4 hardness units, respectively. The average difference between consecutive bins was 5.7 hardness units. The average standard deviation of hardness index within samples was 19.9 units for the unsorted samples, and this reduced to an average of 18.3 units within each bin for the sorted samples. Thus, the SKNIR system was effective at sorting samples by hardness and also narrowed the distribution of hardness within sorted samples.

**TABLE III**  
Average Hardness Index in Unsorted Samples and After Sorting Using a Single-Kernel Near-Infrared System<sup>a</sup>

Sample ID	Unsorted Sample	Sorted Sample			
		Bin 1	Bin 2	Bin 3	Bin 4
115	56.0 (22.8)	46.5 (22.6)	51.8 (21.3)	59.3 (24.8)	67.8 (25.2)
262	79.0 (15.4)	77.5 (12.7)	81.1 (12.6)	82.6 (14.0)	85.2 (13.1)
354	68.3 (18.1)	61.6 (20.4)	70.7 (17.5)	70.5 (16.7)	74.6 (13.7)
356	64.6 (22.4)	52.8 (22.6)	59.0 (24.4)	68.8 (19.9)	74.6 (17.2)
358	66.3 (20.9)	55.3 (21.1)	66.6 (20.0)	74.7 (18.4)	76.8 (17.2)
359	64.0 (23.2)	47.9 (21.9)	57.0 (20.7)	66.6 (22.6)	77.3 (19.4)
365	58.9 (25.3)	43.6 (20.0)	53.5 (20.9)	59.6 (24.4)	67.3 (22.3)
444	65.0 (24.4)	53.3 (25.5)	63.2 (24.5)	69.6 (23.0)	76.6 (18.7)
511	71.9 (23.1)	61.3 (21.5)	64.5 (25.0)	72.7 (22.7)	82.4 (17.6)
513	67.8 (23.4)	54.7 (24.1)	61.2 (21.9)	70.9 (19.5)	77.3 (16.6)
530	68.1 (16.9)	64.0 (13.0)	65.4 (18.3)	69.5 (15.0)	71.4 (17.1)
NE01643	59.2 (15.6)	57.4 (10.7)	53.8 (15.0)	63.3 (14.1)	60.2 (16.9)
Infinity	63.6 (13.8)	63.2 (11.3)	65.6 (12.8)	66.0 (14.0)	67.1 (15.3)
Hallam-04Y	60.3 (13.5)	55.9 (11.3)	57.4 (17.0)	62.1 (13.7)	63.9 (17.5)
441	64.4 (23.7)	55.5 (23.7)	62.7 (22.7)	62.4 (24.0)	77.9 (20.2)
507	60.4 (24.3)	51.7 (19.9)	60.2 (18.6)	65.7 (21.7)	76.0 (15.6)
Hallam-02Y	44.7 (14.7)	36.5 (11.5)	44.4 (13.7)	47.7 (12.8)	52.3 (15.6)
NI98439-02Y	43.1 (16.9)	33.6 (13.3)	42.2 (13.8)	45.0 (12.9)	50.8 (15.4)

<sup>a</sup> Numbers in parentheses indicate standard deviation.

**TABLE IV**  
Percentage of Waxy Proso Millet Kernels in Unsorted Samples and After Sorting Using an Automated Single-Kernel Near-Infrared System

Sample Set	No. Samples	Unsorted Waxy Kernels (%)	Sorted Waxy Kernels (%)
Calibration set	20	87.4–99.8	100.0
	1	93.6	94.7
	1	95.5	95.2
	1	97.9	94.7
Test set (white)	10	78.8–99.4	100.0
	1	95.8	95.0
	1	64.8	95.0
	1	97.4	94.7
	1	88.3	90.0
	1	89.1	90.0
	1	90.4	90.0
	1	89.7	88.2
Test set (red)	6	93.0–99.8	100.0
	1	88.9	95.5
	1	93.9	95.0

### Sorting Proso Millet by Waxy Character

Although the SKNIR system was developed for wheat, it was able to sort millet kernels by installing an air bypass valve in the vacuum line to reduce suction and prevent multiple millet seeds, which are much smaller than wheat seeds, from being picked up by the vacuum ports. The length of the V-shaped trough was also reduced to keep the millet seed centered under the fiber optics. The calibration to separate samples containing amylose (wild-type) from amylose-free (waxy) samples resulted in  $R^2 = 0.65$  and SEC $V = 0.29$  when using six PLS factors. A cross-validation showed that all waxy samples and 89.5% of the wild-type samples were correctly identified. The regression coefficients (Fig. 2) showed important wavelengths at  $\approx 985$ , 1180, 1440, and 1650 nm that correspond to starch absorption regions reported by Williams (2001). These results were obtained by classifying spectra averaged from multiple kernels from one sample, but further analysis showed how the calibration performed when sorting individual kernels.

For the waxy samples, sorting to select out waxy kernels increased the waxy kernels in 42 of the 48 waxy samples (Table IV) and increased the percentage of waxy kernels in these samples from an average of 94% in the unsorted samples to 98% in the sorted samples. Six samples showed an average decrease in waxy kernels from 94.5 to 93%. This slight decrease may be due to sampling error because only 48 kernels were tested per sample and due to the subjectivity involved when the staining is not definitive on

some samples. The calibration set samples had an increase in waxy kernels from 96 to 99%, the white test set samples had an increase from 91 to 97%, and the red test set samples had an increase from 95 to 99% in the unsorted and sorted samples, respectively. Thus, the calibration performed well for the calibration set and test set, including samples with red kernels. It was not expected that the kernel color would affect the sorting accuracy because the SKNIR system does not measure visible wavelengths.

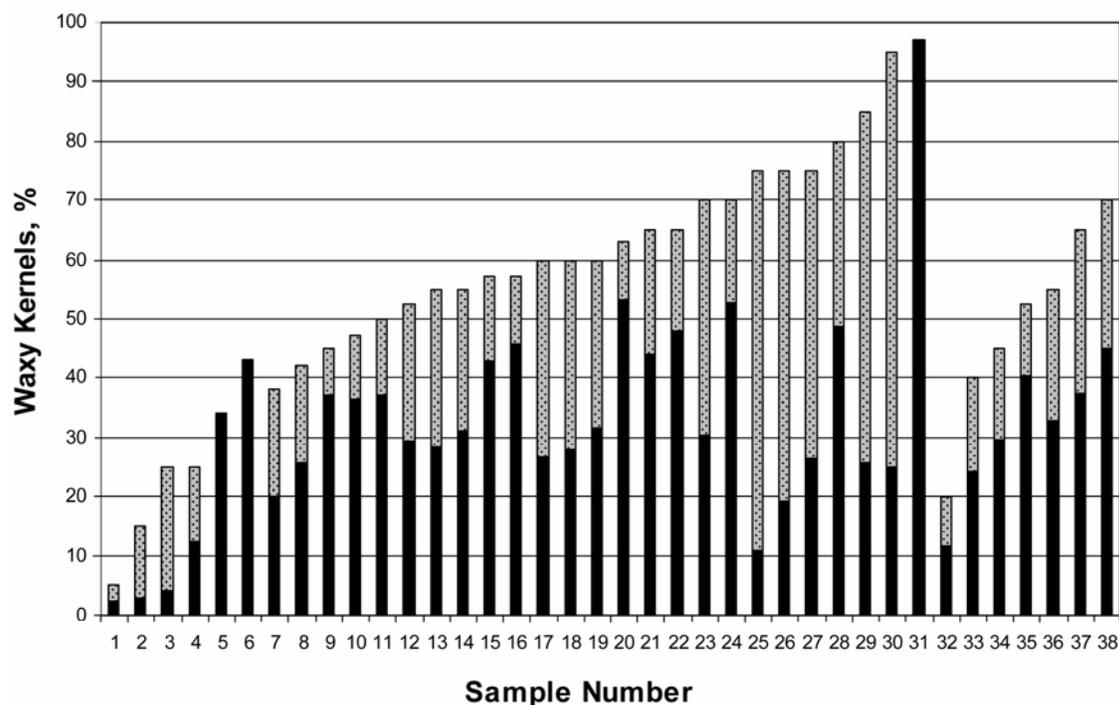
For the segregating samples, sorting increased the waxy kernels in 35 of the 38 segregating samples (Fig. 3). The waxy kernels in the unsorted segregating samples averaged 32%, and this was increased to 55% after sorting. The red samples had an increase in waxy seed from 32% in the original sample to 50% in the sorted samples, and the white samples showed an increase from 32 to 56%. Again, there appeared to be little influence of kernel color on the sorting efficiency, and the system was effective at increasing waxy kernels in sorted millet samples. Some samples responded well to the sorting process, while others show little or no increase in waxy seeds. The reason for this discrepancy in sorting efficiency should be investigated in future tests.

Although the system was demonstrated by sorting wheat by protein content and hardness, and millet by waxy character, the system may have applications in selecting other characteristics such as vitreousness, sprout damage, scab damage, or internal or parasitized insects in wheat. The system could also have applications to other small grains such as sorghum, rice, or barley, or to other biological materials such as fly pupae that have morphological characteristics similar to single grain kernels as demonstrated by Dowell et al (2005).

### CONCLUSIONS

The SKNIR system was effective at sorting kernels based on measured levels of selected characteristics. Wheat samples were sorted into protein content fractions, with the highest and lowest fractions differing by  $\approx 3.1$  percentage points. Samples were sorted by hardness index, with the highest and lowest fractions differing by 17 hardness units. The system was also effective at increasing the waxy seeds in proso millet advanced lines that are predominately waxy, and also in segregating population samples.

After calibrations were developed and installed, the system required little user skill and attention. This system will provide breeders with a tool to nondestructively select desirable characteristics from segregating populations and to increase the purity of heterogeneous advanced or released lines. Experiments are cur-



**Fig. 3.** Percentage of segregating white (samples 1–31) and red (samples 32–38) proso millet kernels in unsorted samples (black bars) and after sorting (shaded bars) using an automated single-kernel near-infrared system.

rently underway or being designed to study the effects of genetics and environment on kernels with selected characteristics obtained using this technology. The system could also be used by various other grain industry segments to measure the distribution of quality characteristics within samples for marketing, storage, or processing purposes.

#### ACKNOWLEDGMENTS

We gratefully acknowledge Robert Rousser, instrument maker, USDA ARS; Duane Walker, retired electrical engineer, USDA ARS; Donghai Wang, assistant professor, Kansas State University; and Yankun Peng, assistant professor, Michigan State University, for their work on developing prototypes of the SKNIR system. We also thank Perten Instruments for providing the commercial SKNIR instrument for this research; the USDA Grain Inspectors, Packers and Stockyards Administration for providing samples used for protein content calibration; and Thomas Robison and Melanie Berry for assisting in spectral data collection. We also thank Donghai Wang; Robert Bowden, research leader, USDA ARS; and Bo Allvin, products and applications manager, Perten Instruments, for reviewing early versions of this manuscript.

#### LITERATURE CITED

AACC International. 2000. Approved Methods of the American Association of Cereal Chemists, 10th Ed. Methods 39-25, 46-11A, and 55-31. The Association: St. Paul, MN.

Baltensperger, D., and Cai, Y. Z. 2004. Minor millet. Pages 261-268 in: Encyclopedia of Grain Science. C. Wrigley, H. Corke, and C. E. Walker, eds. Elsevier Science: Amsterdam.

Bushuk, W., Briggs, K. G., and Shebeski, L. H. 1969. Protein quantity and quality as factors in the evaluation of bread wheats. *Can. J. Plant Sci.* 49:113-122.

Chung, O. K., Maghirang, E. B., Park, S. H., Caley, M. S., Dowell, F. E., and Seabourn, B. W. 2004. Quality parameters of four wheat fractions singulated and sorted based on kernel hardness. Abstract 417. Published online at <http://www.scisoc.org/aacc/meeting/2004/abstracts/>, 2004. AACC International: St. Paul, MN.

Delwiche, S. R., and Hruschka, W. R. 2000. Protein content of bulk wheat from near-infrared reflectance of individual kernels. *Cereal Chem.* 77:86-88.

Dowell, F. E. 1998. Automated color classification of single wheat

kernels using visible and near-infrared reflectance. *Cereal Chem.* 75:142-144.

Dowell, F. E. 2000. Differentiating vitreous and nonvitreous durum wheat kernels by using near-infrared spectroscopy. *Cereal Chem.* 77:155-158.

Dowell, F. E., Throne, J. E., and Baker, J. E. 1998. Automated nondestructive detection of internal insect infestation of wheat kernels by using near-infrared reflectance spectroscopy. *J. Econ. Ent.* 91:899-904.

Dowell, F. E., Parker, A. G., Benedict, M. Q., Robinson, A. S., Broce, A. B., and Wirtz, R. A. 2005. Sex separation of tsetse fly pupae using near-infrared spectroscopy. *Bull. Ent. Res.* 95:249-257.

Evers, A. D., and Bechtel, D. B. 1988. Microscopic structure of the wheat grain. Pages 47-95 in: *Wheat: Chemistry and Technology*, Vol. 1, 3rd Ed. Y. Pomeranz, ed. AACC International: St. Paul, MN.

Graybosch, R. A., and Baltensperger, D. D. 2004. Evaluation of the waxy endosperm trait of proso millet. Annual Meeting Abstracts. CD-Rom No. 4188. ASA-CSSA-SSSA: Madison, WI.

Huebner, F. R., and Gaines, C. S. 1992. Relation between wheat kernel hardness, environment, and gliadin composition. *Cereal Chem.* 69:148-151.

Hunt, W. H., Fulk, D. W., Elder, B., and Norris, K. 1977. Collaborative study on infrared reflectance devices for determination of protein in hard red winter wheat, and for protein and oil in soybeans. *Cereal Foods World* 22:534-536, 538.

Levi, I., and Anderson, J. A. 1950. Variations in protein contents of plants, heads, spikelets, and individual kernels of wheat. *Can. J. Res. Sect. F.* 28:71-81.

Maghirang, E. B., and Dowell, F. E. 2003. Hardness measurement of bulk wheat by single-kernel visible and near-infrared reflectance spectroscopy. *Cereal Chem.* 80:316-322.

Malloch, J. G., and Newton, R. 1934. The relation between yield and protein content of wheat. *Can. J. Res.* 10:774-779.

Martens, H., and Naes, T. 1989. *Multivariate Calibration*. John Wiley and Sons: Chichester, UK.

Martin, C. R., Rousser, R., and Brabec, D. L. 1993. Development of a single-kernel wheat characterization system. *Trans. ASAE* 36:1399-1404.

Morris, C. F. 1992. Impact of blending hard and soft white wheats on milling and baking quality. *Cereal Foods World* 37:643-648.

Morris, C. F., DeMacon, V. L., and Giroux, M. J. 1999. Wheat grain hardness among chromosome 5D homozygous recombinant substitution lines using different methods of measurement. *Cereal Chem.* 76:249-254.

- Nielsen, J. P., Pedersen, D. K., and Munck, L. 2003. Development of nondestructive screening methods for single-kernel characterization of wheat. *Cereal Chem.* 80:274-280.
- Osborne, B. G., and Fearn, T. 1983. Collaborative evaluation of near infrared reflectance analysis for the determination of protein, moisture, and hardness in wheat. *J. Sci. Food Agric.* 34:1011-1017.
- Pasikatan, M. C., and Dowell, F. E. 2004. High-speed NIR segregation of high- and low-protein single wheat seeds. *Cereal Chem.* 81:145-150.
- Pedersen, J. F., Bean, S. R., Funnell, D. L., and Graybosch, R. A. 2004. Rapid iodine staining techniques for identifying the waxy phenotype in sorghum grain and waxy genotype in sorghum pollen. *Crop Sci.* 44:764-767.
- Pomeranz, Y., Bolling, H., and Zwingelberg, H. 1984. Wheat hardness and baking properties of wheat flours. *J. Cereal Sci.* 2:137-143.
- Rittiron, R., Saranwong, S., and Kawano, S. 2004. Useful tips for constructing a near infrared-based quality sorting system for single brown-rice kernels. *J. Near Infrared Spectrosc.* 12:133-139.
- Shadow, W., and Carrasco, A. 2000. Practical single-kernel NIR/visible analysis for small grains. *Cereal Foods World* 45:16-18.
- Simmonds, D. H., Barlow, K. K., and Wrigley, C. W. 1973. The biochemical basis of grain hardness in wheat. *Cereal Chem.* 50:553-562.
- Stuber, C. W., Johnson, V. A., and Schmidt, J. W. 1962. Grain protein content and the relationship to other plant and seed characters in the parents and progeny of a cross of *Triticum aestivum* L. *Crop Sci.* 2:506-508.
- Sunderman, D., Wise, M., and Sneed, E. M. 1965. Interrelationships of wheat protein content, flour sedimentation value, farinograph peak time, and dough mixing and baking characteristics in the F<sub>2</sub> and F<sub>3</sub> generations of winter wheat, *Triticum aestivum* L. *Crop Sci.* 5:537-540.
- Williams, P. C. 2001. Implementation of near-infrared technology. Pages 145-169 in: *Near Infrared Technology in the Agricultural and Food Industries*. P. Williams and K. Norris, eds. AACC International: St. Paul, MN.
- Zayas, I. Y., Martin, C. R., Steele, J. L., and Katsevich, A. 1996. Wheat classification using image analysis and crush-force parameters. *Trans ASAE* 39:2199-2204.

[Received December 29, 2005. Accepted May 11, 2006.]