

PREDICTION OF KERNEL DENSITY OF CORN USING SINGLE-KERNEL NEAR INFRARED SPECTROSCOPY

P. R. Armstrong, J. G. Tallada

ABSTRACT. Corn hardness is an important property for dry and wet-millers, food processors, and corn breeders developing hybrids for specific markets. Of the several methods used to measure hardness, kernel density measurements are one of the more repeatable methods to quantify hardness. Near infrared spectroscopy (NIRS) provides an attractive method to measure kernel density as it can also measure other compositional attributes. Some commercial instruments do measure density of bulk samples. Single-seed NIRS, however, may provide additional information and capabilities by measuring density of individual kernels. This has potential applications for breeders or quality control wishing to look at variance within a sample and for sorting. This study examined the accuracy of NIRS to predict density from single seeds of corn. Absorbance spectra (904 to 1685 nm) were collected on single seeds from 67 food hybrids and 40 commodity hybrids. Moisture adjusted density measurements, using 12-g samples, were made using a gas pycnometer and used as the reference method in the development of the prediction equation. The best prediction model developed from partial least squares regression between averaged spectra and density values had a standard error of cross (SECV) validation of 0.018, coefficient of determination (R^2) of 0.79 and the ratio of the standard deviation to the standard error for the cross-validation model (RPD) of 2.1. Predictions for a validation set of 35 samples yielded a standard error of prediction (SEP) equal to 0.016, $R^2 = 0.76$ and the ratio of the standard deviation to the standard error for the cross-validation model (RPD) = 1.9. Other models developed using different spectral pretreatments yielded very similar statistics. Ten samples were subsequently sorted into low, medium, and high density fractions based on spectroscopic predictions. Pycnometer measurements on the fractions verified they were correctly sorted by density and are correlated to starch content ($r = 0.42$) and oil content ($r = -0.39$).

Keywords. Corn hardness, Kernel density, NIRS, Partial least squares regression.

The hardness of corn kernels is a critical quality parameter defining the suitability for different processes and the quality of finished products. Physically, the size and shape of the kernels along with its mass, density, resistance to milling, and compression define the hardness of corn (Fox and Manley, 2009). Pomeranz et al. (1984) cited hardness affecting several factors such bulk density, storability, insect and fungal infestation, and susceptibility to breakage and thus impacting processing operations (milling quality, energy requirement, wet and dry milling yield characteristics). Dry millers prefer hard kernels to maximize large flaking grits for breakfast foods and snack products (Siska and Hurburgh, 1996; Mestres et al., 1995). On the other hand,

wet millers require softer grains because of shorter steeping times and easier separation of starch and protein (Wu and Berquist, 1991).

Fox and Manley (2009) made an extensive literature review of the different methods used for testing hardness in maize kernels. These included particles size index, Stenvert test, kernel density, tangential abrasive dehulling, rapid visco-analysis, Roff milling index, compression tests, near-infrared spectroscopy and vitreousness. An interesting point in their discussion is that even with such wide assortment of techniques, there has been no accepted universal standard for assessing hardness in maize. There could be several reasons for this such as repeatability of the method, ease, and cost of analysis, need for a non-destructive method and end-use processing methods matching a particular hardness method.

Near-infrared reflectance spectroscopy (NIRS) has been shown as a versatile technique not only because many compositional parameters can be measured simultaneously but also because of its speed and minimal preparation of samples for analysis. A disadvantage of this technique for predicting hardness lies in the fact that calibration models would be dependent on measuring a parameter that has direct or indirect relation to hardness measurements such as density (Siska and Hurburgh, 1996), TADD, Tangential abrasive dehulling device, (Wheling et al., 1996; Lee et al., 2005) and the Stenvert test (Armstrong, et al., 2007). Pomeranz et al. (1984) measured high correlation between

Submitted for review in September 2011 as manuscript number FPE 9378; approved for publication by the Food & Process Engineering Institute Division of ASABE in June 2012.

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density and near infrared (NIR) reflectance at 1680 nm for four hybrid groups of corn. Near infrared transmittance studies (Robutti, 1995; Siska and Hurburgh, 1996; Lee et al., 2005) also found useful predictions of density but most studies have dealt with the shorter wavelength region of the NIR (600 to 1100 nm). Williams et al. (2009) used hyperspectral imaging to distinguish between hard and soft endosperm within a kernel and to classify kernels as hard, intermediate, or soft. They also showed that spectral differences between the two endosperm types were most pronounced at 1450 nm.

Single kernel density measurements should provide an effective tool for breeders to measure hardness. Thompson and Goodman (2006) utilized density measurements for selecting kernels to increase kernel density in a backcrossing program for two inbreds of maize. They used floatation and the mass difference in air and suspension in water as methods to determine density on small 20- to 30-g samples. Both methods were effective in selecting kernels to increase density during backcrossing. These density measurements are somewhat tedious though and selecting the 20- to 30-g subsamples may be somewhat random. If single-kernel density could be measured easily, screening samples would be much more effective. Dowell et al. (2009) has also shown single-kernel selection using NIRS could be used to increase hardness in wheat.

A gas pycnometer instrument for measuring porosity, true and apparent density in corn, wheat, and sorghum has been demonstrated by Chang (1988). In his study, he cautioned that the density measurements for whole kernels are not true density because the pore spaces within the seeds are not reached by the measuring gas. Additionally, moisture within the grains can naturally affect a number of properties such as bulk density (test weight), kernel density, and breakage susceptibility. To account for this, Dorsey-Redding et al. (1990) developed a mathematical moisture correction model for density measurements on samples having moisture contents in the range of 11% to 22% (wet basis). The objectives of this study were to develop non-destructive measurement of corn kernel density using single-kernel NIRS and determine if hybrid samples can be segregated by density.

MATERIALS AND METHODS

CORN SAMPLES

Two sets of samples were used in the study. The first set consisted of 40 samples of various yellow corn hybrids obtained from the Iowa State University Grain Quality Laboratory. These samples were typical commodity corn samples obtained during the 2009 growing season in Iowa. Bulk analyses of protein, starch, oil, and pycnometer density were provided. Reference measurements for the Iowa samples were measured by the Iowa State Grain Quality Lab (GQL) using the following standards, AOAC 990.03 for protein, AOAC 920.39 for oil, and Corn Refiners Association A-20 for starch. Measurement for two of these samples were obtained using an Infratec 1241, FOSS (Eden Prairie, Minn.), calibrated and maintained by

the GQL as opposed to using the above standards. Standard error of prediction (SEP) values for their calibrations were 0.53%, 0.32%, 0.74% for corn protein, oil, and starch, respectively. Seed density was also measured by Iowa State using an AccuPyc 1330 pycnometer (Micromeritics Instrument Corp., Norcross, Ga.). The second set consisted of 67 commercial white and yellow corn samples obtained from Frito-Lay Inc. (Gothenburg, Neb.). These samples were part of an internal evaluation of food corn quality by Frito-Lay in 2010. Chipped or broken seeds and dockage were manually removed from all samples prior to lab tests.

COLLECTION OF SINGLE-SEED NIR SPECTRA

A novel single-kernel NIRS instrument, principally designed to rapidly measure kernel composition at a rate up to three per second was used for this study. A conceptual description and the method of operation are described by Armstrong (2006) and its use is also documented by Spielbauer et al. (2009) and Tallada et al. (2009). The instruments major components were an NIR spectrometer, fiberoptic cable, light tube assembly, control board, and computer. The NIR spectrometer (NIR256-1.7T1-USB2/3.1/50um SNIR 1074, Control Development, Inc., South Bend, Ind.) has a thermoelectrically cooled 256-pixel InGaAs diode array sensor with a spectral measurement range of 904 to 1685 nm. The light tube assembly used a 12-mm internal diameter glass tube illuminated by 48 tungsten light bulbs (Part 1150, Gilway Technical Lamp, Woburn, Mass.) equidistantly arranged in six rows along an aluminum tube concentric to the glass tube. A 2-m long BIF600-VIS-NIR (400 to 2500 nm) bifurcated fiber-optic assembly (Ocean Optics, Dunedin, Fla.) with a 600-um core diameter was attached at both ends of the light tube with the common end connected to the spectrometer. The assembly was oriented at 45° to horizontal to allow a kernel to slide down the glass tube as a spectrum was taken. A photo-electric switch mounted at the upper end of the glass tube was used to trigger the spectrometer when a kernel passed through it and slide down the tube. A Microsoft Visual C++ 6.0 program was written and used for processing spectral data and other control functions. The program was interfaced to the spectrometer via a software library supplied by the spectrometer manufacturer.

The instrument was allowed to warm up for at least one hour to stabilize light output and spectrometer electronics prior to spectra collection. Periodic dark background measurements were made to correct for instrument signal drift. Similarly, measurements of an illuminated white reference (Spectralon®, Labsphere, North Sutton, N.H.) were used for the reflectance computation. Reflectance spectra for seeds were collected and saved for processing using a 43-ms integration period.

REFERENCE MEASUREMENT OF KERNEL DENSITY

A helium gas pycnometer (model SPY2, Quantachrome Corp., Syosset, N.Y.) was used to measure density values for Frito-Lay samples and to verify previous density measurements of Iowa State University samples. The principle behind the instrument and general operation was

described by Chang (1988). In this study, the small holding cell was used to hold about 10 to 12 g of corn. Measurements were taken twice for each sample with an allowable tolerance of 0.01 g/cm³ for replicate measurements. The sample mass was measured using a 10-mg resolution analytical balance. Grain moisture content was measured separately for each sample in duplicate using the standard air-oven method (*ASABE Standard S352.2*, 2008) at 103°C for 72 h. Moisture was corrected using the modified Dorsey-Redding et al. (1990) equation as was done by Siska and Hurburgh (1996):

$$D_f = D_i - 0.00289 (M_f - M_i)$$

where D_f and D_i are the final and initial density values (g/cm³) for corn at M_i moisture content corrected to M_f (14% wet basis).

NIRS KERNEL DENSITY PREDICTION MODEL DEVELOPMENT

Fifty single-kernel spectra were collected from a subset of each sample using the methods previously described. A composite absorbance spectrum was computed for each sample by first applying a mean centering spectral pre-treatment on the individual seed spectrum to account for baseline shift and bring the spectra to a common axis, and then averaging the 50 spectra. Only the range 950 to 1650 nm was considered because of the low signal-to-noise ratio near spectra edges. Thus, a set of 107 mean absorbance spectra were matched with their mean reference kernel density values for model development.

Partial least squares regression (PLS) analysis was performed using the TOMCAT statistical package (Daszykowska, 2007) to develop a prediction model for density from the mean-centered spectral data. A standard normal variate (SNV) spectral pre-treatment was also examined during model development. Full cross-validation regression modeling of the entire set (n=107) was initially used to establish a preliminary number of factors for the model. To further test prediction ability, the samples were divided into calibration and validation samples. This was done by sorting reference density values into ascending order and selecting every third sample for validation samples (n=35) with the remaining used to develop the calibration model (n=72). This assured a broad range of reference values in both the calibration and validation set.

NIRS SAMPLE SORTING BY KERNEL DENSITY

Ten sample sets from the validation set were sorted into high and low fractions using the developed PLS model. These samples represented a range of low, medium, and high density kernels. The procedure to set sorting thresholds was to first determine the NIRS predicted density for 48 kernels, calculate the mean and standard deviation, and set thresholds at the mean, plus and minus one-half the standard deviation. Kernels were then measured and sorted into low, medium, and high density fractions based on these thresholds. Because of single-kernel prediction variability, the average of three measurements for each kernel was used for sorting. Each

sorted fraction contained a minimum of 10 g of kernels in order for pycnometer measurements to be taken. Pycnometer measurements were then made on the low, medium, and high density fractions. Pycnometer measurements were duplicated and averaged for all readings. Sorted sample fractions were measured for bulk composition using a Perten DA7200 (Perten Instruments, Springfield, Ill.) instrument to determine if fractions were compositionally different. The DA7200 is a bulk sample, near-infrared reflectance instrument with calibrations for protein, oil, starch, and density provided by the manufacturer.

RESULTS AND DISCUSSION

NIRS SAMPLE SET COMPOSITION

A summary of composition and density values for the Iowa and commercial hybrid corn samples are shown in table 1. Although the measured density values had low coefficient of variation, the experimental range from 1.21 to 1.36 g/cm³ represent a reasonable span of kernel density. Simple correlations between protein, starch, and kernel density are shown for Iowa samples that had reference measurements performed. Protein had reasonable correlation with density while starch had low correlation. Correlations between composition parameters are shown in table 2 and shows good correlation of protein with density and starch, and starch and oil, starch being inversely correlated in all cases. Other research has also shown starch to be inversely correlated with other parameters to varying degrees (Dorsey-Redding et al., 1990; Ngonyamo-Majee et al., 2008).

NIRS KERNEL DENSITY PREDICTION MODEL

Table 3 shows descriptive statistics obtained for PLS regression models to predict kernel density from composite NIR absorbance spectrum. A cross validation model was completed for all 107 samples as well as a calibration model, 72 samples, to predict 35 validation samples. Mean centered (MC) spectral pre-treatment was used on all spectra, with and without the SNV pre-treatment. The performance statistics are similar for both pre-treatments. Using the Iowa samples alone in an earlier study had resulted in much better model statistics than what was obtained using this larger sample set. The incorporation of the Frito-Lay samples resulted in poorer model prediction performance. Nevertheless, this should improve the robustness of the model. Model statistics for the validation set using only MC spectra ($R^2 = 0.74$, SEP = 0.018, RPD =

Table 1. Summary of statistical composition of samples.

Component ^[a]	N	Range	Mean	SD ^[b]	CV%
Iowa State University Samples					
Density (g/cm ³)	40	1.21-1.35	1.27	0.035	2.8
Protein (%)	40	6.43-15.29	9.46	2.05	21.8
Starch (%)	35	63.70-73.68	70.05	2.63	3.8
Oil (%)	40	3.29-9.87	5.12	1.68	32.8
Frito-Lay Samples					
Density (g/cm ³)	67	1.26-1.36	1.30	0.027	2.1

^[a] Protein, starch, and oil are on a dry matter basis.

^[b] SD – Standard deviation; CV – Coefficient of variation; R^2 – Coefficient of determination.

Table 2. Correlation coefficients between Iowa State composition values in table 1.

	Starch	Oil	Pycnometer Density
Protein	-0.69	0.09	0.77
Starch		-0.76	-0.28
Oil			-0.23

1.8) suggest it is acceptable for rough screening of samples. RPD is the ratio of the standard deviation of the sample measurements and the standard error of the model. Prediction values are plotted against reference density in figure 1. Specific wavelength regions that strongly influence prediction as shown by correlation coefficients from regression (fig. 2), are 960*, 1000, 1125, 1180 to 1280, 1366, 1401*, and 1650 nm (Numbers with an asterisk are wavelengths close to those typically associated with water adsorption). The broad region 1180 to 1280 encompasses some wavelengths associated with protein. In general though, density is a physical property determined in part by chemical composition and by macro and micro-

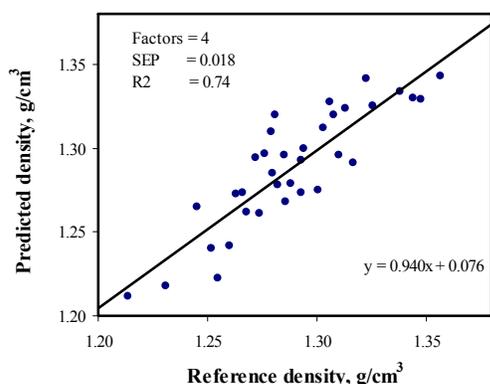


Figure 1. Plot of predicted vs. reference corn density for 35 validation samples.

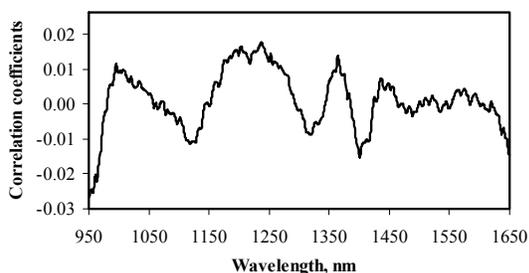


Figure 2. Correlation coefficients for the PLS prediction model for corn density using mean centered spectra.

Table 3. Summary of PLS model statistics for predicting kernel density, g/cm³ from single kernel spectra.^[a]

Modeling	N	Density Range	Mean	SD	Factors	Std Error	RPD	R ²
Mean Center Only								
Cross validation	107	1.212-1.361	1.289	0.033	4	0.017	1.9	0.72
Calibration	72	1.212-1.361	1.289	0.034	4	0.016	2.1	0.78
Validation	35	1.214-1.357	1.289	0.032	4	0.018	1.8	0.74
Mean Center and SNV								
Cross validation	107	1.212-1.361	1.289	0.033	4	0.017	1.9	0.73
Calibration	72	1.212-1.361	1.289	0.034	4	0.016	2.1	0.79
Validation	35	1.214-1.357	1.289	0.032	4	0.017	1.9	0.76

^[a] Mean – mean density of samples; SD – standard deviation of density samples; Factors – number of PLS factors; Std Error – standard error of PLS model; RPD – ratio of the sample standard deviation to the standard error for the model; R² – coefficient of determination of PLS model.

chemical structure. In this regard, density prediction by NIRS may depend on a combination of compositional and structural characteristics, similar to particle size measurement by NIRS. Particle size measurement is basically a measurement of light scattering and is not necessarily wavelength dependent (Pasikatan, 2001).

While sample screening may be useful, several commercial NIRS instruments have this ability. A density calibration developed by Siska and Hurburgh (1996) for an Infratec 1225 Grain Analyzer reported a standard error of prediction (SEP) of 0.0164 gm/cm³ and R² = 0.76. The samples used for calibration intentionally covered a wide temperature range to make predictions more robust. Their SEP is about the same as the error reported for the single kernel instrument in table 3. The standard error of cross validation for density measurement with a Perten DA7200 is 0.012 gm/cm³ (personal communication, Perten Instruments, 2010).

Fox and Manley (2009) explained that there can be a fairly good correlation between kernel hardness and protein and starch composition. This study was able to verify a good linear correlation between kernel density and protein (r = 0.77) for Iowa State samples (table 1), but the correlation with starch was weak (r = -0.28). Dorsey-Redding et al. (1990) found a weak but still significant relationship of density with protein (r = 0.39 to 0.33). The existence of correlation between kernel density and protein suggest indirect prediction of density since protein is commonly predicted using NIR spectroscopy. This result is similar to that encountered by Kovalenko et al. (2006) for predicting amino acids in corn given the fact that amino acid contents are strongly correlated with protein content. They suggested that to verify a prediction model for an amino acid that does indeed predict the amino acid, the model R² value should substantially exceed the squared correlation coefficient between an amino acid and protein values. They reasoned that this test requires that the model's prediction ability to exceed any correlation existing between an amino acid and protein. For the density model, the coefficient of determination (0.73) greatly exceeded the squared correlation coefficient between density and protein (0.52). Based on these criteria, density should not be predicted entirely from an inter-relationship with protein. It is not clear how density is predicted by NIRS but it must be related to compositional parameters that affect the physical structure and overall hardness of the kernels.

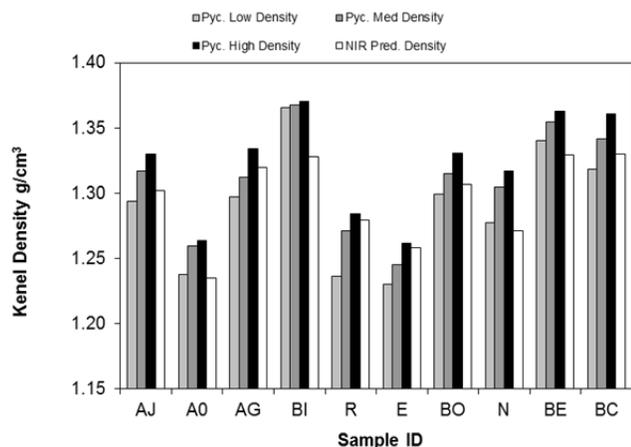


Figure 3. Pycnometer density measurements of sorted samples and from NIRS model predictions.

NIRS DENSITY SORTING

Samples sorted by density were consistently segregated into low, medium, and high density fractions with the average density lying somewhere within this range (table 3). The weight percentage of each density fraction accumulated during sorting shows some variance between these fractions. A graph of the sorted fractions is shown in figure 3. The BI sample shows the least amount of difference between high, medium, and low values and indicates that there is little density variance within the sample. However, the standard deviation of density predicted by NIRS shows this sample had somewhat higher

variation compared to other samples. Because of the error in predicting density with NIRS it is believed that the BI sample variance is actually small based on pycnometer density measurements. To further substantiate this, the standard deviation of NIRS predictions was compared to the difference between the high, medium, and low pycnometer density measurements. It would be expected that these measurements would have some correlation with each other but none was found.

Sorted fractions shows densities of the fractions are consistent with pycnometer readings and single kernel NIRS average predictions (table 4). Composition of sorted fractions are shown in table 5 and were used to derive correlations between compositional parameters, table 6. Correlations between different compositions shows pycnometer density and protein correlation was very poor ($r = -0.19$) and contrasts to that found for the Iowa State samples in table 1, ($r = 0.77$). Correlations between pycnometer density and starch or oil are somewhat improved over Iowa State samples.

Using the average NIR predicted density on a subsample, prior to sorting, was considered the best method in obtaining a workable threshold for density sorting as the error in predicting density makes it difficult or impossible to select the threshold based on a pre-determined density value. Sorting samples into smaller subsets may be possible but was not attempted in this study. The NIRS prediction model developed should have value for screening between samples, but more importantly, for sorting within a sample which would be useful for breeders and agronomists. Singh et al. (2005) examined the effect of field nitrogen levels on

Table 4. Pycnometer density measurements of sorted samples and from NIRS model predictions.

Sample ID ^[a]	AJ	AO	AG	BI	R	E	BO	N	BE	BC
Pycnometer Density										
Low density fraction	1.29	1.24	1.30	1.37	1.24	1.23	1.30	1.28	1.34	1.32
Medium density	1.32	1.26	1.31	1.37	1.27	1.26	1.32	1.31	1.36	1.34
High density fraction	1.33	1.26	1.33	1.37	1.28	1.26	1.33	1.32	1.36	1.36
Weight Percentage % of Sorted Fractions										
Low	33	42	31	23	45	42	29	24	41	39
Medium	39	31	30	36	26	27	36	35	29	25
High	28	27	39	41	29	31	35	41	30	36
NIRS Density Predictions										
NIRS averaged prediction from cross validation model	1.30	1.24	1.32	1.33	1.28	1.26	1.31	1.27	1.33	1.33
Standard Deviation of NIRS predicted values	0.03	0.04	0.05	0.06	0.06	0.06	0.06	0.04	0.04	0.05

^[a] Letters were used for sample identification and have no significance other than identification.

Table 5. Dry basis composition of sorted fractions measured by DA7200.

Sample ID Fraction	AJ			AO			AG			BI			R		
	Low	Med	High	Low	Med	High	Low	Med	High	Low	Med	High	Low	Med	High
Protein (%db)	8.20	7.94	7.67	10.09	10.21	10.18	7.24	7.74	8.45	9.70	8.21	7.60	7.86	8.67	8.87
Oil (%db)	4.35	6.21	7.47	5.59	5.62	5.88	5.50	5.45	4.98	5.59	4.87	3.42	5.38	4.32	4.16
Density	1.24	1.27	1.28	1.17	1.19	1.19	1.27	1.27	1.28	1.24	1.25	1.29	1.19	1.30	1.34
Starch (%db)	69.3	69.7	72.2	65.6	65.9	66.2	70.6	70.4	69.6	66.4	66.8	71.9	68.7	71.2	72.5
Sample ID Fraction	E			BO			N			BE			BC		
	Low	Med	High	Low	Med	High	Low	Med	High	Low	Med	High	Low	Med	High
Protein (%db)	8.96	8.76	8.39	8.81	9.48	9.92	8.05	8.58	9.64	7.09	8.62	9.27	8.66	8.69	8.98
Oil (%db)	6.42	6.32	5.89	3.75	3.98	5.10	4.57	5.21	5.64	3.87	4.02	4.71	5.96	3.91	3.73
Density	1.18	1.21	1.25	1.22	1.25	1.27	1.25	1.27	1.29	1.26	1.31	1.33	1.20	1.29	1.33
Starch (%db)	66.9	66.8	68.4	70.7	68.5	68.5	70.3	69.8	70.1	70.0	71.3	73.5	67.2	68.4	73.3

Table 6. Correlation coefficients between the fraction compositions measured by the DA7200 (table 5) and Pycnometer density (table 4).

	Starch ^[a]	Oil	Density	Pycnometer Density
Protein	-0.44	0.10	-0.25	-0.19
Starch		-0.42	0.83	0.42
Oil			-0.45	-0.39
Density				0.63

^[a] Numbers in bold are significant at $\alpha = 0.05$.

oil, protein, and extractable starch. Findings suggest nitrogen levels strongly influenced protein and extractable starch levels and thus some control of composition can be achieved through agronomic practices. Kernel density traits might potentially be controlled in a similar manner provided a better understanding of what compositional traits affect density. Sorting of hybrids by density and measuring composition of these fractions may help establish better correlations with chemical composition and provide insight on the effects of agronomic and genetic factors. Current commercial NIR instruments can screen bulk samples for density but not sort samples into fractions.

CONCLUSIONS

Measurement of average kernel density using the single-kernel NIRS prediction model provides reasonable accuracy for discrimination between samples. The model developed using partial least squares regression between averaged spectra and density values had a SECV of 0.018 and R^2 of 0.79. A validation set of 35 samples yielded a standard error of prediction SEP of 0.016, and R^2 of 0.76. The variance of NIRS density predictions for single kernels within a sample may not give an accurate measure of true variance. The real value of the single-kernel method is the ability to sort a sample into density fractions using the study's procedure which will allow the examination of the structural and compositional changes between sorted fractions. Pycnometer measurements show sorted fractions were effectively sorted and the density of the fractions were correlated to starch content ($r = 0.42$) and oil content ($r = -0.39$). From a scientific perspective, a better understanding of how kernel density is predicted with NIRS may provide improved measurement.

REFERENCES

Armstrong, P. R., J. E. Lingenfelter, and L. McKinney. 2007. The effect of moisture content on determining corn hardness from grinding time, grinding energy, and near-infrared spectroscopy. *Appl. Eng. in Agric.* 23(6): 793-799.

Armstrong, P. R. 2006. Rapid single kernel NIR measurement of grain and oil-seed attributes. *Appl. Eng. in Agric.* 22(5): 767-772.

ASABE Standards. 2008. S352.2: Moisture measurement- Grain and seeds. St. Joseph, Mich.: ASABE.

Chang, C. S. 1988. Measuring density and porosity for grain kernels using a gas pycnometer. *Cereal Chem.* 65(1): 13-15.

Daszykowska, M., S. Serneelsb, K. Kaczmareka, P. Van Espenb, C. Crouxc, and B. Walczaka. 2007. TOMCAT: A MATLAB toolbox for multivariate calibration techniques. *Chemometrics and Intelligent Laboratory Systems* 85(2): 269-277.

Dorsey-Redding, C., C. R. Hurburgh Jr., L. A. Johnson, and S. R. Fox. 1990. Adjustment of maize quality data for moisture content. *Cereal Chem.* 67(3): 292-295.

Dowell, F. E., E. B. Maghirang, and P. S. Baenziger. 2009. Automated single-kernel sorting to select for quality traits in wheat breeding lines. *Cereal Chem.* 86(5): 527-533.

Fox, G., and M. Manley. 2009. Hardness methods for testing maize kernels. *J. Agric. Food Chem.* 57(13): 5647-5657.

Kovalenko, I. V., G. R. Rippke, and C. R. Hurburgh. 2006. Determination of amino acid composition of soybeans (Glycine max) by near-infrared spectroscopy. *J. Agric. and Food Chem.* 54(10): 3485-3491.

Lee, K. M., T. Herrman, J. Lingenfelter, and D. S. Jackson. 2005. Classification and prediction of maize hardness-associated properties using multivariate statistical analyses. *J. Cereal Sci.* 41(1): 85-93.

Mestres, C., F. Matencio, and A. Louis-Alexandre. 1995. Mechanical behavior of corn kernels: Development of a laboratory friability test that can predict milling behavior. *Cereal Chem.* 72(6): 652-657.

Ngonyamo-Majee, D., R. D. Shaver, J. G. Coors, D. Sapienza, and J. G. Lauer. 2008. Relationships between kernel vitreousness and dry matter degradability for diverse corn germplasm: II. Ruminant and post-ruminant degradabilities. *Anim. Feed Sci. and Tech.* 142(3-4): 259-274.

Pasikatan, M. C., J. L. Steele, C. K. Spillman, and E. Haque. 2001. Near infrared reflectance spectroscopy for online particle size analysis of powders and ground materials. *J. Near Infrared Spectrosc.* 9: 153-164.

Perten Instruments. 2010. Personal communications. Perten Instruments North America, Springfield, Ill.

Pomeranz, Y., C. R. Martin, D. D. Traylor, and F. S. Lai. 1984. Corn hardness determination. *Cereal Chem.* 61(2): 147-150.

Robutti, J. L. 1995. Maize kernel hardness estimation in breeding by near-infrared transmission analysis. *Cereal Chem.* 72(6): 632-636.

Singh, M., M. R. Paulsen, L. Tian, and H. Yao. 2005. Site-specific study of corn protein, oil, and extractable starch variability using NIT spectroscopy. *Appl. Eng. in Agric.* 21(2): 239-251.

Siska, J., and C. R. Hurburgh Jr. 1996. Corn density measurement by near-infrared transmittance. *Trans. ASAE* 38(6): 1821-1824.

Spielbauer, G., P. R. Armstrong, J. W. Baier, W. B. Allen, K. Richardson, B. Shen, and A. M. Settles. 2009. High-throughput near-infrared reflectance spectroscopy for predicting quantitative and qualitative composition phenotypes of individual maize kernels. *Cereal Chem.* 86(5): 556-564.

Tallada, J. G., N. Palacios-Rojas, and P. R. Armstrong. 2009. Prediction of maize seed attributes using a rapid single kernel near infrared instrument. *J. Cereal Sci.* 50(3): 381-387.

Thompson, D. L., and M. M. Goodman. 2006. Increasing kernel density for two inbred lines of maize. *Crop Sci.* 46(5): 2179-2182.

Wheling, R. L., D. S. Jackson, and B. R. Hamaker. 1996. Prediction of corn dry-milling quality by near-infrared spectroscopy. *Cereal Chem.* 75(5): 543-546.

Williams, P., P. Geladib, G. Fox, and M. Manley. 2009. Maize kernel hardness classification by near infrared (NIR) hyperspectral imaging and multivariate data analysis. *Analytica Chimica Acta* 653(2): 121-130.

Wu, Y. V., and R. R. Berquist. 1991. Relation of corn grain density to yields of dry milling products. *Cereal Chem.* 68(5): 524-544.