SHORT COMMUNICATION

Nondestructive Single-Kernel Oil Determination of Maize by Near-Infrared Transmission Spectroscopy

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The utility of near-infrared transmission spectroscopy (NITS) for the nondestructive prediction of oil content in single maize kernels was explored. Calibration models were developed from spectral information gathered between 850 and 1050 nm. Nuclear magnetic resonance (NMR) spectroscopy was employed as a reference method to determine the actual oil content of samples used for calibration development and testing. Various positionings of the kernels in the light path and calibration math treatments were explored. The best NITS calibration yielded a 1.2% standard error of cross validation, which was over four times the standard error of NMR reproducibility. Although not as accurate as NMR, NITS does have utility in selecting kernels with the highest oil content from a segregating population.

KEY WORDS: Maize, near-infrared transmission spectroscopy, oil.

The grain of normal maize hybrids contains 3–4% oil on a dry weight basis (1). However, long-term selection programs have resulted in germplasm with oil contents greater than 19% (2). Numerous breeding programs now have the goal of introgressing the high oil trait from selected germplasm into elite maize inbreds to produce high oil hybrids with competitive agronomic performance (high yield, standability, disease and insect resistance).

Silela *et al.* (3) demonstrated that the rate of oil content gain was significantly greater if breeding selection occurred on a single kernel basis, as opposed to using oil values from composite samples containing all kernels on an ear. To date, researchers have utilized wide-line nuclear magnetic resonance (NMR) spectroscopy to nondestructively determine single-kernel oil content in maize. Experiments conducted by Alexander *et al.* (4) demonstrated a highly positive correlation ($r = 0.99$) between NMR and solvent-extracted oil content determinations in maize. The utility of near-infrared transmission spectroscopy (NITS) for the rapid, nondestructive analysis of the oil, protein and starch content of bulk (500+ kernel) maize grain samples has been demonstrated by Hurburgh (Iowa State University, Ames, IA, personal communication), Koeltzow (FGIS, Kansas City, MO, personal communication) and Orman and Schumann (5). Single-seed moisture determinations have been achieved with NITS for maize (6), soybean (7) and sunflower (8). In this study, we explore the accuracy of NITS for the nondestructive determination of oil in individual maize kernels.

MATERIALS AND METHODS

Individual maize kernel spectra were obtained with a model 1255 Infratec scanning monochrometer near-infrared spectrophotometer equipped with a single-seed adapter (NIR Systems, Silver Springs, MD). Absorbance (log 1/transmission) values were collected at 2-nm intervals between 850 and 1050 nm. Spectra were obtained for all kernels in two positions—embryo side up (toward the light source) and embryo side down (toward the detector). In both cases the tip of the kernel was positioned toward the center of the sample cup holder.

All calibration models were constructed by using ISI software (NIR Systems) with principal component regression (PCR). To select kernels to serve as a calibration set, 10 kernels were randomly chosen from each of 93 bulk maize samples. The bulk samples represented a group of chemically and spectrally diverse germplasm previously used to construct a bulk (500+ kernel) NITS oil calibration on the same spectrophotometer.

The 930 resulting spectra, gathered with the embryo positioned upward, were reduced based on spectral diversity to a calibration set of 73 seeds, by means of the Infratec Calibration Maker software (NIR Systems). Initially, nine independent PCR calibration models were constructed from the 73 kernel calibration set. Calibrations were made with embryo up, embryo down, and averaged embryo up/down spectra. For each set of spectra, zero, first and second derivative math was explored. In addition, 10 spectra were gathered with the embryo positioned upward for each kernel in the calibration set, removing the kernels from the holder and replacing them between each scan. Zero, first and second derivative PCR calibration models were developed from the average of the 10 spectra for each kernel. All kernels used in this study had a low and uniform moisture content—between 8 and 10%. This is typical of maize grain dried and stored for breeding research use.

Single-kernel oil determinations were obtained through the National Center for NMR applications (B. Hawkins and C. Riednour, Colorado State University, Ft. Collins, CO) with a modified NT-200 NMR spectrophotometer operating at 187 MHz. Essentially, the method of Alexander (4) was used, wherein 200 data points were collected on resonance at the echo maxima by using a Carr-Purcell-Meiboom-Gill pulse train. The pulse spacing between 22 μs 180° pulses was 200 μs, with every other echo being sampled. Kernels were centered in a six-turn coil made with #16 gauge copper wire and spaced such that its length was 18 mm with a 13-mm inside diameter. Eight scans were collected for each determination with transmitter and receiver phase cycling. The resulting decays were fit to an exponential function by means of a nonlinear least-squares procedure, and the resulting pre-exponential factor was used as the measure of the oil signal. Calibration was done with weighed portions of pure corn oil and the identical experimental procedure.

RESULTS AND DISCUSSION

The performance of PCR calibration models based on zero, first and second derivative math is shown in Table 1 for

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TABLE 1

Near-Infrared Transmission Spectroscopy Calibration Model Performance for Embryo Up, Embryo Down, and Embryo Up/Down Averaged Spectra (n = 73; % oil range = 13.5)

Embryo position	Math[a]	SECV[b]	R[c]
Up	0,5,5	1.3	0.75
Up	1,5,5	1.3	0.75
Up	2,10,10	1.2	0.69
Down	0,5,5	1.5	0.66
Down	1,5,5	1.5	0.66
Down	2,10,10	1.5	0.65
Up/down	0,5,5	1.4	0.72
Up/down	1,5,5	1.3	0.74
Up/down	2,10,10	1.4	0.72

[a]Derivative, derivative gap, boxcar smooth (ref. 9).
[b]Standard error of cross validation (ref. 10).
[c]Coefficient of determination.

embryo up, embryo down, and embryo up/down averaged spectra. The type of derivative math used had little effect on calibration model performance. Calibrations made from spectra with the embryo positioned up had smaller standard errors of cross validation (SECV) (10) and larger coefficients of determination (R) than calibrations constructed from embryo down spectra. For example, with first derivative math, the embryo up calibration yielded an SECV of 1.3% and an R of 0.75, whereas the embryo down calibration had an SECV of 1.5% and an R of 0.66. Similar results were obtained when zero and second derivative math calibrations were compared. Calibrations developed from embryo up/down averaged spectra performed intermediate to calibration models from spectra with the embryo positioned only up or down. It was concluded that spectra collected from kernels positioned with the embryo down yielded no useful information beyond that obtained from spectra gathered with the embryo positioned upward.

In an attempt to improve the performance of the embryo up models, calibrations were developed from the average of 10 spectra obtained for each kernel in the calibration set. The kernels were removed from the spectrophotometer holder and reinserted between each scan. This multiple scanning did not improve calibration performance (Table 2). For the first derivative PCR calibrations, the SECV remained at 1.3% for both models, but the R values were 0.75 and 0.72 for models developed from the single and the ten averaged spectra, respectively. As with the calibrations developed from single spectra, derivative math selection had little effect on calibration model performance.

The performance of NITS calibrations developed for bulk (500+kernels) sample and single-kernel oil analysis are compared in Table 3. These results demonstrate that calibration methods that have been used to accurately predict the oil content of bulk maize samples do not perform as well as NMR for single-kernel predictions. The bulk-sample NITS calibration model predicted the oil content of maize grain almost as accurately as did NMR (Table 3). The SECV of the bulk-sample NITS calibration was 0.5%, relative to a standard error of 0.3% for NMR reproducibility. The SECV of 1.3% for individual-kernel NITS oil prediction is four times greater than that of the

TABLE 2

Near-Infrared Transmission Spectroscopy Calibration Model Performance for Single and 10 Averaged Spectra per Kernel; All Samples Positioned Embryo Up (n = 73; % oil range = 13.5)

Type of spectra	Math[a]	SECV[b]	R[c]
Single	0,5,5	1.3	0.75
Single	1,5,5	1.3	0.75
Single	2,10,10	1.2	0.69
10 Averaged	0,5,5	1.4	0.68
10 Averaged	1,5,5	1.3	0.72
10 Averaged	2,10,10	1.4	0.70

[a]Derivative, derivative gap, boxcar smooth (ref. 9).
[b]Standard error of cross validation (ref. 10).
[c]Coefficient of determination.

TABLE 3

Near-Infrared Transmission Spectroscopy Single-Kernel *vs.* Bulk-Sample Principal Component Regression Calibration Performance for % Oil Prediction

	Single-kernel[a]	Bulk-sample
Calibration math[b]	1,5,5	1,5,5
Number of samples	73	93
% Oil range	13.5	8.7
SECV[c]	1.3	0.5
SE lab[d]	0.3	0.3
R[e]	0.75	0.97

[a]Calibration developed from individual spectra of kernels positioned embryo up.
[b]Derivative, derivative Gap, boxcar smooth (ref. 9).
[c]Standard error of cross validation (ref. 10).
[d]Standard error of nuclear magnetic resonance oil determination.
[e]Coefficient of determination.

NMR standard error. However, the major goal of most breeding experiments is only to identify the best performing individuals from a widely segregating population. Although the accuracy of NMR is desirable, many breeding programs currently use NITS to predict the chemical composition of bulk samples. NITS offers an optional means to select kernels with the greatest oil content without the need to purchase and maintain an additional instrument. For most plant-breeding applications, quickly choosing the right kernels for generation advance is more important than determining the absolute value of a constituent.

To assess the ability of NITS to select kernels with the highest oil content from a diverse population, a set of 48 kernels, not used in the calibration set and representing an even distribution in oil content between 4.0% and 14.5%, was utilized. Individual spectra and 10 averaged spectra were gathered for each of the 48 kernels. Oil content was then predicted for each kernel from the first derivative PCR calibration developed from single spectra and 10 averaged spectra, respectively. For each data set, the top 25% of the population for oil content was identified and compared to those samples that would have been chosen from NMR data. The calibration developed from 10 averaged spectra produced 75% agreement with the NMR selections, while the calibration developed from single spectra had only 58% agreement. Hence, even though both the calibrations had similar SECV and R values, the model developed from the averaged spectra performed better on the diverse test set.

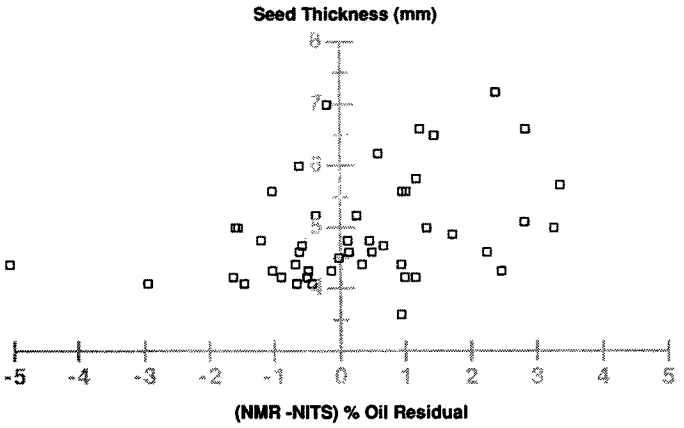

FIG. 1. Plot of [NMR − NITS (first derivative PCR with single spectra calibration)] % oil residual as a function of seed thickness (n = 48). NMR, nuclear magnetic resonance; NITS, near-infrared transmission spectroscopy; PCR, principal component regression.

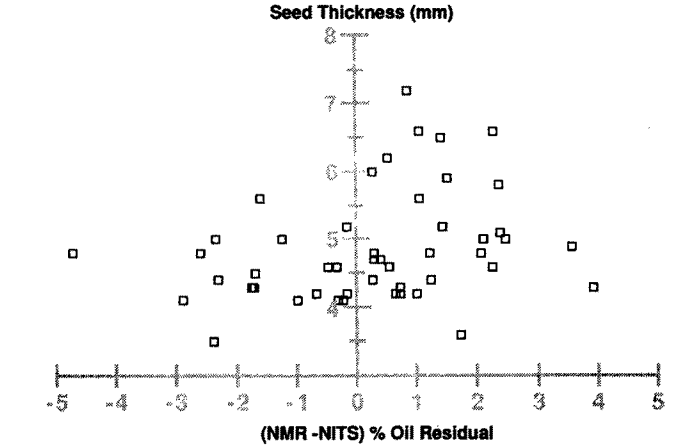

FIG. 2. Plot of [NMR − NITS (first derivative PCR with 10 average spectra calibration)] % oil residual as a function of seed thickness (n = 48). NMR, nuclear magnetic resonance; NITS, near-infrared transmission spectroscopy; PCR, principal component regression.

To examine the effect of kernel size on NITS prediction performance, the residuals between NMR and NITS predicted oil values for the 48-sample test set were plotted as a function of seed thickness. Figures 1 and 2 display residual plots for first derivative PCR NITS calibrations developed from single spectra and the 10 spectra averaged per kernel, respectively. The distribution of the residuals appears to be independent of seed size, except that for large kernels, with thicknesses greater than 5.5 mm, the residuals are always positive (NMR values greater than the NITS predictions). Construction of new PCR first derivative calibration models based only on kernels from the original calibration set with thicknesses between 4.0 and 5.5 mm (n = 50) did not improve calibration performance. It was concluded that variation in kernel thickness is not limiting NITS oil content prediction performance.

In its current state, NITS does not predict the oil content of single maize kernels as accurately as NMR. However, for selecting kernels with the highest oil content from a segregating population, NITS has utility. In addition, NITS offers the potential to predict protein and starch in individual maize kernels, which is not possible with NMR. Software and/or hardware modifications to existing transmission spectrophotometers to gather multiple spectral scans per kernel automatically have the potential to improve single-kernel constituent prediction performance.

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